The Past, Present and Future of Dutch Teratological Collections

From enigmatic specimens to paradigm breakers

Lucas Boer

The Past, Present and Future of Dutch Teratological Collections

From enigmatic specimens to paradigm breakers

Lucas Boer

Colophon

Author: Lucas Boer

Cover: Double herm bust of José Nicolás de Azara and Anton Raphael Mengs by Giovanni Volpato (1785) which is looking into the past and the future. The overlay of the human eye can be designated as the observer of the present. The blackness of the pupil represents the many unanswered questions in teratological research. Designed by Lucas Boer and Tim Rijnhout.

Lay-out: Theo Hafmans Fotografie & Lucas Boer

Printed by: Ipskamp Printing

ISBN: 9789081835633

Publication of this thesis was financially supported by the Department of Anatomy from the Radboud university medical center and the friends foundation of the Museum for Anatomy and Pathology in Nijmegen (The Netherlands). The work presented in this thesis was carried out within the Radboud Institute for Health Sciences (RIHS) graduate school.

The Past, Present and Future of Dutch Teratological Collections

From enigmatic specimens to paradigm breakers

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. dr. J.H.J.M. van Krieken,
volgens besluit van het college van decanen
in het openbaar te verdedigen op woensdag 18 september 2019
om 14.30 uur precies

door

Lucas Leendert Boer

geboren op 27 april 1988
te Oss

Promotoren:

Prof. dr. D.J. Ruiter
Prof. dr. R-J. Oostra (Amsterdam AMC)

Copromotoren:

Dr. A.N. Schepens-Franke
Dr. W.M. Klein

Manuscriptcommissie:

Prof. dr. H.G. Brunner (voorzitter)
Prof. dr. F.P.H.A. Vandenbussche
Prof. dr. R.R. van Rijn (Amsterdam AMC)

Voor baby Mylena († 2016)

Table of Contents

1 General introduction and outline of the thesis

This thesis concerns past, present and future descriptive and explorative perspectives of Dutch teratological collections. These collections consist of fetuses and newborns with congenital anomalies. The research executed throughout this PhD tract has crystallized in both historically and medically oriented topics. The reason for this thesis was born after inventorising the teratological collection of the Museum for Anatomy and Pathology of the Radboud university medical center in Nijmegen (The Netherlands) in 2012. This collection was poorly documented and somewhat neglected. Both scientific and esthetic attention was essential to fully exploit its educational and museological potentials. In the following years of subsequent research a novel and publicly accessible teratological exhibition was realized and opened in 2017: a unique working environment for (post)academic and public education (See addendum). Besides investigating the teratological collection in Nijmegen, collaboration was sought to scientifically explore other Dutch teratological collections. Interestingly, a profound literature dearth—and therewith a knowledge gap—appeared to be present on the topic of (institutionalized) teratological collections. Although many European museums retain and house such valuable collections, they are rarely scientifically and/or educationally exploited and predominantly exist for historical reasons, which is why these collections are often seen and interpreted with archaic thoughts, norms and values.

Each chapter of this thesis is devoted to teratological collections and/or specimens and focuses on historical considerations with embryological and accompanying (dys)morphological aspects. In addition, teratological collections are studied to find answers in etiological and pathogenetic clues in order to test certain currently used etiopathogenetic paradigms. Finally, contemporary techniques are used to unravel the internal characteristics and/or diagnosis of teratological specimens itself.

What follows is an introduction on multiple general facets of teratology. In addition, the scientifically exploitable characteristics of teratological collections is denoted and further delineated in the various included chapters. Finally the aims and outline of this thesis are given.

Since much of the work presented in this thesis is derived from research in teratological collections—mostly affiliated with a medical university museum—it is logical and perhaps necessary to start with some basic museological contemplations.

Museum

The concept of a museum

The word "museum" finds it origin from the ancient Greek word *Μουσεῖον* (Mouseion) which designated a place or temple dedicated to the Muses: the patron divinities and inspirational goddesses of literature, science and art in Greek mythology—better known as the Ancient University or Museum of Alexandria. [1] In subsequent times no noteworthy museological activities arose and it was not until the 18th century that the first publicly accessible collections arose. [2] Observers were being confronted with completely unknown objects, curious natural objects, artefacts and organisms, many of which originated from the newly discovered territories in Africa, the Far East and the New World; an entirely new perspective opened. [3] These collections were primarily the property of dedicated naturalists or wealthy aristocrats. The often underlying reason to collect certain specimens was the desire to foster (scientific) curiosity and wonder about the diversity of things created by nature. [4] These mostly privately owned collections were seen as "cabinets of curiosities" and well known among the wealthy and cultivated of that time. Besides that these cabinets were seen as places where objects were housed, they were an inspirational environment and the center of literary gatherings where fresh and continuous dialogues were established and even relationships were formed. [4] A dialogue between the (privileged) visitor, the owner of the cabinet and the professional was a predominant existing factor. [5] Over time, these anecdotally collected cabinets were bought by or donated to institutions after their (original) collectors deceased. Historical cabinets became the foundation of the present museums; inheriting the past epochs of collecting. These museums show historical reflections, different time spirits, memories, morals, norms and values—captured inside the objects itself or in the often abundantly present treatises.

Nowadays, a museum is a (governmental) non-profit institution or organisation in the service of society and its development. A museum is open to the public and is dedicated to acquire, collect, preserve, interpret, investigate, communicate and (permanently or temporarily) exhibit their tangible and intangible heritage of humanity and its environment for the purposes of education, study and enjoyment. [6] According to the International Council of Museums (ICOM), the most comprehensive directory Museums of the World covers more than 55,097 museums in 202 countries. [7] However, estimations exist that produce a figure close to 80.000 museums throughout the world. [8] This enormous amount of museums indicates a great social

importance and apparent inborn tendency to archive and display the worlds material and immaterial (cultural) substances. These public places can be seen as an entertaining and enlightening way to spend the day, but on a more altruistic thought these institutions can be considered as "storehouses of knowledge," and cultural heritage for the future offspring. Every "modern" and "self-respecting" museum should have three major attributes that identify its role as a museum which closely intertwine the relationship between collecting, exhibiting and researching its collections. [9] These three activities conventionally connects a museum to the community in three ways: 1) museums preserve, document and collect evidence for present and future generations, 2) museums create novel exhibitions to contribute to community development and education, and 3) museums perform or stimulate research which keeps their knowledge alive and involves them in projects with social impact. [10] Finally, it could be stated that research is (or should be) the inspirational *Alma Mater* of any exhibition or collection and the collection itself should be the main motive for a visit. [11] Finally, these priceless collections should be preserved and bequeathed for future generations to ensure their sustainability. [12]

How to create a novel and contemporary exhibition?

Since much of the work presented in this thesis originated during the creation of a novel teratological exhibition in the Anatomical Museum of the Radboud University in Nijmegen, some general exhibitional considerations should be given.

The focus of a new exhibition should be grounded and centered on a general but innovative idea that is powerful enough to yield a multiplicity of derived ideas and should either be presenting something new, or put something—which already exists or is known—in a new perspective. [13] These new perspectives will ideally provoke and create wonder, curiosity and knowledge. [14] The general idea, as those derived from it, should be attractive and interesting, with widely linkable implications to other (scientific) entities such as e.g. history, literature and art. [15] Novel museological exhibitions should present ideas to its visitors, or help these visitors to understand ideas through a process of assisted discovery. [3] A high-quality exhibition should assist the observer in the formulation of his or her own questions. This does not imply that all (arising) questions have straightforward answers. To extend the impact of the exhibition some challenging questions should be left open to stimulate visitors to think about after they left the exhibition or museum. Afterwards, these open endings could provoke dialogues in a different (community) setting.

Novel exhibitions should be question-inspiring and thought-provoking. [16] Furthermore, an exhibition should ideally promote surprise, mystification, astonishment and create ground for extended inquiry. [17] Additionally, novel presentation techniques should be offered to contrast or stimulate certain situations or particular (exhibited) aspects. Digital complements can promote a paradox, can help to foster inner reflection or create a dialogue with other observers. Furthermore, a public exhibition should promote that visitors are welcomed to leave their comment, [14] especially when exhibitions are created which potentially awake strong emotions and opinions—as could be the case in exhibiting teratological specimens. [3] Finally, a qualitatively valuable exhibition should be an unfinished intellectual dialogue that aims to change the visitors' knowledge, attitudes and behaviors and changes the way he or she perceives the world, albeit only minimally. [18]

Teratology

Etymologizing teratology

It was the French zoologist Isidore Geoffroy Saint-Hilaire (1805-1861) who introduced the term "teratology." [19] Teratology stems from the Greek words *το τέρας* (monster or marvel) and *ὁ λόγος* (word, reason, thought, but often read as "the study of") freely interpreted as the study of monster or marvel. Historically rationalized, *το τέρας* implies that the subject—e.g. the child—is seen as a monster. However, on the other hand it concerns a marvel; this dually translatable word indicates the exact sensitivity and stigmatized nature that is often present in many teratological collections. Observers are shocked to see "monstrous births," although they are curious and fascinated by the "wondrous creations" nature can produce. This twofold interpretation is still actual and makes teratological collections—as well as exhibiting other human remains—prone for stigmatization and (ethical) debate. [20] The challenge for a medical museum—exhibiting human remains including teratological specimens—is to diminish this dichotomy and exhibit their specimens in a way that observers become intrigued and leave the stigmatized emotions aside as much as possible.

The study of congenital anomalies

The study of abnormal prenatal development and congenital malformations is generally covered by two overlapping disciplines: teratology and dysmorphology. Teratology is a medico-biological discipline that deals with the pathogenesis and epidemiology of congenital anomalies. [21] A contemporary description of teratological research can be defined as a science that focuses on the causes, patterns, mechanisms and manifestations of developmental deviations of either structural or functional nature. [22] Dysmorphology is a medical, mostly paediatric and clinical genetic, discipline focusing on the symptomatology of physically apparent patterns of congenital anomalies and its clinical diagnosis. [23] Congenital anomalies or developmental defects comprise all structural and functional deficits detected in the implanted embryo, fetus or in the neonate, infant, child and adult. They might be macro- or microscopic, on the surface or within the body, sporadic or familiar, hereditary or nonhereditary and single or multiple. [24] The majority of scientific studies of congenital anomalies is executed in a clinical (research) setting. However, the study of congenital anomalies can also be done in a more historical and museological setting.

A historical perspective in the study of congenital anomalies

Natural phenomena always intrigued mankind—the birth of a child with any malformation was, and still is, subject of wonder and unbridled fantasy. [25] Throughout mankind congenital anomalies tantalized human inquisitive powers. [26] These capricious births were more than just singular cases of rare congenital anomalies, they were initially perceived and considered as omens, hybridizations, divine interventions or even punishments of supernatural origin and were thus rather mystically and vaguely assigned. [27] However, they defied an incorporation into a scientific discipline only until approximately the last few decades: indistinctly described phenomena were rationalized and placed into a framework of exact sciences. [28]

From the second half of the 15th century the rise of the printing press—and with that descriptive sciences—helped spread scientific knowledge like never before. It was during the 16th century that the earliest, although subjective, descriptions of congenital anomalies appeared. [27] A plethora on quintessential prodigy books flourished during the 16th and 17th century: anomalous births were wondrously and enigmatically perceived and abundantly depicted. [25] Among the many treatises a few are noteworthy in this perspective. In the credulous chronicle from Conrad

Lycosthenes (1518-1561) entitled *Prodigiorum ac ostentorum Chronicon* [29] more than 1500 woodcuts of congenital anomalies embellishes this compendium. Most depicted illustrations are hybridizations of imaginative and fabulous conglomerates of both human and animal origin. Interesting is that some depictions show reasonable accurate representations.

In 1573 the French surgeon Ambroise Paré (1510-1590) published *Des Monstres et Prodiges*. [30] In this work an astonishing number of hybrid prodigies of both animal and human characteristics are depicted. Paré catalogued thirteen causes of malformations and listed—among others—the glory of god, the craft of the devil, demons and witches, and maternal imagination as the cause of congenital anomalies. Besides these rather enigmatic causes, some more rational thoughts were already contemplated and included hereditary and accidental illnesses, trauma of the pregnant woman and the narrowness of the womb as causes of congenital anomalies. [25] Initially written for midwives, in the posthumously appeared work of Jacob Rueff (1500-1558), the fifth part of the *De Conceptu et Generatione Hominis* [31] consists out of a comprehensive depiction of congenital anomalies. Residents from that period were morbidly interested in anomalies, remnants of the religious and superstitious basis of medicine are seen in the uncritical mingling of accurately depicted true malformations along with imaginary monstrosities. [32] The rise of scholars inclined to use reason in an attempt to explain the world which included congenital anomalies. Different works are noteworthy and include that of Johann Georg Schenck von Grafenberg (1560-1620) with his book entitled *Monstrorum historia memorabilis.* [33] Attempts are made to rationalize congenital anomalies, although emblematic and fabulous drawings flourished throughout this work.

In 1634, *De Monstrorum causis, natura et differentiis* [34] from Fortunio Liceti (1577-1657) appeared who described and categorized different anomalies according to their dysmorphological appearance—instead of categorizing according to the cause of the anomaly. This work can be seen as the first of its kind and flourishes with elegant copperplates. In the posthumously appeared *Monstrorum Historia* [35] from Ulisse Aldrovandi (1522-1605) attempts are made to scientifically categorize congenital anomalies based on certain patterns or site of abnormality and included more than 350 woodcut illustrations of anomalies in humans, animals and plants. Besides excellent depictions of actual specimens, many apocryphal ones appeared.

Interestingly, the common characteristic to all above-mentioned treatises and chronicles are the uncritical juxtaposition of intertwined fabulous imaginative creatures drawn from hearsay of both human and animal origin and the depiction of relative accurate depictions of genuine malformations (Fig. 1).

Fig. 1. Depictions from the early 17th century work of Fortunio Licetus (1577-1657) entitled: *De Monstrorum causis, natura et differentiis.* [34] The upper print shows imaginative hybrid creations of human and animals. However, besides the many fictional and fabulous depictions, some relative accurate engravings are present. The lower left drawing does not immediately imply a known congenital anomaly, as for the lower right depiction it is clear that it concerns a parapagus dicephalus.

While superstition and fantastical explanations of congenital anomalies preponderated, teratology as a scientific discipline incorporated the heydays of "enlightened science" and it was during the mid-seventeenth to early 18th century that curiosity began to replace the superstition-filled era in which congenital anomalies were perceived until then. The formation of numerous scholarly societies grew, which in turn led to more detailed medical studies: objective studies of birth defects would rise and studies would be placed in a more rational scientific investigational point of view. It was between 1812-1818 when Johann Friedrich Meckel, the Younger (1781-1833) published his three books entitled *Handbuch der pathologischen Anatomie* [36] and developed for the first time an extremely accurate system of human malformations with details of all gradations of all than known anomalies—the first systematic and methodological investigation on congenital anomalies was born and it is this work that is now credited as the beginning of scientific teratology. [27] Meckel was convinced that knowledge of normal embryogenesis must precede a comprehension of the process of abnormal development. As stated earlier, it was the French zoologist Isidore Geoffroy Saint-Hilaire (1805-1861) who introduced the term "teratology." [19] Therefore, he can be seen as the patriarch in scientifically and systematically describing the field of teratology, many of which have been perpetuated. The aforementioned periods were followed by an interval in which vast amounts of literature on descriptive and experimental teratology appeared. In subsequent decades further development of knowledge and society led to the recognition that the causes of congenital anomalies are manifold. [22] During the mid-twentieth century major progress was made in e.g. experimental embryology, epidemiology, teratogenesis, cytogenetics and the characterization of clinical syndromes. [27] One well-known example—out of the multitude of examples—which increased the interest in clinical teratology and understanding of inducement of developmental defects is the thalidomide episode during the early 1960's. [37] It became clear that agents which produced minimal toxicity in the adult could induce severe embryotoxicity. [38]

In current times, prenatal and postnatal diagnostics such as conventional karyotyping, chromosomal micro-array (CMA) and quantitative Fluorescence-Polymerase chain reaction (QF-PCR) can detect the underlying chromosomal abnormality up to 40%. This means that in 60% of the cases the underlying genetic lesion remains undefined. [39] With advances in genomic technologies, the diagnostic yield of testing with for instance whole exome and genome sequencing could increase the prognostic implications for the fetus. [40] This premise is further delineated in the general discussion of this thesis.

Epidemiology and etiopathogenesis of congenital anomalies

The length of normal human gestation lasts between 37 and 42 weeks and can be divided into generally accepted phases: fertilization, blastocyst formation, blastocyst implantation, embryological development and fetal development. [41] The embryo is most vulnerable and sensitive to toxic substances between 3.5 and 9.5 weeks after fertilization during organogenesis. However, shared molecular determinants, spatial contiguity and close timing of morphogenetic events during blastogenesis imply that most malformations which arise during blastogenesis—up until the first four weeks of development—are polytopic and usually of unknown etiology. [42] Contrarily, defects of organogenesis tend to be monotopic malformations. [43] Substances which potentially interfere with intra-uterine growth are called teratogens. [44] The most common patho-mechanisms of teratogens recognized so far are oxidative stress, vascular disruption, hyperacetylation of histones, cholesterol and retinoic acid imbalances, alteration of folate metabolism and folate antagonism and endocrine disruptions. [45] The causes of congenital anomalies vary markedly but they mostly remain indecipherable. [39] A large study by Toufaily *et al.* (2018) [46] identified the apparent etiological factors for 7020 infants with malformations among 289,365 births and concluded that they were genetic in 21.2%, multi-factorial in 20.8%, uterine factors and twinning in 3.2%, environmental factors in 3.4% and unknown in 51.5%. The collectively recorded prevalence of all congenital anomalies is between 2 and 2.4% of all live births. [47:48] However a transitional region exists—rather than a sharp boundary—between normal morphology and congenital anomalies which includes anatomical variations. [21] Although these variations deviate from the standard pattern of the human building plan, they are not necessarily disadvantageous to the affected subject.

A crude distinction can be made between endogenous and exogenous induced factors that can affect the developing embryo or fetus. [49] They can individually cause an anomaly, although a combination of both can also be the cause of the developmental defect. Endogenous or intrinsic factors include more than 5000 presently known genetic defects (mutations) and many chromosomal imbalances in which there is a net gain or loss of genetic material such as e.g. meiotic nondisjunctions with subsequent aneuploidies. [50] Aneuploidy is present in approximately 0.6% of newborns and nearly 70% of spontaneous abortions, indicating that they are very common in humans. [51] Exogenous or extrinsic factors include the enormous variety of environmental inputs such as nutritional deficiencies or excesses and a wide variety of (maternal) infections and chemicals. [44] Many toxic substances and infections

can be transmitted via the placenta and can subsequently render the fetus either deprived of or overexposed to certain agents; causing a cascade of errors and in some cases a developmental defect or delay. [22] It is generally recognized that approximately 70 extrinsic substances could potentially interact with human development and that more than 1200 chemical and physical agents produce developmental defects in experimental animals. [52] How many of these agents actually produce developmental defects in humans remains elusive. [53]

Due to e.g. ecological or geographical factors, the distribution of congenital anomalies is unequally divided over the global population. Therefore prevalence's of specific anomalies can differ profoundly among different populations. For instance, certain genetic conditions are significantly more prevalent in geographically or culturally isolated communities in which ancestral mutations are preserved in sequent generations. If a small group in a population splinters off from the original population and forms a new one, genetic alterations can become part of a stationary and increasingly homogeneously "pool of genes." The new colony may have less genetic variation than the original population. This is well-known as the "founder effect" as introduced by Mayr (1942). [54] This genetic drift will eventually creates inbred communities that are vulnerable to extinction or predisposed to often very specific and rare congenital anomalies. A well-known example of this inbred is seen in Vadoma people of the Bantwana tribe living in near seclusion along the Zambezi River Valley in Zimbabwe which are better known as the ostrich people (Fig. 2). The condition these secluded people inherited is known as ectrodactyly, a terminal limb defect, with prevalences in the general population between 1-9 in 100,000 live births. [55] Besides that ecological and geographical issues can cause deviant prevalences for congenital anomalies, exogenous factors such as e.g. intoxications and maternal infections can also develop in community-specific prevalences with additional relations to resource-constrained and socioeconomic stratification. [21]

Fig. 2. Photograph of the Vadoma people of the Bantwana tribe from the North of Zimbabwe affected by the severe terminal limb defect ectrodactyly, better known as the ostrich people or two-toed tribe.

Institutionalized teratological collections

Historical teratological specimens and contemporary teratological research

As described earlier, the early decades of the 19th century are seen as the golden age of descriptive and gross teratology in Europe; morphological studies of malformations attained levels of excellence rarely seen today. [25] Especially those specimens presented with dramatic dysmorphologies and rarely occurring birth defects attracted special attention and interest. [27] In this period, thousands of teratological fetuses were collected throughout Europe and some exquisite collections are still present and maintained in different Dutch museums. Among others, *Museum Vrolik* (Amsterdam), *Museum Anatomicum* (Leiden) and *Museum Bleulandinum* (Utrecht) are places in which many dysmorphological fetuses are kept. Due to the lack of available prenatal diagnostics—and thereby the additional absence of a possible intervention during pregnancy—it is inherent to these collections that a nowadays rare glance is revealed when a developmental defect proceeds well into the third trimester. In most cases a subsequent perinatal death due to the anomalies' severe nature occurred—leaving the deceased fetus or newborn destined to become included in a so-called "cabinet of curiosity." For some

observers, these collections are imbued with negative feelings ranging from intangible, repulsive, sensitive to horrendous and often difficult to watch. These understandable feelings make these collections prone to neglect, stigmatization and matter for provocative disrespectfulness of their onlookers (author's personal experience). The often made—almost naturally present—analogy with a cabinet of rarities frequently amplifies the observers' emotions. Besides these already strong emotions, the fact that many specimens are near or full-term newborns creates awareness of the fact that these museologically exhibited objects are children of bequeathed and bereaved parents that became objectified museological elements over time. However, each specimen bares a (past) story—which is unfortunately in most cases unknown due to the normative process of anonymization of these decedents in the past. Without a story these specimens remain silent objects which only show failing embryological development but could be prone to un-acceptance and even misbehavior of their observers. Historically rationalized, these deceased children were seen as inert and impersonal entities. In today's society they are increasingly depicted as personified entities. [56] Donation of a decedent child to a university is almost unthinkable within a modern context.

Except the "emotional-filled" reaction of the visitors, wonder, curiosity and sheer fascination often accompanies the mind of the spectator. Besides this "observers influenced" stigmatization, economic downturns, space crunches and institutional apathy are topical elements old medical museums—and therewith teratological collections—have to deal with. This makes extant collections unique, although vulnerable time capsules that show the perceptions, attitudes and superstitions of past epochs. [57] Moreover, some institutions were closed due to legal reasons; the way in which the material was exhibited had become incompatible with current safety standards or the ethics regarding the way these objects were collected initially was rejected from a modern point of view. [12]

On the other hand these collections are important to treasure because they can provide "critical missing links" in for instance anomalies that are so rare that only a few specimens have ever been described adequately; the only existing examples are those present in these old medical collections. It is imaginable that, through the course of many decades of collecting, multiple anomalies of the same type are brought together, creating the unique possibility that research can be performed on very rarely occurring birth defects avoiding the N=1 phenomena (or restriction) which is often present in describing rare anomalies in individual clinical case studies. It becomes possible to further expand the clinical heterogeneity or spectrum of an anomaly. Furthermore, these collections are useful for research in the evolution of diseases [12] and can be used to describe e.g. historical concepts. [58]

Interestingly, teratological specimens are often only scantily or, in most cases, not at all described and substantiated developmental or embryologically oriented etiopathogenetic questions concerning their possible origin are usually lacking. Therefore, these old museological collections are filled with unanswered questions which can be explored to unravel embryological and etiopathogenetic oriented issues concerning rarely occurring birth defects. Moreover, teratological collections can yield valuable epidemiological information and have potential for radiological imaging and even for molecular studies. Finally, these collections can be inexhaustibly used to teach teratology and embryology to a broad audience, (bio)medical students, medical specialists (in training) from a variety of disciplines and to lay people. These potentials are further denoted in the various included chapters since they were the rationale to conduct the studies described in this thesis.

Aims and outline of this thesis

The overall objective of this thesis was to explore and describe past, present and future aspects of Dutch teratological collections in a predominantly historical and medical perspective. Exploiting these—often neglected—collections could potentially give insight in (erroneous) historical assumptions, the recognition of very rarely occurring congenital anomalies and an elaboration on etiopathogenetic or embryologically oriented questions. Dutch teratological collections and therewith teratological specimens are the centerpiece throughout this thesis.

The thesis begins with a general introduction (chapter 1) on multiple general facets of museology and teratology, the latter especially regarded from a historical premise. In addition, the objective and an overview of the different studies presented in this thesis are provided. The actual research in this thesis is divided into three parts, denoted in 7 chapters.

Part 1: The past of Dutch teratological collections

In the first part we describe historical aspects of Dutch teratological collections and we provide an overview of the historical and contemporary legacy of the teratological specimens collected by the 17th century Dutch anatomist Frederik Ruysch (chapter 2), currently present in The Peter the Great Museum of Anthropology and Ethnography (Kunstkamera) in Saint Petersburg (Russia). In chapter 3 we focus on describing and rediagnosing

the teratological collection of the *Museum Anatomicum* of the University of Leiden (The Netherlands). This vast anatomical collection contains the oldest Dutch teratological specimens currently known.

Part 2: The present of Dutch teratological collections

In this part we describe the present aspects of Dutch teratological collections and provide an overview of diagnosing and interpreting congenital conditions of the skeleton in a paleodysmorphological and paleoteratological context (chapter 4). Chapter 5 consists of an extensive appraisal about sirenomelia. This rare congenital anomaly remains subject of ongoing etiological and pathogenetic controversies. A museological specimen was used to expand its heterogeneous phenotypical spectrum and efforts were made to create awareness that sirenomelia could, in some cases, be placed in the VACTERL(-H) receptacle. In chapter 6 we describe the currently postulated etiopathogenetic theories in the enigmatic genesis of conjoined twins. We point out to their weaknesses and include a novel view on the possible genesis of conjoined twins which could be the link among the everlasting dogmas between partial fission and secondary fusion.

Part 3: The future of Dutch teratological collections

In the third part of this thesis, concerning the future potentials of teratological collections, we use radiological imaging and genetic diagnostics in order to describe their internal characteristics and revise their historical diagnosis. We performed 3T Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) on 41 teratological fetuses of the teratological collection of the Radboud university medical center in Nijmegen (The Netherlands). Hereby, their museological and education potentials were further exploited (chapter 7). In chapter 8 we performed genetic testing aiming at the pathognomonic mutation in tooth material from a 180-year-old museological achondroplastic skeleton from the teratological collection of the *Vrolik Museum* housed in the Academic Medical Center in Amsterdam (The Netherlands).

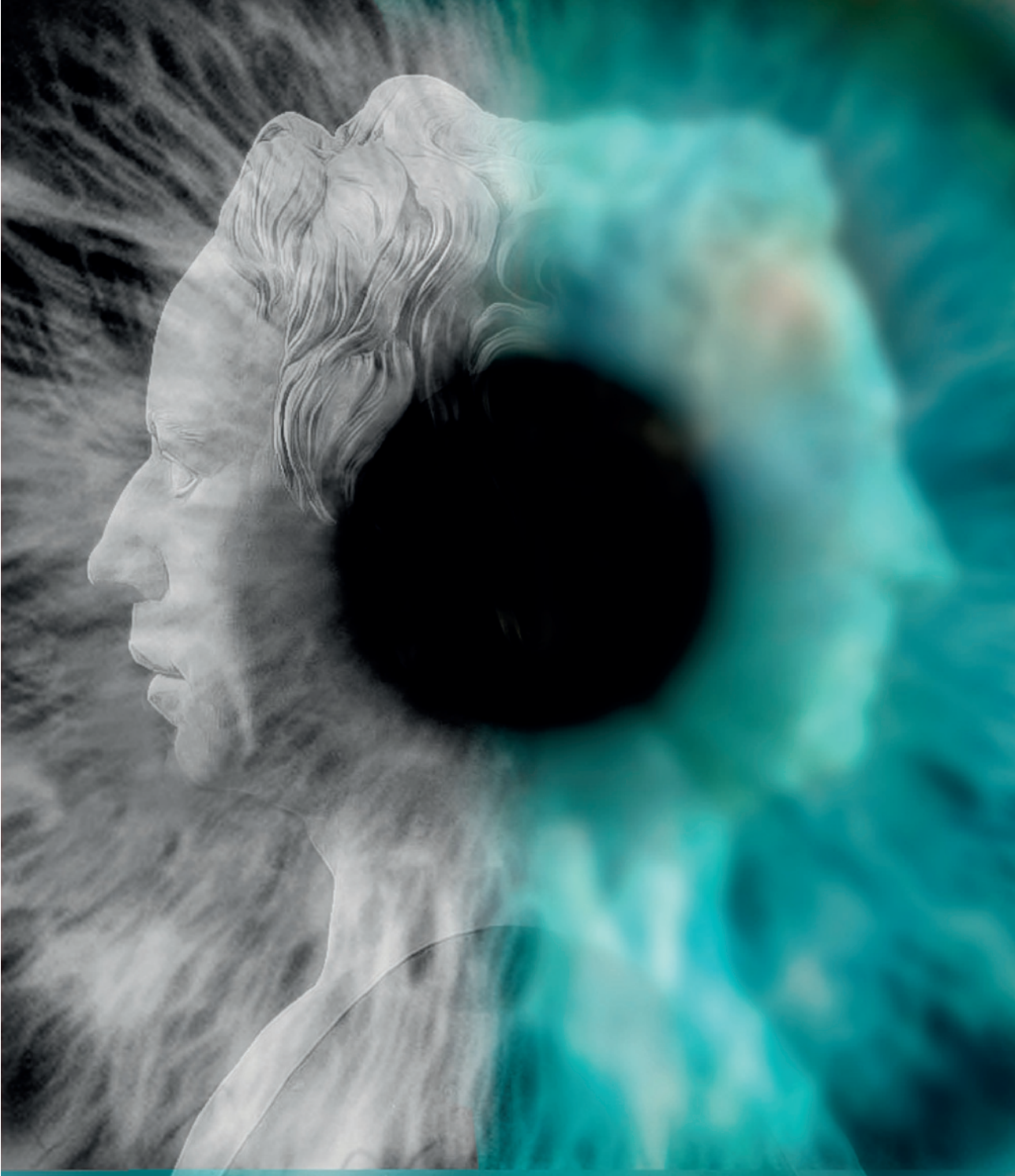
In chapter 9 a supplementary discussion and future perspectives of Dutch teratological collections are given. Finally the thesis is summarized in English and Dutch in chapter 10.

References

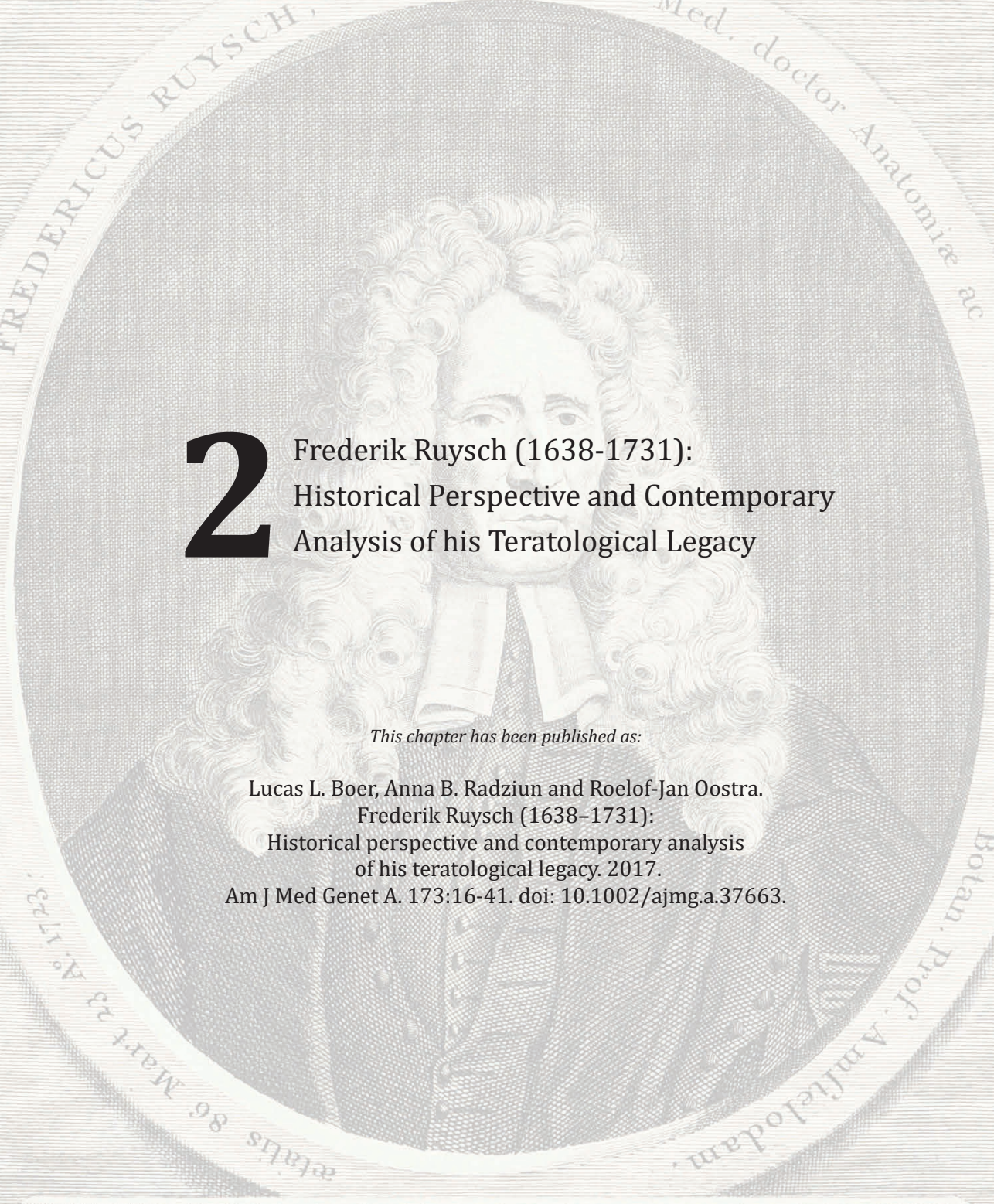
1. Argyle AW. 1974. The Ancient University of Alexandria. The Classical Journal. 69:348-50.
2. Whitehead PJP. 1970. Museums in the history of zoology. Museums Journal. 70:50–57 and 70:155–160.
3. Valdecasas AG, Correia V, Correas AM. 2006. Museums at the crossroad: Contributing to dialogue, curiosity and wonder in natural history museums Museum Management and Curatorship. 21:32–43.
4. Findlen P. 1994. Possessing nature museums, collecting, and scientific culture in early modern Italy. Berkeley, CA: University of California Press.
5. Daston L, Park K. 1998. Wonders and the order of nature, 1150–1750. New York: Zone Books.
6. Alexander EP, Alexander M, Altamira R. 2008. Museums in Motion: An Introduction to the History and Functions of Museums. 2nd edition. Lanham MD AltaMira Press.
7. De Gruyter Saur. 2012. Museums of the World. Berlin. Boston.
8. Lord GD, Blankenberg N. 2015. Cities, Museums and Soft Power. Rowman & Littlefield. American Alliance of Museums Press.
9. Kotler N, Kotler P. 2000. Can museums be all things to all people? Missions, goals, and marketing's role. Museum Management and Curatorship. 18:271–287.
10. Stapp CB. 1998. Museums and community development. Curator. 41:229–34.
11. Jacobsen J. 2010. "A research vision for museums" Curator: The Museum Journal. 53:281–289.
12. Charlier P, Hervé C. 2017. What new life for the Parisian (and European) anatomical and pathological collections? struggle for medical education. Clinical Anatomy. 30:432–33.
13. Gardner GS. 1985. The Design of Educational Exhibits. Compiled by R. S. Miles in collaboration with M.B. Alt, D.C. Gosling, B.N. Lewis, and A.F. Tout. Curator: The Museum Journal. 28:149–51.
14. Walter CH. 2002. "Creating Extraordinary Learning Environments," Curator. 45: 277-288.
15. van Speybroeck D, van Krieken J, van Daal W, Ruiter D. 2008. Memento of the post-mortem. J Pathol. 215:103-7.
16. Castaño FJG, Reinoso JG, Castiñeira FG, Montenegro EC, Carballo JMP. 2005. Computers & Education. 45:105-121.
17. Fergusson R, Manser M, Pickering D. 2000. The new Penguin thesaurus. London: Penguin Books.
18. Mujtaba T, Lawrence M, Oliver M, Reiss MJ. 2018. Learning and engagement through natural history museums. Stud Sci Educ. 54:41-67.
19. Saint-Hilaire IG. 1836. Histoire générale et particulière des anomalies de l'organisation chez l'homme et les animaux, ou, Traité de teratology. J.B. Baillière, Paris.
20. Edgar HJH, Rautman ALM. 2014. Contemporary Museum Policies and the Ethics of Accepting Human Remains. Curator 57: 237-24.
21. Oostra RJ, Boer LL, Alie E. van der Merwe. 2016. Paleodysmorphology and Paleoteratology: Diagnosing and Interpreting Congenital Conditions of the Skeleton in Anthropological Contexts. Clin Anat. 29: 878-91.

22. Ujházy E, Mach M, Navarová J, Brucknerová I, Dubovický M. 2012. Teratology - past, present and future. Interdiscip Toxicol. 5:163-8.
23. Opitz JM, Gilbert EF. 1982. Pathogenetic analysis of congenital anomalies in humans. Pathobiol Annu. 12:301-49.
24. Kalter H. 2003. Teratology in the 20th century: environmental causes of congenital malformations in humans and how they were established. Neurotoxicol Teratol. 25:131-282.
25. Bates AW. 2005. Emblematic monsters: unnatural conceptions and deformed births in early modern Europe. Clio Med. 77:3-334.
26. Pachajoa H, Rodriguez C. 2011. Possible case of sirenomelia in the Tumaco-Tolita pottery pre-Columbian culture, 2000 years before the epidemic focus of sirenomelia in Cali-Colombia. Am J Med Genet A. 155A:2327-8.
27. Beckwith JB. 2012. Congenital malformations: from superstition to understanding. Virchows Arch. 461:609-19.
28. Morin A. 1996. "La tératologie de Geoffroy Saint-Hilaire à nos jours" [Teratology from Geoffroy Saint-Hilaire to the present]. Bulletin De l'Association Des Anatomistes (in French). 80: 17–31.
29. Lycosthenes K. 1557. Prodigiorum ac ostentorum chronicon. Henricum Petri, Basel.
30. Paré A. 1573. Deux livres de chirurgie. I. De la generation de l'homme. II. Des monstres tant terrestres que marins avec leurs portraits. André Wechel, Paris.
31. Rueff J. 1580. De conceptu et generations hominis: de matrice et eius partibus. Frankfurt am Main: Sigismund Feyerabend.
32. Longo LD. 1995. De conceptu et generatione hominis. Am J Obstet Gynecol. 173:1349-50.
33. Schenck JG. 1609. Monstrorum historia memorabilis. Matthiae Becker, Frankfurt.
34. Liceti F. 1634. De Monstrorum Caussis, Natura, et Differentiis, Libri Duo, 2nd edn. Paulum Frambottum, Padua.
35. Aldrovandi U. 1642. Monstrorum Historia. Nicolai Tebaldini, Bologna.
36. Meckel JF. 1812–1818. Handbuch der pathologischen Anatomie. Carl Heinrich Reclam, Leipzig.
37. McBride WG. 1961. Thalidomide and congenital abnormalities. Lancet. 2:1358.
38. Franks ME, Macpherson GR, Figg WD. 2004. Thalidomide. Lancet. 363:1802-11.
39. Mone F, Quinlan-Jones E, Kilby MD. 2018. Clinical utility of exome sequencing in the prenatal diagnosis of congenital anomalies: A Review. Eur J Obstet Gynecol Reprod Biol. 231:19-24.
40. Blackburn HL, Schroeder B, Turner C, Shriver CD, Ellsworth Dl, Ellsworth RE. 2015. Management of incidental findings in the era of next-generation sequencing. Curr Genomics. 16:159-174.
41. Schoenwolf G, Bleyl S, Brauer P, Francis-West P. 2009. Larsen's Human Embryology. (4th ed.) Philadelphia: Churchill Livingstone Elsevier.
42. Opitz JM. 1993. Blastogenesis and the "primary field" in human development. Birth Defects Orig Artic Ser. 29:3-37.
43. Martínez-Frías ML, Frías JL, Opitz JM. 1998. Errors of morphogenesis and developmental field theory. Am J Med Genet. 76:291-6.
44. Flamier A, Singh S, Rasmussen TP. 2017. A standardized human embryoid body platform for the detection and analysis of teratogens. Lako M, ed. PLoS ONE. 12:e0171101.

45. Giavini E, Menegola E. 2012. Biomarkers of teratogenesis: suggestions from animal studies. Reprod Toxicol. 34:180-5.
46. Toufaily MH, Westgate MN, Nasri H, Holmes LB. 2018. Malformations among 289,365 Births Attributed to Mutations with Autosomal Dominant and Recessive and X-Linked Inheritance. Birth Defects Res. 110:92-97.
47. Dolk H, Loane M, Garne E. 2010. The prevalence of congenital anomalies in Europe. Adv Exp Med Biol. 686:349–64.
48. Feldkamp ML, Carey JC, Byrne JLB, Krikov S, Botto LD. 2017. Etiology and clinical presentation of birth defects: population based study. BMJ. 30:357:j2249.
49. National Research Council (US) Committee on Developmental Toxicology. Scientific Frontiers in Developmental Toxicology and Risk Assessment. Washington (DC): National Academies Press (US); 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK225678/
50. Theisen A, Shaffer LG. 2010. Disorders caused by chromosome abnormalities. The Application of Clinical Genetics. 3:159-174.
51. Morton CC. Lee. 2009. C. Cytogenetics in Reproduction in Yen & Jaffe's Reproductive Endocrinology (Sixth Edition)
52. Holmes LB. 2012. Common Malformations. New York: Oxford University Press.
53. Shepard TH. 1998. Catalog of Teratogenic Agents. 9th Ed., editor. Baltimore: Johns Hopkins University Press.
54. Mayr E. 1942. Systematics and the Origin of Species. Columbia University Press, New York.
55. Farrell HB. 1984. The two-toed Wadoma--familial ectrodactyly in Zimbabwe. S Afr Med J. 65:531-3.
56. Morgan LM. 2002. "Properly disposed of": a history of embryo disposal and the changing claims on fetal remains. Med Anthropol. 21:247-74.
57. Jones DG, Gear R, Galvin KA. 2003. Stored human tissue: an ethical perspective on the fate of anonymous, archival material. Journal of Medical Ethics 29:343-347.
58. Tourreil V, Giran G, Benmoussa N, Paternoster G, Conan P, Charlier P, Khonsari RH. 2018. Three Skulls Dating from the French Revolutionary Years Diagnosed with Tinea Capitis: A Paleopathologic Approach. J Craniofac Surg. 29:1713-1716.



The Past of Dutch Teratological Collections



2 Frederik Ruysch (1638-1731): Historical Perspective and Contemporary Analysis of his Teratological Legacy

This chapter has been published as:

Lucas L. Boer, Anna B. Radziun and Roelof-Jan Oostra.
Frederik Ruysch (1638–1731):
Historical perspective and contemporary analysis
of his teratological legacy. 2017.
Am J Med Genet A. 173:16-41. doi: 10.1002/ajmg.a.37663.

Abstract

The Peter the Great Museum of Anthropology and Ethnography (Kunstkamera) in Saint Petersburg is the oldest museum in Russia. It keeps the remains of the anatomical collection of the world-famous 17th century Dutch anatomist Frederik Ruysch. This unique collection was bought and shipped in 1717 by Czar Peter the Great, and presently still comprises more than 900 specimens, a modest number of which concerns specimens with congenital anomalies. We searched for teratological clues in the existing collection and in all his descriptions and correspondence regarding specimens and cases he encountered during his career as *doctor anatomiae* and chief instructor of the surgeons and midwives in Amsterdam. A total of 63 teratological specimens and case descriptions were identified in this legacy, including some exceedingly rare anomalies. As it turns out, Ruysch was the first to describe several of the conditions we encountered, including intracranial teratoma, enchondromatosis and Majewski syndrome. Although his comments pose an interesting view on how congenital anomalies were scientifically perceived in early 18th century Europe, Ruysch mostly refrained from explaining the causes of the conditions he encountered. Instead, he dedicated himself to careful descriptions of his specimens. Almost 300 years after his demise, Ruysch's legacy still impresses and inspires both scientists and lay men.

Introduction

Frederik Ruysch (1638–1731) (Fig. 1) was a Dutch professor in anatomy and botany at the *Athenaeum Illustre*, the predecessor of the University of Amsterdam, The Netherlands. During his career as a *doctor anatomiae*, Praelector of the Amsterdam Guild of Surgeons (Fig. 2) and chief instructor of midwives, he built up a collection of over 2,000 anatomical, pathological, zoological, and botanical specimens, which he preserved either dried or embalmed. [1] He exhibited his collection, open to the public, in his three-story dwelling at the *Bloemgracht* in downtown Amsterdam. Already in the 17th century, Ruysch became famous for his meticulous technique of postmortem vascular injections. He developed a mercuric sulfide-based injection mixture originating from cinnabar—a naturally occurring red colored mineral. This injection fluid gave his specimens, especially the many preparations of fetuses and infants, a reddish, almost lively expression (Fig. 3). With this technique the smallest blood vessels could be visualized and dissected, a groundbreaking technique in the 17th century. [2]

When Czar Peter the Great (1672–1725) came to power in Russia at the end of the 17th century, he was determined to modernize Russia. Peter the Great had a prestige project: the new city of Saint Petersburg. To achieve this modernization, he let the Dutch republic inspire him; the Golden age in Holland, a perfect model for Russia. [1] Following his visits to Ruysch's anatomical collection in Amsterdam in 1697 and again in 1716, he purchased it for his planned cabinet of curiosities, for an astonishing amount of 30,000 guilders. Upon arrival in 1717, the 2,000 specimens were initially stored in his summer palace before they were exhibited in the Peter the Great Museum of Anthropology and Ethnography (Kunstkamera). After the grand opening of the Kunstkamera in 1719, in the former mansion of the boyar Alexander Kikin, it started to function as a public museum, the first of its kind in Russia. [3] The new building of the Kunstkamera on the banks of the Neva river in Saint Petersburg (Fig. 4) opened its doors in 1728 and presently houses nearly two million items, including the remnants of the anatomical collection of Frederik Ruysch.

The collection that was bought by Peter the Great (from here on referred to as Ruysch's first collection) consisted of twelve subsequently composed cabinets (*Thesauri Anatomici*), comprising human, animal and plant specimens which, upon completion, were described by Ruysch in Latin and issued as illustrated catalogues. Ten of these cabinets were numbered (*Thesaurus Anatomicus I–X*), one comprised his earliest specimens (*Musaeum Anatomicum Ruyschianum sive Catalogus Rariorum*) and one consisted only of

animal specimens (*Thesaurus Animalium*). Together with numerous clinical observations, treatises and correspondences they were collectively re-issued in 1721 as *Opera Omnia Anatomico-Medico-Chirurgica* (Collected anatomical, medical and surgical works), [4] from here on referred to as *Opera Omnia* or Collected Works (CW). Until 1728, later editions were amended with subsequent contributions, including observations, letters and two additional cabinets, named *Curae Posteriores* (Subsequent Exercises) and *Curae Renovatae* (Renewed Exercises) that Ruysch composed after he had sold his first collection. A biography on Ruysch by Johan Friedrich Schreiber was added in 1732. In 1744, 13 years after Ruysch's death, the CW were translated into Dutch by Ysbrand Gysbert Arlebout (*Alle de Ontleed-, Genees- en Heelkundige Werken*). [5] Only a small part, concerning the 100 clinical observations, was translated into French [6] and English. [7]

Soon after he died in 1731 his second collection, that he had been composing since 1717, was auctioned. The auction catalogue, titled *Catalogus Musaei Ruyschianii*, listed almost 1,300 indexed specimens in no less than nine cabinets, including the two *Curae* collections that had appeared in later editions of the CW, albeit in reversed order. [8] The present whereabouts of these specimens are unknown.

Although all specimens had made it safely from Amsterdam to Saint Petersburg and the original order of display in their respective cabinets was initially preserved, the matching between specimens and their descriptions got lost in later years during subsequent rearrangements of the exhibition. Over the years, about half of the specimens perished, disappeared or were donated to other institutes. [9-11] Today, the 916 specimens ascribed to Ruysch that have stood the tooth of time are being restored and investigated by the Ruysch Research Group, an international consortium of Russian and Dutch historians, anatomists and surgeons that focus on the matching of specimens and descriptions. We here report on the proceedings regarding the specimens of human congenital malformations and the diagnoses that we made. Additionally, we discuss the scientific and public opinions of Ruysch and his contemporaries towards congenital malformations and the present day value of his legacy.

Fig. 1. Portrait of Frederik Ruysch at the age of 85, copperplate. Jan Wandelaar, 1723.

Fig. 2. "The anatomical lesson of Frederik Ruysch" (oil painting on canvas). Depicted are the board of the Surgeons Guild, Frederik Ruysch presenting the umbilicus of the "*subjectum anatomicum,*" and his son Hendrik Ruysch holding a dried neonatal skeleton. Jan van Neck, 1683. Amsterdam Museum.

Fig. 3. Specimen 4070-174: A child's head with artificial glass eyes and red colored skin resulted from postmortem cinnabar-containing vascular injections. From the collection of the Peter the Great Museum of Anthropology and Ethnography (Kunstkamera), Russian Academy of Sciences.

Fig. 4. Peter the Great Museum of Anthropology and Ethnography (Kunstkamera) on the banks of the Neva river in Saint Petersburg (Russia).

Material and methods

In this survey we included the complete legacy of Frederik Ruysch regarding specimens and descriptions of human cases of congenital anomalies. Animal specimens were left out of this survey. We first investigated the specimens of congenital anomalies, attributed to Ruysch and present at the Kunstkamera in St. Petersburg, and diagnosed the conditions they presented with by means of external inspection. In some cases the specimens were taken out of their jars for restoration purposes, which made it possible to investigate them in more detail. To refer to specimens in the extant collection we used the numbers that they got assigned during the last re-inventory in 1947. [10] These numbers consist of two parts, the first of which (4070) refers to the Ruysch collection and the second one (1–935) to the specimen itself. Images and details of the specimens can be found at the website of the Kunstkamera.

Secondly, we explored all parts of the CW, as well as the auction catalogue of his second collection (see Table I) for descriptions and pictures of congenital anomalies. Most of these descriptions referred to specimens that were part of the first or second Ruysch collections but several concerned case observations that Ruysch made in his anatomical and obstetric practice without resulting in anatomized preparations. Some specimens and case descriptions were mentioned more than once in the CW. Unlike the Dutch text, the Latin text of the complete set of works (*Opera Omnia*) was published without a continuous page numbering. To refer to descriptions of specimens and cases we therefore used the page numbering of the separate works, named by codes derived from their (abbreviated) titles (see Table I). These separate works, as well as the two volumes of the CW, have been published as e-books on Google Books and can be inspected and downloaded for free.

Thirdly, we tried to match the descriptions and pictures with the specimens still present in the Kunstkamera collection. All (references to) specimens and descriptions were reduced to "unique cases," meaning that if more than one description of, or references to, a case and/or specimen was found, they were counted as one collective case. This also concerns cases consisting of multiple specimens. Regarding the specimens, cases and descriptions, we defined the following categories:

1. Specimens of the first collection with matching descriptions.
2. Specimens of the first collection without matching descriptions.
3. Descriptions referring to either the first or the second collection without matching specimens.
4. Descriptions of case observations that did not result in anatomized specimens.

Finally, we searched the literature for references to Ruysch's specimens and case descriptions.

Table I: Contents of Frederik Ruysch's written legacy

Original title (abbreviated)	Title code[a]	Year	Contents
Opera Omnia, volume 1:		*1721-1728*	
Historia vitae		1732	Bibliography on Ruysch
Dilucidatio valvularum	*Dil Valv*	1665	Treatise on vascular valves & 26 clinical observations
Observationum anatomico-chirurgicarum	*Obs Cent*	1691	100 clinical observations
Museum anatomicum Ruyschianum	*Mus Anat*	1691	Descriptions of collected specimens
Epistola anatomica I		1695	Correspondence with Gaubius
Epistola anatomica II		1695	Correspondence with Gaubius
Epistola anatomica III		1695	Correspondence with Gaubius
Epistola anatomica IV		1696	Correspondence with Campdomercus
Epistola anatomica V		1696	Correspondence with Frentz
Epistola anatomica VI		1696	Correspondence with Graetz
Epistola anatomica VII		1696	Correspondence with Graetz
Epistola anatomica VIII		1696	Correspondence with Graetz
Epistola anatomica IX		1697	Correspondence with Goehlicke
Epistola anatomica X		1697	Correspondence with Keerwolff
Epistola anatomica XI		1698	Correspondence with Wolf
Epistola anatomica XII		1699	Correspondence with Ettmüller
Epistola anatomica XIII		1700	Correspondence with Wedel
Epistola anatomica XIV	*Ep Anat XIV*	1701	Correspondence with Van Reverhorst
Epistola anatomica XV		1704	Correspondence with Graetz
Epistola anatomica XVI		1713	Correspondence with Vater
Responsio ad Godefridi Bidloi libellum		1721	Answer to Bidloo on his treatise
Adversariorum anatomico-medico-chir. decas prima	*Adv Dec I*	1717	10 clinical remarks
Adversariorum anatomico-medico-chir. decas secunda	*Adv Dec II*	1720	10 clinical remarks
Adversariorum anatomico-medico-chir. decas tertia	*Adv Dec III*	1723	10 clinical remarks
Opera Omnia, volume 2:		*1721-1728*	
Thesaurus animalium primus		1710	Descriptions of collected animal specimens
Thesaurus anatomicus I	*Thes Anat I*	1701	Descriptions of collected specimens
Thesaurus anatomicus II	*Thes Anat II*	1702	Descriptions of collected specimens
Thesaurus anatomicus III	*Thes Anat III*	1703	Descriptions of collected specimens
Thesaurus anatomicus IV	*Thes Anat IV*	1704	Descriptions of collected specimens
Thesaurus anatomicus V		1705	Descriptions of collected specimens
Thesaurus anatomicus VI	*Thes Anat VI*	1705	Descriptions of collected specimens
Thesaurus anatomicus VII	*Thes Anat VII*	1707	Descriptions of collected specimens
Thesaurus anatomicus VIII	*Thes Anat VIII*	1709	Descriptions of collected specimens
Thesaurus anatomicus IX	*Thes Anat IX*	1714	Descriptions of collected specimens
Thesaurus anatomicus X	*Thes Anat X*	1716	Descriptions of collected specimens
Curae posteriores	*Cur Post*	1724	Descriptions of collected specimens
Curae renovatae	*Cur Reno*	1728	Descriptions of collected specimens
Tractatio anatomica de musculo in fundo uteri	*Trac Anat*	1723	Treatise on morphology of uterus and placenta
Epistola problematica Bohlii		1726	Correspondence with Bohlius
Epistola problematica Hecqueti		1726	Correspondence with Hecquet
Epistola problematica Vateri		1726	Correspondence with Vater
Epistola problematica Boerhavi		1722	Correspondence with Boerhaave
Other works:			
Catalogus Musaei Ruyschiani	*Cat Mus*	1731	Auction catalogue of Ruysch's second collection
Observations Anatomiques et Chirurgicales,		1734	French translation of the 100 clinical observations
Ruysch's Practical Observations in Surgery and Midwifry		1751	English translation of the 100 clinical observations

[a] Title codes of the relevant sections of the Collected Works are used in the text and in Table II to refer to specimen and case descriptions.

Table II. Human Congenital Anomalies Found in the Legacy of Frederik Ruysch.

Case	Category[a]	Diagnosis	Specimen(s)[b]	Description(s) in CW[c]	Figures
1	3	Acardiac twin		Thes Anat IX, p.17, 41; Adv Dec II, p.42	5
2	3	Cleft lip and palate		Thes Anat VI, p.34; Adv Dec I, p. 21	
3	3	Gastric teratoma		Adv Dec III, p.2-7; Cur Post, p.23	6
4	1	Intracranial teratoma	4070-906,-672,-681	Thes Anat II, p. 16-18, 43, 44	7
5	3	Omental teratoma		Obs Cent, p.23, 24; Mus Anat, p.36; Thes Anat III, p. 27; Cur Post, p. 11; Cat Mus, p.54	8
6	3	Ovarial teratoma		Thes Anat I, p.29, 30, 33	9
7	3	Umbilical haemagiomyxoma		Thes Anat IX, p.10	
8	1	Lateral conjoined twins	4070-913	Thes Anat VII, p. 3; Adv Dec I, p.20	10A
9	2	Lateral conjoined twins	4070-911		
10	2	Lateral conjoined twins	4070-914		10B
11	2	Lateral conjoined twins	4070-916		10C
12	1	Ventral conjoined twins	4070-912	Adv Dec I, p.21, Adv Dec II, p.42	10D
13	4	Membranous rectal atresia		Adv Dec II, p.43	
14	4	Rectal agenesis		Adv Dec II, p.43	
15	4	Hirschsprung's disease		Obs Cent, p.86	
16	3	Meckel's diverticulum		Mus Anat, p.63; Thes Anat VII, p.3, 7	11
17	3	Meckel's diverticulum		Thes Anat VII, p.7	
18	3	Meckel's diverticulum		Cur Post, p.22	
19	3	Multiple liver cysts		Cat Mus, p.70	
20	3	Holoprosencephaly		Cur Reno, p.10; Adv Dec I, p.21	
21	3	Hydatidiform mole		Cur Post, p.7	
22	3	Hydatidiform mole		Cur Post, p.15	
23	3	Hydatidiform mole		Cat Mus, p.62	
24	4	Hydatidiform mole	4070-666		
25	4	Hydatidiform mole	4070-669		
26	4	Hydatidiform mole	4070-670		
27	4	Hydatidiform mole	4070-673		
28	4	Hydatidiform mole	4070-674		12
29	4	Hydatidiform mole	4070-675		
30	3	Hydrocephaly		Mus Anat, p.65; Thes Anat III, p.11	
31	3	Hydrocephaly		Thes Anat IX, p.19	
32	1	Limb malformation	4070-907	Thes Anat VIII, p.16	13
33	2	Limb malformation	4070-39		14A
34	2	Limb malformation	4070-908		14B
35	1	Anencephaly	4070-915	Adv Dec I, p.20	15
36	3	Anencephaly		Thes Anat VIII, p.3; Adv Dec I, p.20	16
37	3	Anencephaly		Adv Dec I, p.20	
38	3	Anencephaly		Cat Mus, p.85	
39	3	Meningo(myelo)cele		Obs Cent, p.33	
40	4	Meningo(myelo)cele		Obs Cent, p.33	17
41	4	Meningo(myelo)cele		Obs Cent, p.33	
42	4	Meningo(myelo)cele		Obs Cent, p.35	
43	4	Meningo(myelo)cele		Obs Cent, p.35	
44	3	Ciliary chondrodysplasia		Mus Anat, p.186; Adv Dec I, p.20	18
45	4	Enchondromatosis		Ep Anat XIV, p.9-18	19
46	4	Disorder of sex development		Thes Anat VIII, p.17; Adv Dec I, p.22	
47	3	Fallopian tube duplication		Thes Anat IV, p.20	
48	2	Hypospadias	4070-600		20
49	4	Imperforate hymen		Obs Cent, p.42	
50	4	Transverse vaginal septum		Obs Cent, p.27	21
51	4	Ureteral duplication		Dil Valv, p.18	
52	4	Ureteral duplication		Dil Valv, p.25	
53	4	Ureteral duplication		Obs Cent, p.100	22
54	3	Ureteral duplication		Thes Anat VII, p.9	
55	4	Bladder exstrophy		Obs Cent, p.22, 23	23
56	4	Omphalocele		Obs Cent, p.92	24
57	4	Omphalocele		Obs Cent, p.93	
58	4	Omphalocele /gastroschisis		Obs Cent, p.93	
59	4	Axial skeletal anomaly		Dil Valv, p.14	
60	3	Axial skeletal anomaly		Cat Mus, p.90	
61	4	Cranial tumor		Obs Cent, p.50	25
62	3	Other generalized condition		Thes Anat VI, p.34	
63	3	Other generalized condition		Cat Mus, p.60	

[a] See text for category definitions.
[b] Numbers refer to specimens in the extant collection.
[c] Title codes are explained in Table I

Results

In the existing anatomical collection present at the Kunstkamera, we identified 17 specimens that showed one or more congenital anomalies. In the CW and the auction catalogue, we found descriptions of 32 specimens with one or more congenital anomalies. Five of these descriptions could be matched with certainty to a specimen in the existing collection (category 1). Twelve specimens could not be matched with descriptions, mainly because uniquely identifying structures were lacking (category 2). Furthermore, we found descriptions of 27 cases without matching specimens (category 3), ten of which concerned specimens of the second collection (including the *Curae*), and 19 case descriptions that did not refer to anatomical specimens (category 4). In total we found 63 unique cases, most of which could be diagnosed with reasonable certainty, in that a single diagnosis or a confined group of related diagnoses was applicable to the presented condition. If two or more divergent diagnoses were equivalently applicable, we considered the condition to be indiscernible. In Table II the cases, numbered 1-63, in each of the defined categories are listed according to the diagnoses we made. The characteristics of these cases are given below. Unless stated otherwise they concerned Ruysch's first collection.

Acardiac twin

Case 1. As part of his ninth cabinet (the 24th specimen) and in his second set of ten clinical remarks Ruysch described and depicted a placenta showing a marginally located protruding mass with an appended leg and foot with three toes (Fig. 5), delivered together with a healthy newborn. [12,13] Ruysch noted that inside the fatty tissue mass a femoral bone was present that was completely hollow. Only a thin cortex was noticeable with complete absence of spongious bone. Additionally, there was absence of any muscle tissue. Based on Ruysch's description, this case can either be diagnosed as an acardiac twin or as a placental teratoma. Following Fox and Butler-Manuel, [14] it is generally assumed that acardiac twins have their own umbilical cord and vessels and a rudimentary axial organization, whereas placental teratomas do not. As it appears, however, none of these criteria is truly pathognomonic. [15] Placental teratomas are often smooth surfaced, round or oval shaped with diameters ranging from 2.0–7.5 cm consisting of mature fat tissue, skin and skin appendices, bone and cartilage. Furthermore, placental teratomas are often marginally located on the placenta. However, each of these characteristics may also be applicable to acardiac twins. What made us incline to diagnose

the latter condition is the well recognizable foot, the size of which is seemingly in accordance with the gestational age of the host twin. To the best of our knowledge fetiform structures have never been reported as part of placental teratomas.

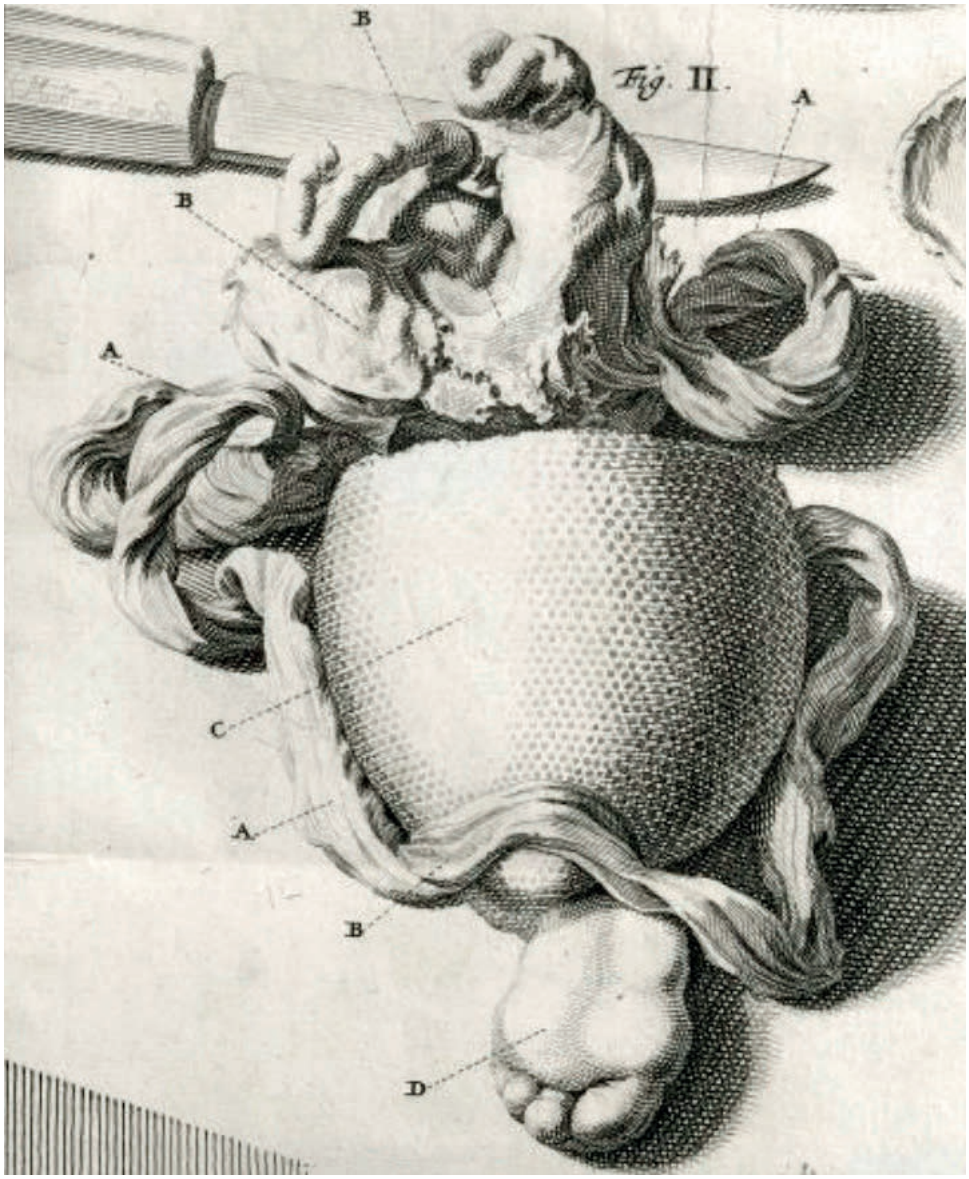


Fig. 5. Case 1. Acardiac twin, copperplate. [12,13]

Cleft lip and palate

Case 2. Ruysch described a miscarriage in which an immature child was seen with a split lip and palate. This description concerns the 52nd of his sixth cabinet and the first set of ten clinical remarks. [16,17] Ruysch stated that there was no need for grief when this child died, as it would have been born with a malformed lip and mouth, which was possibly a condition with a poor prognosis in the 17th century. Ruysch referred only once to this congenital anomaly, that we diagnosed as cleft lip and palate.

Congenital tumors

Gastric teratoma.

Case 3. In his third set of ten clinical remarks and as part of the second cabinet of his second collection (the 160th and 161st specimens), Ruysch described the case history of a man, presenting with a supra-umbilical ulcerating lesion, who was admitted to the hospital in Semarang, Indonesia, on August, 15th, 1716. [18,19] The patient lived for an additional 4 months during which he received extensive treatment for his abdominal ulcer, including daily drainages of the opened ulcer. After the patient died, the body was dissected by the chief of surgery, Cornelis Smit. On exploration, the stomach was found to be filled with a white substance, a bundle of hair and two tissue masses, one attached to the cardia, containing skin covered osseous or cartilaginous structures and four well-recognizable teeth (Fig. 6A). No other abnormalities regarding the autopsy were described, except for the liver, which appeared to be "putrid." The specimen was donated to Ruysch by his friend and colleague Caspar Commelin (1668–1731), professor of exotic botany at the *Athenaeum Illustre*, together with a written statement, signed by all eyewitnesses who were present at the autopsy. Ruysch confirmed the description of Smit and additionally mentioned a finger-like structure that he compared with the foreleg of a small hartebeest (*Alcelaphus buselaphus*), the nail of which resembled a human nail (Fig. 6B). The skin of the putative paw showed multiple fine little hairs resembling the skin of a human hand. Anticipating on the skepticism of his peers concerning the extraordinarity of this report, he published it together with Smit's statement, thereby defying any allegations of forgery. We diagnosed this condition as gastric teratoma. At least part of the tumor appeared to have been located endogastrically, whereas the ulceration and subsequent fistulization may have been indicative for a proliferative exogastric component in response to an inflammation or a malignant degeneration. In this respect the "putrid"

liver could be interpreted as hepatic metastases. Nails, in addition to hairs and teeth, are common findings in teratomas, and even nail baring finger-like structures have been described, particularly in non-ovarian teratomas, [20] although their fetiform nature is disputed. [21]

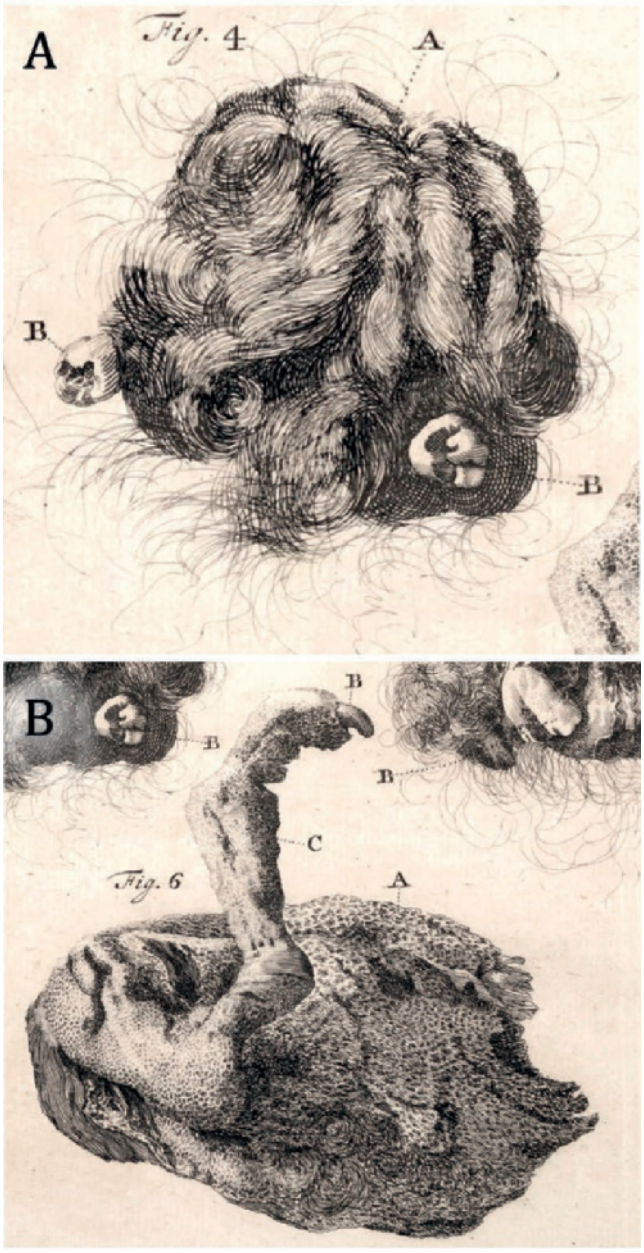


Fig. 6. Case 3. Gastric teratoma, copperplate, presenting with [A] teeth and hair and [B] a nail-bearing finger-like protrusion. [18,19]

Intracranial teratoma

Case 4. This case concerns the specimen of a male neonate with a grossly enlarged head (Fig. 7A and B), which was part of the second cabinet.[22] According to Ruysch, the child whom he considered to suffer from hydrocephalus, was born around 6 months of gestation. His assistance was requested by the midwife who conducted the delivery because expulsion was stagnant. Arriving at the scene, both the child and the placenta had nevertheless already been delivered but the uterine cavity was still occupied, according to the midwife. Subsequently, several shapeless, multi-structured lumps of tissue were expelled in which Ruysch, much to his surprise, recognized remnants of no less than twenty miniature extremities without any other recognizable body parts. Ruysch separately bottled five of these tissue lumps (Fig. 7C and D), two of which have been identified in the extant collection. Inspection of the child's head showed an asymmetrical sac-like enlargement of the cranial vault, with a 3 cm wide roughly edged opening at the top of the sac. Deviating positions of hands and feet indicated that the child was probably stillborn. Apart from the reddish coloring of the skin, resulting from postmortem cinnabar containing vascular injections, no additional abnormalities were found. Inspection of the tissue lumps confirmed Ruysch's descriptions in detail: several more or less well-formed arms and legs were recognizable among various ill-definable tissue types. Some parts of the appendages were de-fleshed, resulting from careless handling by onlookers during the delivery as Ruysch stated, but thereby showing that they contained skeletal elements resembling tubular bones. The size of these parts would have been in accordance with around 3 months of gestation, again confirming Ruysch's estimation. The combination of multi-structured tissue lumps and several limb-like appendages, the size of which is not in accordance with the actual gestational age, is a strong indication for fetiforme teratoma. Considering the consecutive expulsion of the child, the placenta and the tumorous tissue, we think that the latter originated from the sac-like protrusion of the child's head, which ruptured during birth (hence the roughly edged opening), a mechanism that has been reported several times.[23,24] Subsequently, its contents were expelled in the uterine cavity. We therefore diagnosed this condition as intracranial fetiform teratoma.

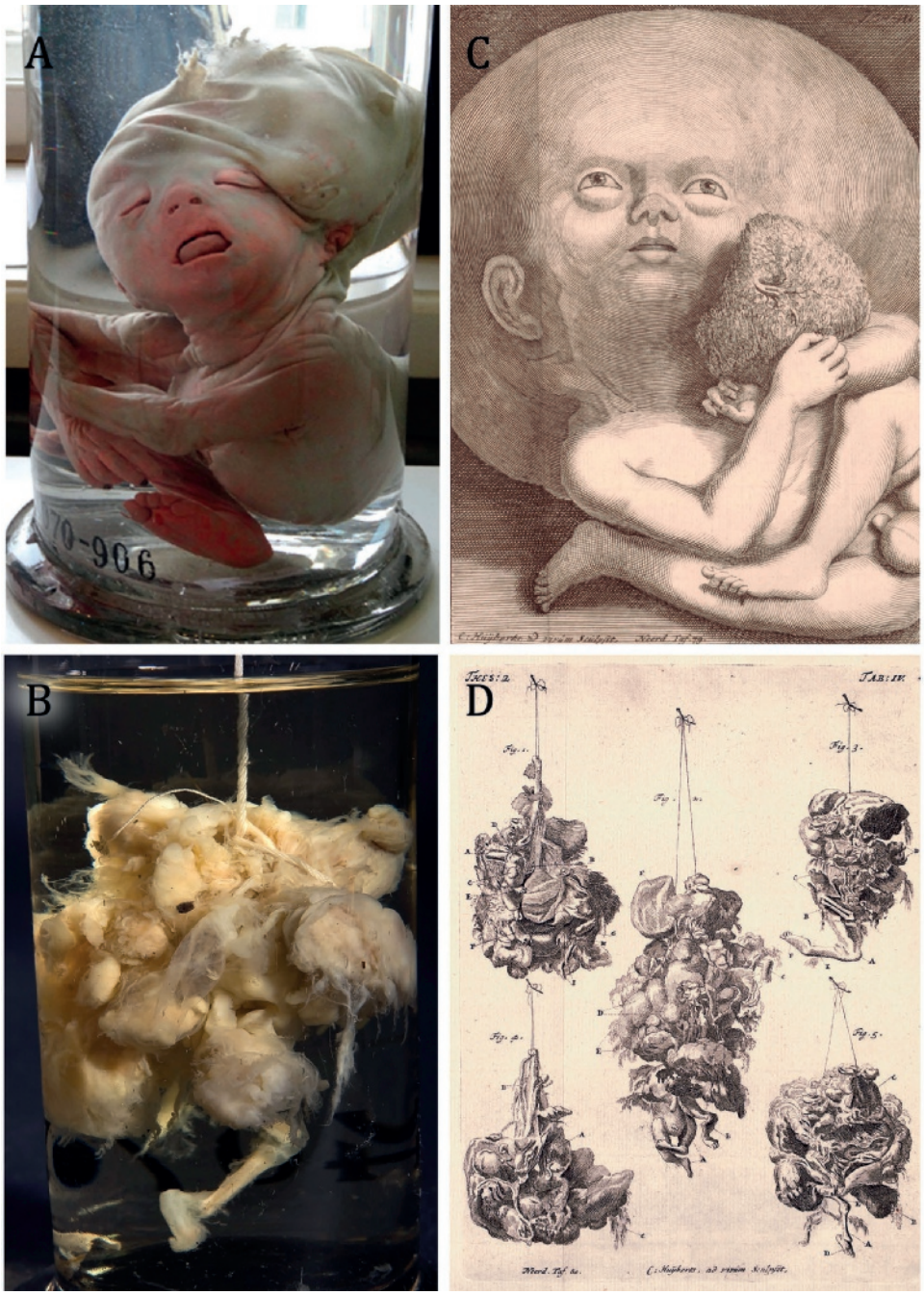


Fig. 7. Case 4. Intracranial fetiform teratoma. [A] Specimen 4070-906: neonate with asymmetrical enlarged head. [B] Specimen 4070-672: detail of the teratoma, presenting with limb-like structures. From the collection of the Peter the Great Museum of Anthropology and Ethnography (Kunstkamera), Russian Academy of Sciences. [C/D] Copperplates. [22]

Omental teratoma

Case 5. In the 18th of the 100 clinical observations Ruysch described the postmortem examination of a woman that he performed in 1683 and depicted the specimen that he subsequently obtained. [25] Prior to her death the patient experienced a supra-umbilical inflammation of the abdominal wall after she had been suffering from ascites for 15 years. Upon surgical relief of the ascitis, large quantities of clear fluid oozed out of the wound, both spontaneously and after repeated drainage. Although initially odourless, the fluid that was evacuated rapidly acquired an unbearable stench, which heralded her death five days later. At autopsy, Ruysch found a tumor as big as a fist, located in the major omentum and additionally attached to the peritoneum. The tumor consisted of a soft, non-smelling, white substance as well as a substantial amount of matted and curled hair (Fig. 8). Ruysch found these hairs to be similar to those growing on a human head, which he concluded by microscopic examination, albeit that no roots were visible. Fragments of the tumor became specimens in four different cabinets including the ninth specimen on the fourth shelf of his earliest cabinet, the 23rd specimen of the third anatomical cabinet, and the 74th and 106th specimens in the second and third cabinets of his second collection. [26,27,19,8] We diagnosed the condition as cystic omental teratoma (dermoid cyst). The reported ascites may have resulted from malignant transformation of an initially benign tumor, [28] although this seems unlikely considering its longstanding existence. Alternatively, the ascites and the inflammation of the abdominal wall were caused by co-occurring, unrelated conditions.

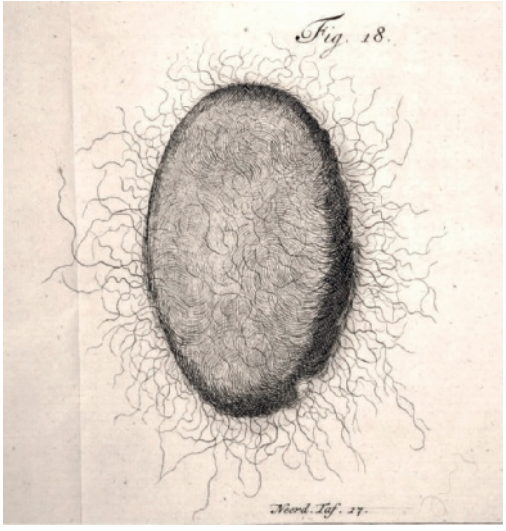


Fig. 8. Case 5. Omental teratoma, presenting with matted and curled hair, copperplate. [25]

Ovarial teratoma

Case 6. A short case description of a 24-year-old woman who suffered from fever and complained about pain in the lower abdominal region is mentioned and depicted as the 17th specimen of the first cabinet. [29] When Ruysch and his son (Hendrik Ruysch) opened the women's body after she had died, they both noticed a hardening of the left ovary. Subsequently, they dissected the dysmorphic ovary, finding four teeth-like structures (Fig. 9). This report can be diagnosed as an ovarian teratoma.

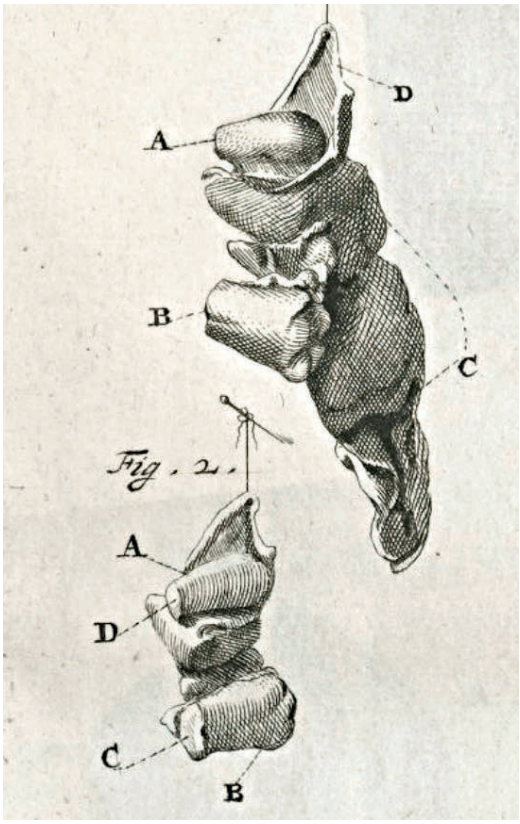


Fig. 9. Case 6. Ovarial teratoma, presenting with four teeth, copperplate. [29]

Umbilical angiomyxoma

Case 7. The third specimen in the ninth cabinet concerned a piece of an umbilical cord with a hardened tumor in it. [12] Because of its position quite proximal to the ventral wall of the otherwise healthy child, the midwife that conducted the delivery probably suspected an umbilical hernia, as Ruysch initially did, and she had cut the cord distal to the tumor. On dissection Ruysch

found the tumor to contain solid structures and to be partially filled with a curious fluid-like substance. What Ruysch described seems to have been a true neoplasm of the umbilical cord, considering the solid and cystic components. The mentioned substance could be interpreted as degenerated Wharton jelly, which is indicative for haemangioma (angiomyxoma). Nevertheless a teratoma, although exceedingly rare, cannot be ruled out in the absence of histology. [30] One can easily understand Ruysch's initial assumption that the tumor was an umbilical hernia, considering the overall rarity of umbilical tumors and its superficial morphological resemblance to umbilical hernias, especially when located in close proximity to the umbilicus.

Conjoined twins

Laterally conjoined twins

Case 8. This case concerns the embalmed and mummified body of a conjoined twin (Fig. 10A). According to Ruysch, who purchased the specimen from an unknown seller for his seventh cabinet, the applied embalming technique was quite different from his own. [31,17] This rendered the specimen very hard and stripped from its natural complexity. He described the specimen as a term neonate with two heads, three arms and two legs. External inspection confirmed this description. We diagnosed the condition as a laterally conjoined twin, more specific; parapagus dicephalus tribrachius dipus. We made the diagnosis laterally conjoined twins in three other specimens as well (Fig. 10B and C) none of which was described or mentioned in the CW (cases 9–11).

Ventrally conjoined twins

Case 12. This case concerns the embalmed and mummified body of a conjoined twin (Fig. 10D). Ruysch described this specimen briefly in both his first and second sets of ten clinical remarks. [17,13] However, this specimen is not mentioned as part of any of the cabinets. According to Ruysch the twin were two prematurely born children joined together at the thorax and abdomen. It surprised him that women can actually give birth to conjoined twins vaginally and apparently survive. In this specific case Ruysch made a special arrangement with the parents in that they were allowed at all times to visit their children at his dwelling. External inspection confirmed Ruysch's description. We diagnosed the condition as a ventrally conjoined twin, more specific: thoracoileopagus tetrabrachius tetrapus.

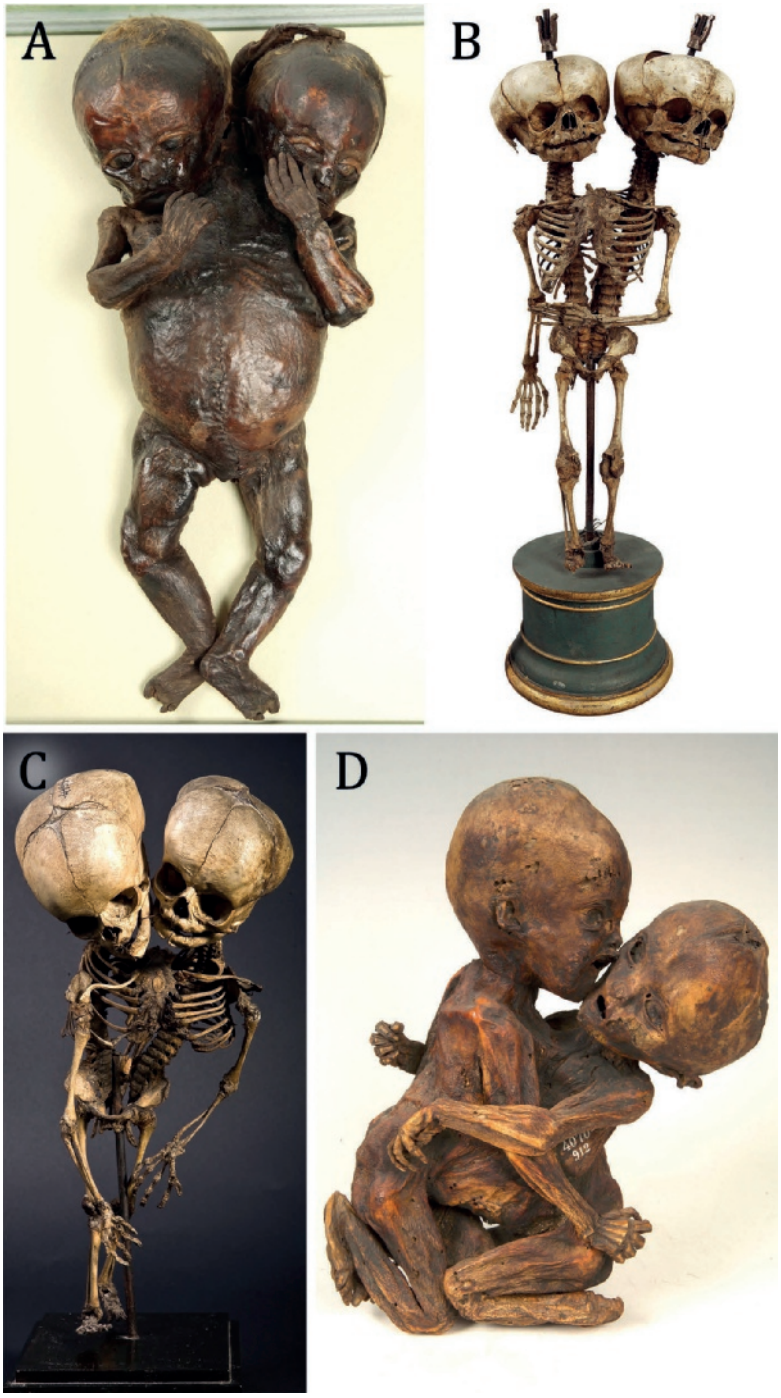


Fig. 10. Conjoined twins. [A] Specimen 4070-913: Parapagus dicephalus tribrachius dipus (case 8). [B] Specimen 4070-914: Parapagus dicephalus tribrachius dipus (case 10). [C] Specimen 4070-916: Parapagus dicephalus tribrachius dipus (case 11). [D] Specimen 4070-912: Thoracoileopagus tetrabrachius tetrapus (case 12). From the collection of the Peter the Great Museum of Anthropology and Ethnography (Kunstkamera), Russian Academy of Sciences.

Gastrointestinal anomalies

Anorectal malformations

Case 13. In the second set of ten clinical remarks Ruysch described a male infant which was seen by him at home, dated August 1718. [13] The affected child was suffering from an anal malformation in which the anus was closed by a very thin, round membrane. Behind this membrane, Ruysch noted the presence of meconium. After 5 days this membrane ruptured spontaneously and meconium oozed out. Unfortunately, the child died after a couple of days. No cause of death was identified. We diagnosed the condition membranous rectal atresia. Ruysch noted that this anomaly could have healed properly after making an incision of the membrane with a trocart. Additionally, Ruysch mentioned two children with congenital absence of the rectum and external opening. Ruysch described that in one of them (case 14) surgeon Pieter Adriaanze pierced the skin at the place where the anus should be located. After piercing the skin the length of an entire small finger could be inserted before Adriaanze reached the intestine. Ruysch concluded that the entire rectum was absent. We therefore diagnosed this condition as rectal agenesis, apparently without fistula.

Hirschsprung's disease

Case 15. Ruysch described a 5-year-old girl with an excessively dilated colon ("*Enormis intestini coli dilatatio*") in the 92nd of his 100 clinical observations. [25] This child complained of abdominal pain and constipation for a long time. All kind of remedies were tried, to no avail. When Ruysch opened the body of the child after its demise, he was astonished by the extent and size of the colon. Most other viscera were hidden under this dilated colon. Unfortunately, this case was not depicted. Although the clinical description is rather incomplete, it is generally assumed that this case report—which has been referred to multiple times in the literature—is the first cited case of aganglionic megacolon (Hirschsprung disease), thus leading to the eponym Ruysch disease (see Discussion).

Meckel's Diverticulum

Case 16. Ruysch described a dried piece of an ileum with a blind ending dilatation (Fig. 11) as part of his earliest collection and mentioned and depicted it again when describing a similar specimen (case 17) as part of his 7th cabinet. [26,31] Ruysch noted that such a diverticulum mostly originated from the ileum although other part of the intestines could be affected as well. If the small intestines were located at the lower abdominal regions, these affected pieces of intestine could herniate into the inguinal region. As part of the second cabinet of his second collection Ruysch mentioned a third similar specimen (case 18). [19] We diagnosed all three cases as Meckel diverticulum.

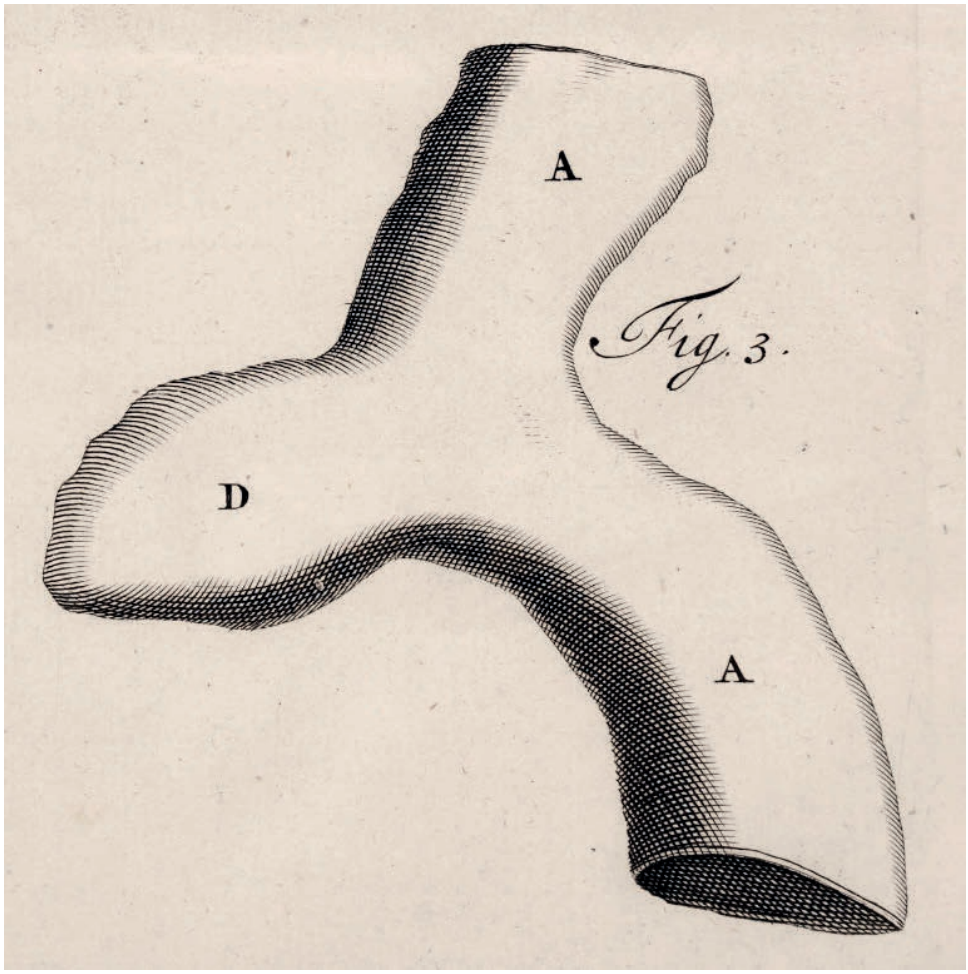


Fig. 11. Case 16. Meckel's diverticulum, copperplate. [26]

Multiple liver cysts

Case 19. The 29th specimen in the seventh cabinet of the second collection concerned part of the liver of a new born child. According to the brief description, it presented with water-filled vesicles. [8] Despite the paucity of information we are inclined to diagnose the condition as multiple liver cysts.

Holoprosencephaly

Case 20. The 74th specimen in the first cabinet of the second collection concerns a case description of a 3-month-old fetus with a snout-like appendage, instead of a nose, which according to Ruysch resembled a male genital organ. [32] Ruysch referred to this case previously in the first set of clinical remarks. [17] Despite the paucity of details in this brief description we are inclined to diagnose the condition as holoprosencephaly (cebocephaly or ethmocephaly).

Hydatidiform moles

The CW contains at least four references (Mus Anat, p.93; Thes Anat III, p.11; Thes Anat VI, p.47; Thes Anat X, p.27) [27,16,33] to specimens that, according to Ruysch, concerned placentas, together with their membranes and umbilical cords, that changed completely into grapelike water-filled vesicles and sometimes proliferated to astonishing amounts (Fig. 12A). Three additional similar specimens (cases 21–23) were described as part of his second collection. [19,8] As he stated on several occasions throughout the CW (Obs Cent, p.34 and 43; Adv Dec II, p.32; Trac Anat, p.16), [25,13,34] he considered them to be the result of postpartum retained and subsequently degenerated, yet initially normal placentas. He claimed this to be demonstrated in placentas that were only partially vesicularized, of which he described four specimens. The extant collection contains six specimens of typical complete hydatidiform moles (cases 24–29), with or without parts of the uterine wall (Fig. 12B). Quite probably, these specimens overlap with the four CW references above (and perhaps with some of the less specific specimen descriptions as well) but a one-on-one matching is impossible since specimen-specific features are lacking. Moreover, there are several other specimens with less typical characteristics of hydatidiform mole that could be partial moles but are more likely to be hydropic abortions. This also counts for the several partially vesicularized specimens mentioned by Ruysch. Convincing cases of partial moles were not found in the extant specimens collection.

Fig. 12. Hydatidiform moles. [A] Copperplate. [26] [B] Specimen 4070–674 (case 28). From the collection of the Peter the Great Museum of Anthropology and Ethnography (Kunstkamera), Russian Academy of Sciences.

Hydrocephaly

Case 30. The ninth and tenth specimen in Ruysch's earliest collection concern preparations of the septum pellucidum and part of the skull of an 8-year-old child who suffered from congenital hydrocephaly. [26] Ruysch noted that the caudal end of the septum pellucidum was extended to the inferior parts of the brain and contained multiple perforations permitting flow of fluid between the ventricles. According to Ruysch the increase of fluid caused the amount of brain tissue to diminish and the sutures of the skull to widen at least the width of a finger. Interestingly, the ninth specimen in the third cabinet concerns a piece of brain derived from the same patient. [27]A second case of hydrocephaly concerns a 6-month-old fetus (case 31), being the 29th specimen in the ninth cabinet. [12] Ruysch used this specimen as part of a display of different natural products, such as corals, casts of blood vessels, bones and urinary- and bladderstones.

Limb malformations

Case 32. This case concerns the specimen of a neonate with asymmetrical limb malformations, affecting all but one of the extremities (Fig. 13A and B). Ruysch had placed it in the back of the eighth cabinet, out of courtesy for his visitors, who might consider its appearance to be too confrontational. [35] He noticed that the right arm was bent markedly, and that the right hand only had three fingers. The right foot contained three toes: a deviating big toe and two normal toes. The left lower limb seemed to be lacking at all, apart from a slight elevation in the hip region. Furthermore, there was a tail-like appendage attached to the abdominal wall. The left upper limb, as well as the head and body, showed no malformations. External inspection confirmed the observations done by Ruysch. In addition to the oligodactyly, the affected extremities were markedly shortened. The facial appearance, which we considered mildly dysmorphic, might have resulted from oligohydramnios but is probably a fixation and storage artifact. The reddish coloring of the skin resulted from postmortem cinnabar-containing vascular injections. Undoubtedly, the most remarkable symptom is the tail-like appendage.

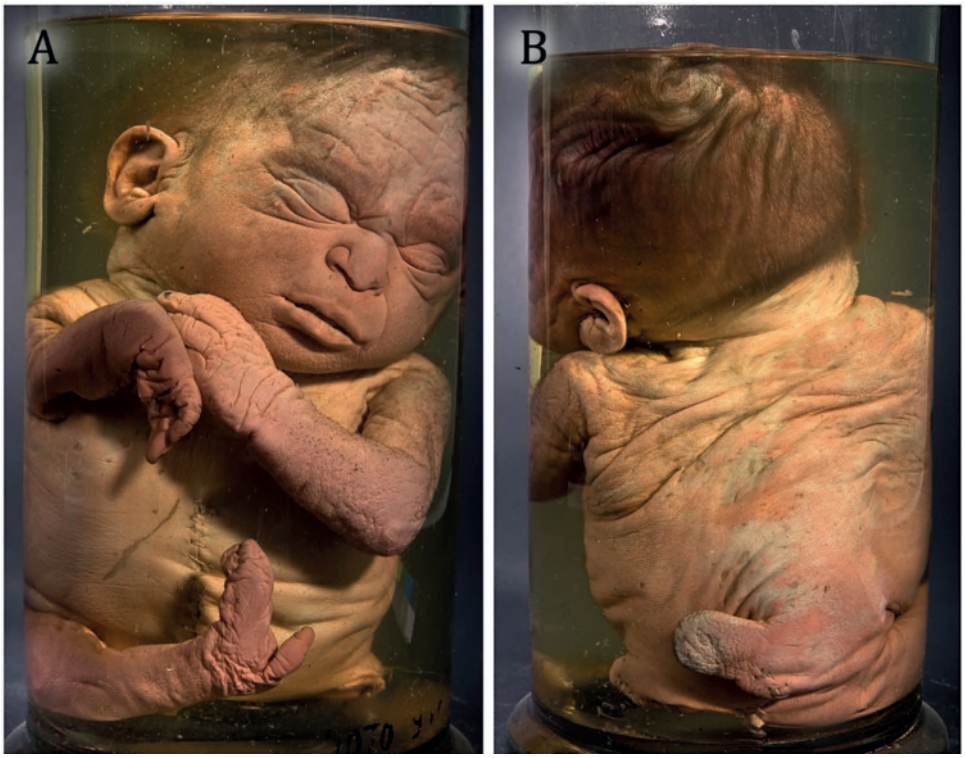


Fig. 13. Case 32. Limb malformation with tail like appendage. Specimen 4070–907, [A] ventral and [B] dorsal views. From the collection of the Peter the Great Museum of Anthropology and Ethnography (Kunstkamera), Russian Academy of Sciences.

In combination with the otherwise nonspecific reduction defects, the only condition that comes to mind is disorganization-like syndrome (MIM 223200). Profound investigation of the specimen, including imaging, would be necessary to confirm this diagnosis, but is unfortunately impossible. Limb malformations (presumably radial aplasia) with oligodactyly were present in two other specimens. These two specimens (cases 33 and 34) were upper extremities of neonates, neither of which was described or mentioned in the CW (Fig. 14A and B).

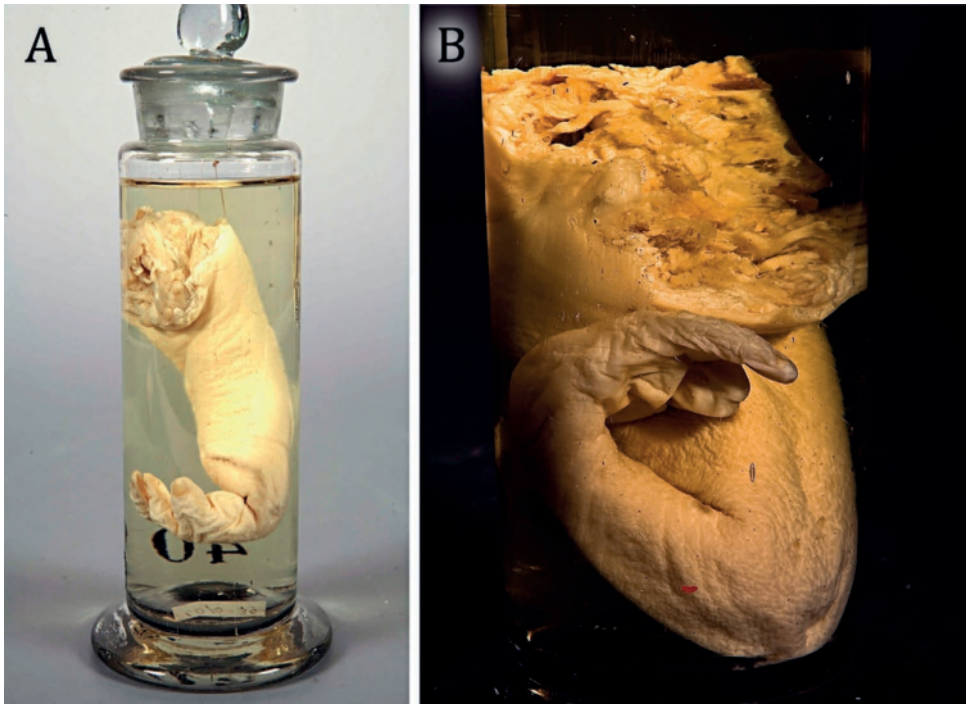


Fig. 14. Limb malformations. [A] Specimen 4070–039 (case 33). [B] Specimen 4070–908 (case 34). From the collection of the Peter the Great Museum of Anthropology and Ethnography (Kunstkamera), Russian Academy of Sciences.

Neural tube closure defects

Case 35. This case concerns the dried skeleton of a neonate (Fig. 15). Ruysch mentioned this specimen quite briefly in his first set of ten clinical remarks in the section on misconceptions but not as part of any of the cabinets. [17] It is one of the three specimens in his first collection of prematurely born children lacking a brain and a cranial cavity, according to Ruysch. Of the other two specimens, he skeletonized one (case 36, see further) and kept the other on embalming fluid (case 37). The latter one has apparently gone lost. On external inspection we confirmed Ruysch's description and found all vertebral arches

to be intact. We diagnosed the condition as anterior neural tube closure defect (holoacrania). The first specimen of Ruysch's eighth cabinet concerned the dried skeleton of a prematurely born infant without a brain and a cranial cavity (case 36). [35] Additionally, he found the cervical and upper thoracic vertebral arches to be cloven. At these levels the spinal cord was absent, whereas it was intact and well formed at lower thoracic and lumbar levels. Ruysch did not depict the specimen himself but instead referred to drawings made by his friend and colleague Theodor Kerckring (1638–1693), a Dutch anatomist and alchemist, who described and depicted this specimen in his *Spicilegium anatomicum*, a collection of 100 anatomical curiosities. [36]

Fig. 15. Case 35. Anterior neural tube closure defect (holoacrania), specimen 4070–915 from the collection of the Peter the Great Museum of Anthropology and Ethnography (Kunstkamera), Russian Academy of Sciences.

Based on the drawings (Fig. 16) and descriptions we made the diagnosis anterior neural tube closure defect (holoacrania with partial rachischisis). A fourth case of anterior neural tube closure defect (holoacrania with or without partial rachischisis) is found as the 24th specimen in the ninth cabinet of his second collection.[8] According to Ruysch it concerned a fetus that lacked a brain and a neck (case 38). It cannot be excluded that this case is the same as the embalmed specimen Ruysch referred to in the first set of ten clinical remarks, since this part of the CW was written after he had sold his first collection, and there seems to be no specimen in the first collection to which the description applies. On the other hand there are more specimens, including specimen 4070–915 (see above), that were not described as part of any of the cabinets of the first collection.

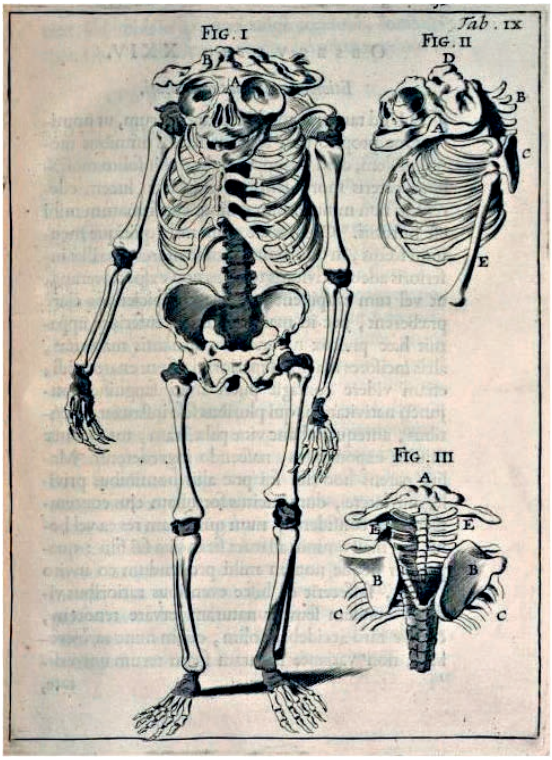


Fig. 16. Case 36. Anterior neural tube closure defect (holoacrania with partial rachischisis), copperplate.

In the 34th, 35th, and 36th of his 100 clinical observations, Ruysch described five cases (although he mentioned to have seen at least ten) of one or more bifid vertebral arches whereas the vertebral bodies appeared morphologically normal (cases 39–43). [25] He found these defects to co-occur with soft tumors of different sizes which were filled with clear fluid,

interpreted by Ruysch as spinal cord tissue that changed into a water-like substance. He compared this with the changing of neural tissue into water occurring inside the head, resulting in hydrocephaly. Most children that Ruysch encountered with this condition had paralyzed legs, could not be treated other than palliatively and died soon after birth, especially when the tumor was perforated. Ruysch therefore recalled the words of one of his anatomical predecessors in Amsterdam, Nicolaas Tulp (1593–1674), who founded the name "spina bifida" for this condition, to carefully handle the tumor and prevent it from being damaged. [37] However, Ruysch observed two infants, one with a sacral lesion, who survived until her first year. Ruysch concluded that this relative long survival period may have been due to the low insertion of the tumor. Among the described observations of posterior neural tube closure defects, we diagnosed three cases of lumbar meningo(myelo)cele, two of which were depicted (Fig. 17), and one sacral meningo(myelo)cele. Additionally, Ruysch mentioned in the 34th observation a specimen of what seemed to have been a cervical meningo(myelo)cele, that supposedly was part of his earliest cabinet. However, none of the specimens in this or any other cabinet matched with that description.

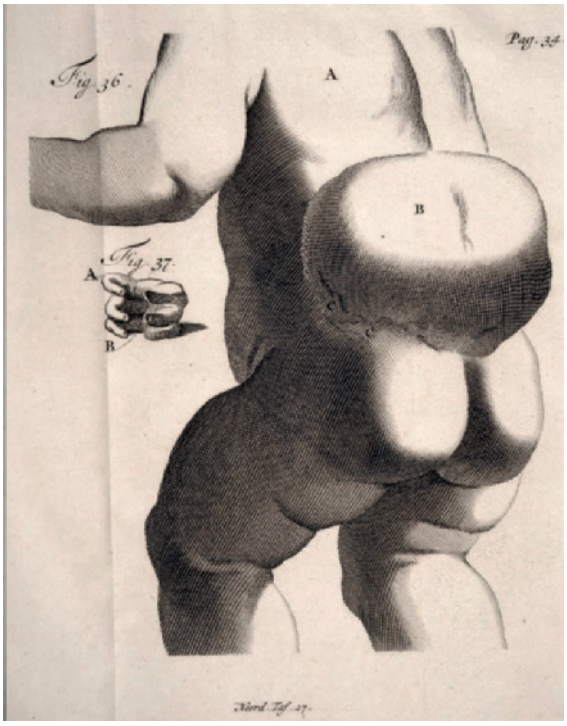


Fig. 17. Case 40. Posterior neural tube closure defect (cystic spina bifida), copperplate. [25]

Skeletal dysplasias

Ciliary chondrodysplasia

Case 44. As part of his earliest collection, Ruysch mentioned the dried skeleton of a neonate, whose body was found floating in the IJ-river in Amsterdam (Fig. 18). [26] It was brought to him for dissection by the city council. On examination Ruysch noted several peculiarities, including a disproportion between the head and the body, short limbs and supernumerary fingers and toes. The frontal fontanel was twice the expected size, the temporal bones were very small, the orbits were somewhat misshapen and the nasal bones were broad and depressed. There was a small median cleft in the upper jaw, whereas the lower jaw showed a median crest-like elevation, absence of the mandibular symphysis and bony protrusions of the rami. Some teeth were already erupting. The thorax showed very short ribs, small shoulder blades and a little sternum. The tubular bones of all extremities were very short, their length not exceeding the width of one or two fingers. The right hand had seven complete fingers, the left hand six, with a round appendage on both the thumb and the 5th finger. The right and left feet contained eight and nine toes respectively. He referred to this specimen additionally in his first set of ten clinical remarks [17] where he stated that there is a certain size or measure in all things: if nature creates things in abundance like supernumerary fingers and toes, this is often useless and prejudicial. Finally, he noticed several carpal, tarsal and metatarsal ossicles in both hands and feet. Ruysch did not depict the specimen himself but again referred to drawings made by Theodor Kerckring for his *Spicilegium Anatomicum*. [36] Interestingly, although Kerckring gave an equally detailed report of his own examination, these findings differ slightly from those made by Ruysch, especially regarding the lower limbs. He noticed the tibiae to be equally thick to the femora, with cartilage on either site.

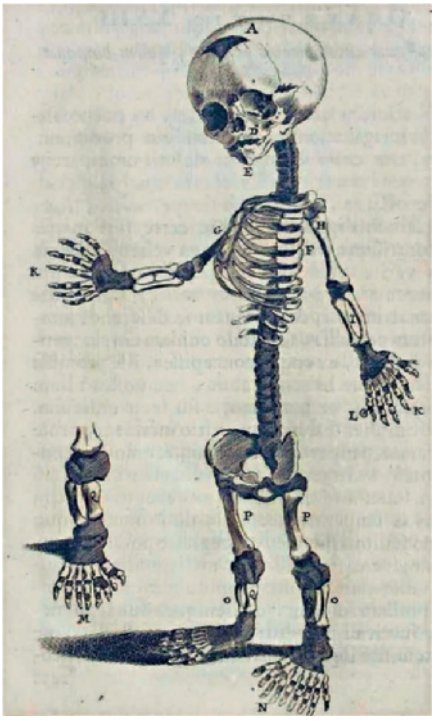


Fig. 18. Case 44. Ciliary chondrodysplasia (Majewski syndrome), copperplate. [36]

Unfortunately, neither of the reports includes any soft tissue characteristics. This case has been referred to several times in the literature (see Discussion), in most instances understandably, but erroneously, assigned to Kerckring. Inspection of Kerckring's images largely confirms the reported findings. The drawing of the skull implies a short cranial base, relative bulging of the forehead, mild hypertelorism, a broad and depressed nasal bridge and a small medial cleft of the maxilla. The polydactyly of most extremities appears to be of both pre- and post-axial nature, with both proximal and distal duplications. The detailed drawing of the right leg suggests that the tibia was significantly shorter than the fibula and somewhat ovoid in shape. The combination of very short ribs and limbs, polydactyly and apparent neonatal death is very suggestive of a lethal skeletal dysplasia, in this case a ciliary chondrodysplasia (short-rib thoracic dysplasia/short-rib polydactyly syndrome). Most findings, including the median cleft, the severe shortening of the ribs and the limbs, the mixed polydactylies and the dysplastic tibiae, are in line with Majewski syndrome (SRTD6, SRPS2, MIM 263520) although the cranial dysmorphology (except for the median cleft) is more suggestive of Verma-Naumoff syndrome (SRTD3, SRPS3, MIM 613091). Neonatal teeth have been reported occasionally in various ciliary chondrodysplasias. [38]

Enchondromatosis

Case 45. Corresponding with his well acquainted colleague Mauritius van Reverhorst (1666–1722), medical doctor and professor of anatomy and surgery at the Leiden University, Ruysch described the amputation of the left forearm in a 16 years old male. [39] The patient was suffering from large tumors in his hands and, to a lesser extent, in his feet. The tumors appeared small at the time of birth but steadily grow over the years to such proportions that especially his left hand became merely a useless, increasingly painful hindrance that he had to support with his other hand because of its weight. Damage to the hand and infection were repeatedly followed by excessive bleeding of one of the tumors. Ruysch considered the shape of the tumors, most of which were solid and very hard, to resemble that of potatoes, tubers of cyclamens or roots of chrysants. Interestingly, the mother of the patient admitted that she found chrysant roots (*Chrysanthemum spp.)* very tasteful and she had been eating them abundantly during the pregnancy. After the amputation, which was post operatively complicated by infection, progressive inflammation and phantom limb pain, Ruysch examined and depicted the hand (Fig. 19) and opened one of the tumors at the request of Van Reverhorst.

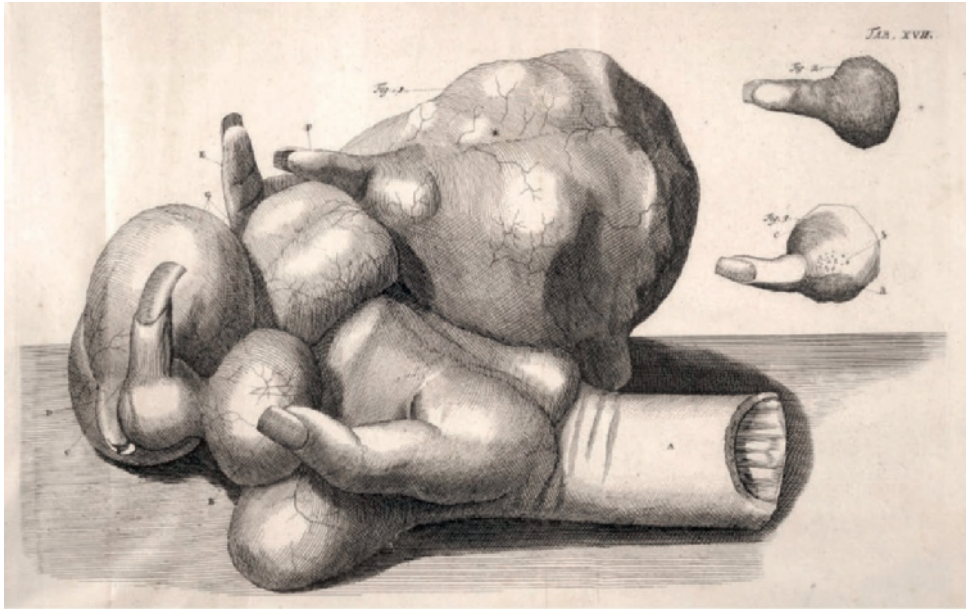


Fig. 19. Case 45. Enchondromatosis, copperplate. [39]

It appeared to contain numerous cartilaginous proliferations, some perhaps even osseous, together with mucous-filled spaces. Although Ruysch stated that he would keep the hand for future examination, it was never admitted to his collection as a specimen. The pictures of the grossly deformed hand suggest that most of the tumors originated from the metacarpals and proximal phalanges. The overall presentation of the condition, the medical history and the cartilaginous nature of the tumors are quite suggestive of enchondromatosis (MIM 166000). If the repetitive and excessive bleeding of one of the tumors is to be interpreted as a haemangioma, Maffucci syndrome (MIM 614569) would be the most probable diagnosis, although metachondromatosis (MIM156250) cannot be ruled out, if there were truly osseous tumors present as well.

Urogenital anomalies

Disorder of sex development

Case 46. In the eighth cabinet and the 18th description of the first set of 10 clinical remarks Ruysch reminisced to have seen several cases of "man-wives," although he stated never to have encountered true hermaphrodites, having both testicular and ovarian tissue, and he discarded reports of alleged cases as pseudohermaphroditism. [35,17] The only case Ruysch actually described concerns a 24-year-old individual with ambiguous genitalia. Although this person had a male facial appearance and claimed to be a heterosexual male, the phallic organ lacked a urethral orifice, which made Ruysch conclude that she was a female. He considered persons like this one to be incapable of reproduction because the sexual organs of either sex were not completely developed. Additionally, two testicle-like organs were located in the groin area but Ruysch assumed that they were little more than lumps of fatty tissue, as he had experienced from the dissection of a sheep with a similar condition. We diagnosed this condition as a disorder of sex development. Since the secondary sex characteristics and the internal sex organs were not described it is impossible to be conclusive on the precise diagnosis. Depending on the nature of the gonads both congenital adrenal hyperplasia and partial androgen insensitivity syndrome seem likely.

Fallopian tube duplication

Case 47. In the 83th specimen of the fourth cabinet Ruysch described the presence of three Fallopian tubes. [40] He stated that this was the only case of multiple Fallopian tubes he had ever seen, although he mentioned that it was possible that both Fallopian tubes were absent, and that unilateral absence occurred as well. This short case can be clearly interpreted as fallopian tube duplication.

Hypospadias

Case 48. This case concerns a specimen of the distal part of a penis presenting with a slit-like urethral orifice at the caudal aspect of the glans, which we diagnosed as distal hypospadias (Fig. 20). Although Ruysch stated to have encountered several patients in which the external urethral meatus was not located at the normal place and referred to these patients when describing similar conditions in animal specimens, [26,35] no descriptions of any human specimens were found. According to Ruysch urethral malposition caused infertility because the expulsion of the sperm was not in line with the cervix of the uterus.

Fig. 20. Case 48. Hypospadias, specimen 4070–600. From the collection of the Peter the Great Museum of Anthropology and Ethnography (Kunstkamera), Russian Academy of Sciences.

Imperforate hymen

Case 49. Ruysch described a case of a 20- year-old virgin who complained of agonizing cyclical abdominal pain in the 32nd of his 100 clinical observations. [25] With the help of obstetrician Andries Boekelman, Ruysch concluded that this re-occurring illness was due to an intra-vaginal membrane which covered the entire vaginal opening. After incision in this membrane, which turned out to be curative, a vast amount of black non-clotted blood was expelled, weighting around four pounds. We diagnosed the condition as haematocolpos resulting from an imperforate hymen.

Transverse vaginal septum

Case 50. Ruysch described a case of an obstructed labor in which an intra-vaginal hymen and a thick concomitant vaginal membrane arrested delivery of the child. He described this case in the 22nd of his 100 clinical observations. [25] During examination of the woman in labor Ruysch noticed an elongated hymen which appeared to be the reason for the stagnant delivery because it was pressing on the child's head. Obstetrician Andries Boekelman and surgeon Pieter Adriaansz assisted Ruysch to surgically remove this hymen. After the surgical procedure another thick membrane was noticeable, again causing a stagnant delivery. Therefore, the second membrane was cut as well, and a healthy newborn was delivered. Ruysch stated that it was possible to become pregnant with a remaining hymen. Interestingly, he thought that the second membrane had arisen after intercourse. This clinical case can be interpreted as a transverse vaginal septum (Fig. 21). Pregnancy in the presence of a seemingly intact hymen can occur due to a micro perforation in the hymen. [41] However, concomitant occurrence of a micro perforated hymen and a vaginal septum is very rare.

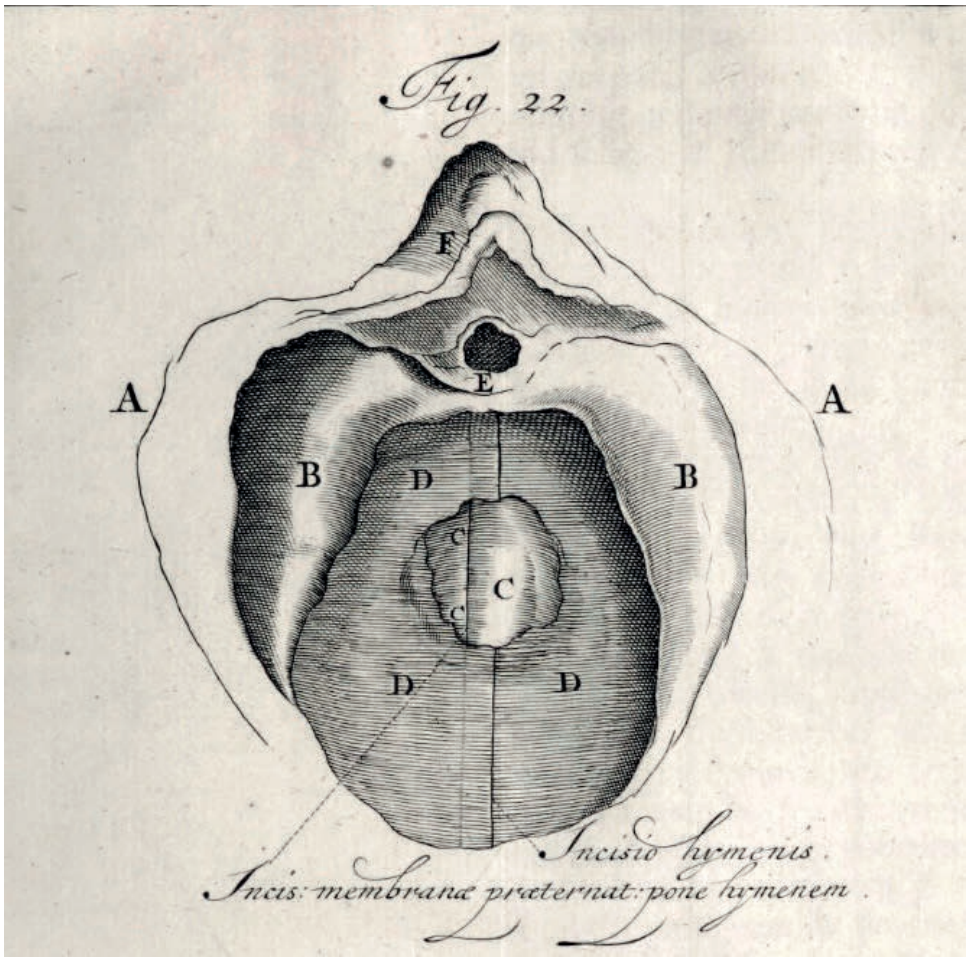


Fig. 21. Case 50. Transverse vaginal septum, copperplate. [25]

Ureteral duplication

Cases 51–54. The CW contains at least four references to cases and specimens that, according to Ruysch, concerned ureteral anomalies (Fig. 22). [42,25,31] Ruysch described both complete duplication of the ureters as well as ureters which unite before entering the bladder. These four cases were diagnosed as (partial) ureteral duplications.

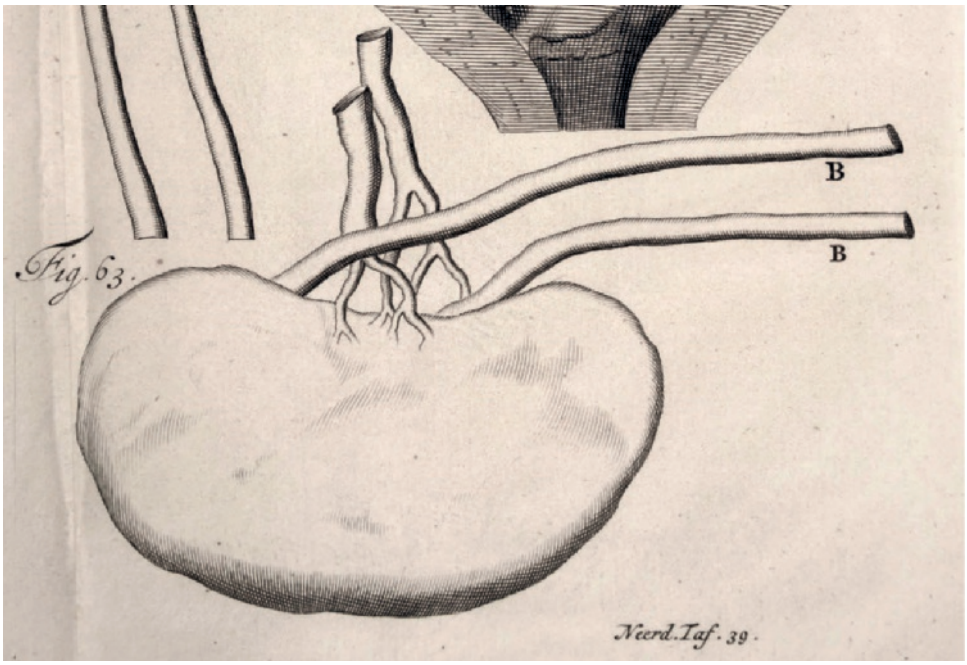


Fig. 22. Case 53. Ureteral duplication, copperplate. [25]

Ventral body wall defects

Bladder exstrophy

Case 55. In the 23rd of his 100 clinical observations Ruysch described the examination of a 6-days-old girl that was born with a malformation of the ventral body wall. [25] The external genitalia seemed to have merged with the umbilicus, thus creating a disorganized mass of tissue with several eminences. In the caudal part of the defect two openings were seen from which urine was continuously oozing, especially when the child cried. No bladder or urethra could be recognized. Additionally, Ruysch asked himself, without giving an answer, whether this anomaly could have been caused by a falling accident that the mother experienced a few weeks before the delivery. The description, as well as a picture of the defect (Fig. 23), fit completely with the diagnosis bladder exstrophy. Ruysch apparently did not recognize the infra-umbilical tissue mass as being the muscular folds on the inner side of the opened and everted bladder wall. The mentioned openings are the ureteral orifices.

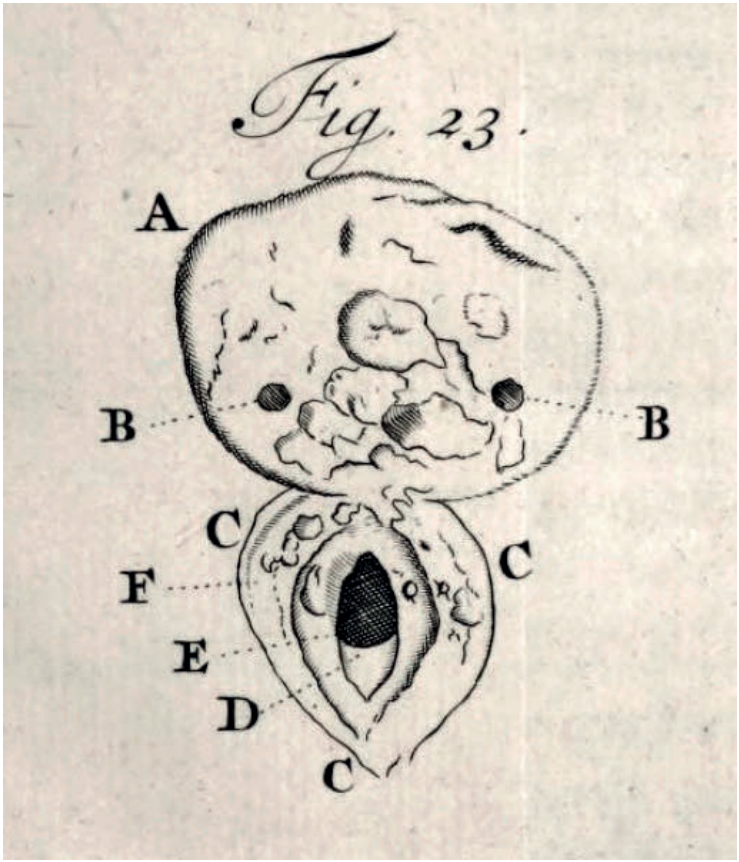


Fig. 23. Case 55. Bladder exstrophy, copperplate. [25]

Omphalocele/gastroschisis.

Cases 56–58. In his 71st, 72nd, and 73rd observations of his 100 clinical observations Ruysch described three cases of ventral body wall defects. [25] In observation 71 Ruysch mentioned the absence of the abdominal skin and muscles around the insertion of the umbilicus (Fig. 24). The intestines, as well as intestinal peristalsis, could be seen through a thin umbilical membrane. Ruysch stated that he encountered this anomaly rather often. However, he never saw recovery and all the affected children died a couple of days after birth. According to Ruysch the term "umbilical hernia" for this condition was chosen incorrectly since there was no true umbilicus noticeable. Instead, there was an outpouching of the peritoneal membranes. We diagnosed this case as an omphalocele. Additionally, in the 73rd observation Ruysch mentioned one other case of omphalocele.

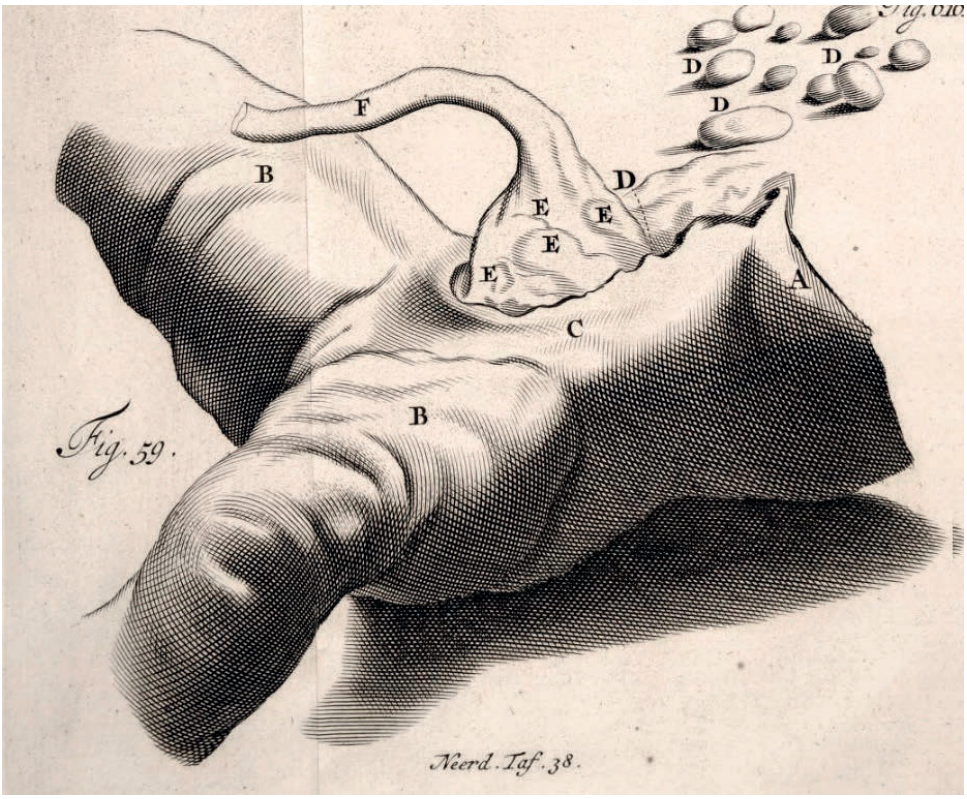


Fig. 24. Case 56. Omphalocele, copperplate. [25]

In his 72nd observation Ruysch described a neonate that was born with its stomach, intestines and spleen located outside its body. In this case, the organs, which bulged through the abdominal wall, were not covered with a membrane. The child lived for a couple of hours, and peristaltic movements of the bowel were noticeable during the short life of the newborn. Ruysch described that these abdominal wall defects were caused by the absence of the abdominal skin and muscles, and not by assumed obstetric malpractice. Unfortunately, Ruysch did not mention anything about the absence or presence of the umbilicus or umbilical cord. With the present information we are inclined to diagnose this case as gastroschisis.

Indiscernible conditions

Axial skeletal anomalies

Case 59. In the first of the 26 clinical reports that were part of his treatise on vascular valves, Ruysch described the bilateral presence of thirteen ribs in a girl who died of cachectic fevers, with two rib pairs being fused. [42] Unfortunately, the brief report does not reveal any details about the nature and location of the rib "fusion," especially regarding the question whether it concerned true fusion (e.g. bridging) or (forked) duplication, nor does it contain any information on the number of (thoracic) vertebrae, or any concomitant anomalies. The differential diagnosis therefore ranges from bifid rib conditions, either isolated or syndromic (e.g. Gorlin–Goltz syndrome), to fused ribs as part of meristic or homeotic segmentation defects of the axial skeleton. If the latter were the case, the cachectic fever that Ruysch mentioned, could be the expression of an end-stage childhood malignancy, which are known to co-occur frequently with abnormal rib counts. [42-44] A comparable description is found in the auction catalogue of the second collection, regarding the 144th specimen in the fourth section of the ninth cabinet. [8] It refers to a specimen of two ribs that, according to Ruysch, are congenitally fused (case 60). Since no other information is given a diagnosis cannot be made.

Cranial tumors

Ruysch described several cases of cranial tumors of the occipital and parietal region, often bigger than the head itself, sometimes even bigger than the child. One of these cases (case 61) was described in the 52th of his 100 clinical observations. [25] These tumors were likely to result in problematic labors and peri-natal deaths. When the tumors were opened they were often filled with fluid, having the same aspect as the fluid present in the ventricles of the brain, but sometimes they appeared to contain more solid, even cartilaginous tissues. The figure that accompanies the observation shows a large parietally located cranial tumor (Fig. 25). Based on the description Ruysch gave of these tumors, the depicted case cannot be diagnosed properly. It is possible though that Ruysch wanted to give an impression of the size of growth of these occipital tumors, without depicting a specific type.

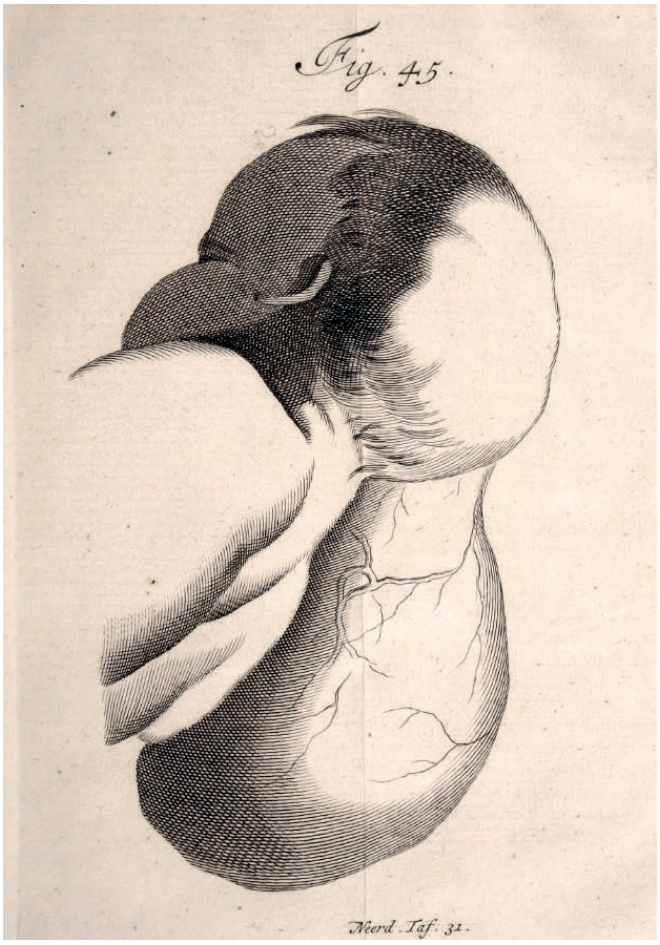


Fig. 25. Case 61. Cranial tumor, copperplate. [25]

Other generalized conditions

Case 62. The 53rd specimen of the sixth anatomical cabinet concerns a very short description of a specimen in which Ruysch described a premature stillborn child with curved arms, hands and feet. [16] Ruysch imagined that this child would have had a very miserable life if it had been born alive. Considering the bowing of long bones of all extremities campomelic dysplasia or osteogenisis imperfecta could be considered, among other diagnoses. With no further information this case cannot be diagnosed properly and stays indefinite. The 13th specimen of the fifth cabinet of the second collection is described without any additional information as a monstrous fetus of about two inch length (case 63), which makes the condition entirely indeterminable. [8]

Discussion

It is generally accepted that the Ruysch collection residing in Saint Petersburg is the first of its kind. Although Ruysch's predecessors and contemporaries were familiar with preparation techniques such as embalming, maceration, corrosion and injection, the magnitude, versatility and reputation of his collection was unprecedented. In the subsequent decades Ruysch became a shining example for ambitious anatomists that aspired a specimen collection of their own, not merely for scientific purposes but mainly to amaze and impress their friends and colleagues. Following the heydays of the private anatomical and naturalia collections in the mid-18th century, a gradual shift took place towards institutionalized collections, that focused on systematic rather than anecdotally collected morphology specimens. Collections like these, primarily meant for the instruction of (bio)medical students and professionals, have been operational up to the present day in many medical schools around the world. What remained, however, is the (partly sensation-driven) fascination of lay people with human anatomy, which is what Ruysch experienced at the time when making his cabinets publically accessible and what makes exhibitions of plastinated anatomical specimens, such as Günter von Hagens' "Body Worlds" so successful 300 years later.

Until the mid-20th century it was unknown to Western Europe whether the collection of Frederik Ruysch, or the remains of it, still existed. Publications regarding the collection, including the mid eighteenth century Latin catalogue [46] apparently went unnoticed by Western European scholars. According to the German anatomist Josef Hyrtl (1810–1894) both the first and second collections had gone lost in their entirety. [47] He insinuated that the sailors, who shipped the specimens of Ruysch to Saint Petersburg, drank the alcohol-based embalming fluid from the bottles, leaving the collection in a deplorable state. [48] This entirely fictive assumption subsequently became a tenacious legend that has persisted up to the present day. As it appeared, however, successive Russian curators took well care of the Ruysch collection although they could not prevent a gradual decline in the number of preserved specimens, from around 1,500 specimens in 1808, 1,350 in 1843, 1,086 in 1903, down to 935 in 1947. [10] Presently, the 916 specimens that survived are constantly being monitored by a team of curators and renovated when necessary.

During the analysis of the extant collection we came across quite a few specimens, both teratological and otherwise, that could not be matched with any of the descriptions, not because they lacked distinctive characteristics (like the hydatidiform moles) but because they had them, without any of the

specimen descriptions being applicable to it. An example of this concerns case 35. In the first set of ten clinical remarks Ruysch almost incidentally mentioned a second skeletonized fetus with an anterior neural tube closure defect, yet none of the cabinet inventories (of both the first and the second collections) listed a specimen to which this description could be applied. This proofs that the collection purchased by Peter the Great contained an unknown number of non described specimens, which at the time was confirmed by the earliest curators of the collection in Saint Petersburg. [10] In addition to that, Russian anatomists, inspired by Ruysch's preparation techniques, made specimens of their own [10] and it is conceivable that in the course of time they became incorporated in the Ruysch collection, for example, to replace specimens that had gone lost.

Considering the modest size of Ruysch's teratological legacy, it contains a surprisingly high number of exceedingly rare conditions, especially concerning congenital tumors. Intracranial teratomas (like case 4) have a reported incidence of 0.43 in one million [23] with only a fraction of those presenting with fetiform structures. In fact, intracranial teratoma with fetiforme remains of multiple individuals, as presented in Ruysch's case, has been reported only twice. [49,50] Gastric teratoma (case 3) accounts for less than 1% of all teratomas in childhood [51] with a 161 reported cases. [52] Only five cases concern adult individuals. [53,54] Omental teratoma (case 5) has been reported in only 31 cases since 1928. [55] Clearly, Ruysch was highly impressed by the shaping powers of nature that could result in tumors containing hairs, teeth, nails and parts of extremities. However, the underrepresentation of more common and explicit congenital conditions, such as cleft lip/palate, limb reduction defects and neural tube closure defects in his collections, may also be a reflection of Ruysch's personal preferences and assumptions. In the description of a neonate with cleft lip and palate (case 2) Ruysch mentioned that he was reluctant to show his visitors frightening specimens like these, unless they insisted. He considered them to be unfit for exposition and should best be buried instead of disharmonizing a fine anatomical exhibition. The purpose of his specimens was to impress his visitors with the beauty of the human body and its tissues and he considered overt "monstrosities" to be in dissonance with this concept, which is why he stored them in the back of his cabinets and is what may have withheld him from proactively collecting this type of specimens. This could account for the fact that, for example, spina bifida, despite being one of the most common malformations and encountered by Ruysch at least ten times, is not represented at all in his collections.

Moreover, Ruysch was aware of the fear among pregnant women to be

confronted with congenital anomalies. According to the maternal imagination theory, which was still a well-grounded conception in the 17th and 18th century, this could be harmful to the unborn child. Ruysch considered this opinion not his to criticize and kept a distance to any verdicts. Although it seems unlikely that Ruysch assumed normally developing fetuses to be susceptible to maternal imaginations, he certainly considered this to be possible with respect to the moment of conception. In the second set of ten clinical remarks Ruysch stressed the importance for parents to explain them the difference between a "monstrosity" and a macerated but otherwise well-formed fetus, since the latter should be much more comforting to them and would prevent the mother from having falsely induced, yet harmful memories during subsequent procreations. [13]

Although admired by friend and foe alike for the beauty of his preparations and his skills in vascular injection, his academic contributions to the medical science were disputed already during his lifetime and shortly thereafter. According to Ginzburg (1953) [10] George Cuvier (1769–1832) was his most important proponent and was marveled by all his works, whereas Karl Ernst von Baer (1792–1876), who admired his specimens was not at all impressed by his scientific output. Hyrtl, who erroneously assumed both Ruysch collections had completely gone lost, had no sympathies for Ruysch's legacy whatsoever and considered his injection specimens to be short lived artifacts resulting from a destructive technique and worthless for detailed histological investigation. [47] Nevertheless, Ruysch did some important anatomical discoveries including the valves of the lymphatic vessels, the intestinal lymph nodes, the "*vasa vasorum*" and the bronchial arterial system. Regarding congenital anomalies, Ruysch displayed a certain modesty. Roughly a century after Vesalius, he must have realized that science was not capable yet of giving adequate answers to all the questions that his teratological specimens generated. Perhaps this is why he dedicated himself to careful descriptions rather than to speculative interpretations and bold opinions and to all sceptics he stated that nature can create all sorts of marvelous things, thereby inviting them to come to his dwelling and see it with their own eyes. This does not mean that he refrained from contributing to the current debates on embryological and teratological topics.

One of these topics was superfetation, the process of ovulation and fertilization during an intact pregnancy resulting in two conceptuses of different developmental stages. [56] In the fourteenth of his 100 clinical observations and as 120th specimen of his sixth cabinet Ruysch described a case, dated 1686, of a pregnant women who gave birth to a healthy newborn.

Six hours later, she delivered a small fetus of around 3 months gestation (Fig. 26). [25,16] Ruysch initially found the placenta to be unusually large and thick considering the gestational age but as he later admitted this enlargement merely concerned clotted blood. Furthermore, the umbilical cord had changed into a concatenation of fluid-filled vesicles. According to Ruysch, the finding of a second concomitant and discordant conceptus was evidence for superfetation.

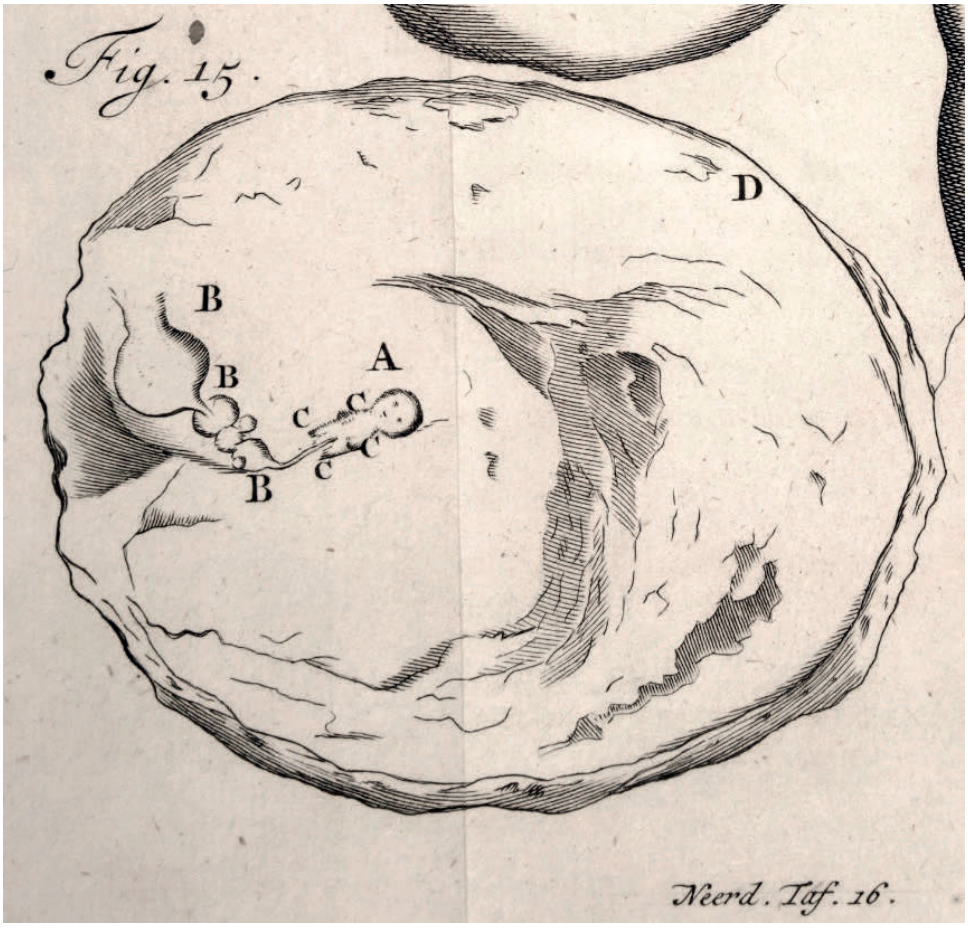


Fig. 26. Discordant twin fetus, presenting with vesiculated umbilical cord and enlarged placenta, copperplate. [25]

The phenomenon of (alleged) superfetation has been abundantly reported in previous times. Gould and Pyle mentioned Ruysch's description and recalled at least thirty other cases, some dating back to classical times. [57] Although true superfetation is known to occur in several mammalian species, either frequently or occasionally, it seems extremely rare in humans and its unequivocal existence has yet to be proven. [56] In most cases of alleged superfetation other causes of discordant development between two (or more)

fetuses seem much more probable (or at least cannot be ruled out), such as superfecundation (successive fertilizations of two or more oocytes originating from a single ovulation), delayed nidation (timing differences in implantation of two or more zygotes), growth discordance in multiple pregnancies due to placental insufficiency or twin to twin transfusion syndrome, and pre- or immature death of a twin sib. [58,56] From Ruysch's description, it seems very likely that the latter was the case. Although not mentioned as such it seems certain that the fetus died long before birth, considering the gestational discrepancy, the hydropic degeneration of the umbilical cord, and the placental hemorrhage, which in first trimester pregnancies has an increased risk of fetal death. [59]

Another phenomenon Ruysch, in his profession of chief obstetric instructor, was confronted with is what in the Latin text of the CW is named "*mola*," which literally means mass or lump. Confusingly, this did not refer to a hydatidiform mole ("*mola hydatidosa*") but to a post-partum intrauterine residue. In the Dutch text it was dubbed "*zuyger*," which would translate to "sucker," referring to its alleged parasitic lifestyle. Nevertheless, the English translation of this text mentioned the Latin "*mola*." [7] According to the midwives instructed by Ruysch, these "*molae*" literally had a life of their own in that they supposedly were able to travel through the room and crawl inside other women via the genitalia to find shelter. Although Ruysch considered these stories to be utter nonsense and exemplary for the general ignorance of the midwives he instructed, he was curious as to what process or phenomenon could underlie these myths. In the 28th and 29th of his 100 clinical descriptions Ruysch suggested that prolonged retained placentas and placental rudiments could be the source of these confabulations. [25] According to Ruysch retained placentas could stay in the uterus for a couple of months without being harmful. By naturally occurring uterine contractions these remnants would be reduced to the size of a chicken egg, reside in the womb during a successive pregnancy and subsequently appear at delivery. As a result of the prolonged compressed state these remnant could take any kind of shape, including that of a frog or another animal. What Ruysch failed to explain however, is the basis for the alleged physical activity of these creatures. Based on his descriptions, we propose that the mythical origin of crawling or flying frog-like "*molae*" lies in unexperienced observations of fetuses born with an anterior neural tube closure defect. It is conceivable that the dramatic presentation of an acraniate and neck-less fetus with bulging eyes gives the impression of a frog, especially to ignorant lay people. Anencephalic infants, most of which are born alive and die within a day, [60] have been reported to show bursts of forceful, jerky and sometimes excessive movements—especially those lacking a brain stem—that are quite unlike normal fetal motion patterns. [61] Again, in the

eyes of ignorants these movements may impress as attempts to crawl or fly.

During the process of rediagnosing the conditions that Ruysch described and depicted, we assumed that the pictures in the CW were accurate representations of the anatomized preparations and case reports, which he described in such great detail that we could make (differential) diagnoses on most teratological descriptions without any significant discrepancy between reality and the depicted figures. Corresponding with Bartholomaeus Keerwolf, Ruysch expressed his dismay for the lack of detail his peers showed for the characteristic morphology of the left cardiac auricle and admitted that a heart which he wanted to be depicted was drawn differently from what the anatomical specimen actually looked like. [62] According to Ruysch, this was mainly due to the multitude of steps which were initiated to create anatomical drawings. However, he did not mention any mistakes in the drawings of the teratological samples.

Several of Ruysch's specimens and case descriptions subsequently re-appeared in the medical literature, most of them referring to conditions not previously attested. Most contemporary authors consider Lebert to be the first one to have described omental teratoma in 1734. However, the German pathologist Hermann Lebert (1813–1878), who lived almost a century later, actually referred in his paper on dermoid cysts to the French translation of the case described by Ruysch (case 5). [63-65] James Ewing also referred to Ruysch's omental teratoma in his text book on tumors. [66] The earliest report of gastric teratoma is generally ascribed to Eustermann and Sentry (1922) [67] although already Lebert in 1852, Lannelonge and Achard in 1886 and Gould and Pyle in 1896 referred to Ruysch's description of case 3 [63,64, 57] and Geyl in 1897, with inclusion of the case report and Ruysch's considerations. [68, 69] Ruysch's description of enchondromatosis (case 45) was referred to by Johannes Müller in 1838, [70,71] whereas Ollier's description of the disease dates from 1890. [72] Ruysch's case descriptions of Meckel diverticulum (cases 16–18), which date from a century before that of Johann Friedrich Meckel the Younger, are also often referred to. Meckel himself, who was the first to realize the developmental nature of the condition, referred to many preceding case reports, including Ruysch's, to whom he ascribed the preponderant ileal location. [73] Interestingly, Ruysch's well documented and depicted case report of an intracranial teratoma (case 4), although often referred to, has never been diagnosed as such prior to our investigations but instead has always been considered a case of hydrocephalus. The first published description of an intracranial teratoma originates from one and a half century later. [74] Ruysch's brief and incomplete description of aganglionic megacolon (case 15) [25] let to the eponym "Ruysch disease" since it is generally accepted that this case report is the first attested case in pre-modern medicine, [75,76] almost two

centuries prior to that of Hirschsprung (1888). [77] Strangely enough, therefore, one of his most sparsely documented case report is the one he became famous for. Possibly, this resulted from the relative inaccessibility of Ruysch's works, which were almost exclusively published in Latin and Dutch. The only exceptions to that are the French and English translations of his 100 clinical observations, which include this very case. One of the other descriptions in the Ruysch legacy that has been frequently referred to concerns the specimen of a neonate with a polydactylous chondrodysplasia, described by both Ruysch and Kerckring (case 44). The earliest known reference is that of Jonathan Hutchinson, who reproduced the drawing of Kerckring in his Archives of Surgery. [78,57,79] He recalled Kerckring's description and showed himself particularly struck by the fact that the dwarfing condition in this case was associated with polydactyly. This convinced him that the nature of this condition was entirely different from that of rickets, which at the time was the default diagnosis for any acquired or congenital shortness or bowing of limbs. In 1940, Nicholls wrote a biography on Theodor Kerckring and referred to the case without mentioning Ruysch's involvement, even though he stated Kerckring to be well acquainted with Ruysch. [80] In the same year, Ellis and Van Creveld described three cases of a polydactylous chondrodysplasia that would subsequently bear their names. They stressed the resemblance between Kerckring's description (again without mentioning Ruysch) and the phenotype of their own cases and since one of these originated from Amsterdam, they even speculated "whether it was entirely coincidence that this child should have been described in the same city as that in which case 2 of the present series was observed many generations later." [81] Finally, in 1971, Majewski and co-workers recalled several historical descriptions, including the one by Kerckring, that resembled their own cases, [82] which was confirmed by Spranger and co-workers, in their paper on short-rib polydactyly syndromes. [83] Although Majewski syndrome still seems the best fitting diagnosis, our analysis of Ruysch's and Kerckring's descriptions illustrates the considerable phenotypic overlap that is contemporarily encountered among the various ciliary chondrodysplasias, that is, the short-rib polydactyly syndromes. [84-86]

Soon after Ruysch sold his first collection to Peter the Great, he started collecting anew, despite being already in his seventies. As stated previously, the auction catalogue that was published after his death in 1731 listed almost 1,300 indexed specimens. [8] In addition to the teratological specimens that were part of the first two cabinets of the second collection—described in the CW as the *Curae*—the subsequent cabinets added to this collection contain only five specimens that unequivocally represent a congenital anomaly and only two of those could be adequately diagnosed. In several other cases however, a congenital condition was suspected but could not be diagnosed

with sufficient certainty. For instance, Ruysch described three specimens of newborn children that were "bereaved from their brains" without clarifying if this was the reason for dissection or the result of it. The general assumption is that when this second collection was auctioned, it was acquired by the Polish king, Friederich August I, who bought it for 20,000 guilders. [87] His son, Friederich August II supposedly donated the collection to the University of Wittenberg, where it came in the custody of the anatomist Abraham Vater. [88,1] Although selling the whole collection, or most of it, to the Polish king may have been Ruysch's intention, it probably never happened. Ginzburg, head of the anthropology department of the Kunstkamera in the middle of the 20th century, casted doubt when he realized that only 5 years after Ruysch had died no more than 59 of the original 1,300 specimens turned up in the museum inventory of the University of Wittenberg [89] and he considered it unlikely that they were bought for 20,000 guilders. [10] Since Vater was a colleague and friend of Ruysch, with whom he corresponded on various anatomical issues, it seems reasonable to assume that he bought the specimens himself or on behalf of his university, without any royal intervention. What remained over time of this sub-collection must have been transported to the University of Halle upon its fusion with the University of Wittenberg in 1817 but a recent inventory did not surface any remaining Ruysch specimens. [88] Specimens of Ruysch's second collection also turned up in the auctioned legacies of other colleagues, including Seba, Gaubius, Deknatel and Houttuyn [90-93] but the present status and whereabouts of these specimens is unknown. Searching for the remains of these cabinets and their specimens could be the next step in this historical quest of unraveling and revealing the entire Ruysch legacy.

Acknowledgements

The authors gratefully acknowledge the help of Prof. Yuri Chistov, Dr. Natalia Kopaneva, and Mrs. Olga Panarina for their cooperation and providing all the possibilities to investigate the extant collection in the Peter the Great Museum of Anthropology and Ethnography (Kunstkamera) in Saint Petersburg, Russia. Furthermore, we thank the members of the Ruysch Research Group, Prof. Otto P. Bleker, Dr. Jozien J. Driessen-van het Reve, Dr. Francisca M.M. Griffioen, Dr. Frank IJpma, Dr. Laurens de Rooy, and Mr. Willem J. Mulder, for their valuable contributions and helpful discussions. We thank Prof. Dirk Ruiter and Dr. Annelieke Schepens-Franke for their critical review of this article, and Mr. Tim Rijnhout and Mrs. Ksenia M. Vozdigan, for reproduction of the images. We thank the Wilhelmina E. Jansen Fonds for their support of the International Ruysch Research Group.

References

1. Kooijmans L. 2004. De doodskunstenaar, de anatomische lessen van Frederik Ruysch. Amsterdam: Bert Bakker.
2. Luyendijk-Elshout AM. 1970. Death enlightened: A study of Frederik Ruysch. JAMA 212:121-126.
3. Radziun A, Chistov Y. 2012. The first scientific collections of Kunstkamera: Exhibition guide. St. Petersburg: MAE RAS.
4. Ruysch F. 1721. Opera Omnia Anatomico-Medico-Chirurgica. Huc usque edita. quorum elenchus sequenti exhibetur, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
5. Ruysch F. 1744. Alle de Ontleed-, Genees- en Heelkundige Werken. Amsterdam: Janssoons-van Waesberge.
6. Ruysch F. 1734. Observations Anatomiques et Chirurgicales, au nombre de Cent par Mr. Frédéric Ruysch, très-célèbre anatomiste. Avec des Figures en Taille-douce qui représentent les parties dans leur grandeur naturelle. Paris:Guillaume Cavelier.
7. Ruysch F. 1751. The Celebrated Dr. F. Ruysch's Practical Observations in Surgery and Midwifry. now first translated from the Latin Into English, by a Physician. Illustrated with Copper-plates. London: T. Osborne.
8. Anonymous. 1731. Catalogus Musaei Ruyschiani, sive permagnae, elegantissimae, nitidissimae, incomparabilis et vere regiae collectionis [...].Amsterdam: Janssoons-van Waesberge.
9. Ternowsky WN. 1927. "Fredericus Ruysch" aus dem Anatomischen Institut der Universität in Kasan. Bulletin du VI. Congres de la Société Internationale d'Histoire de la Médicine: 334-338.
10. Ginzburg VV. 1953. Anatomicheskaia kollektsia F. Riuischa v sobraniiakh Petrovskoi Kunstkameri. Sbornik Museia Antropologii I Etnografii 14:263-305.
11. Ginzburg VV. 1959. Muzej kafedry normal'noi anatomii voenno-meditsinskoi ordena Lenina Akademii im. S.M. Kirova. Archiv Anatomii, Gistologii I Embryologii Leningrad 11:114-124.
12. Ruysch F. 1714. Thesaurus anatomicus nonus, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
13. Ruysch F. 1720. Adversariorum anatomico-medico-chirurgicorum decas secunda, in qua varia notatu digna recensentur, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
14. Fox H, Butler-Manuel R. 1964. A teratoma of the placenta. J Pathol Bacteriol 88: 137–140.
15. Stephens TD, Spall R, Urfer AG, Martin R. 1989. Fetus amorphus or placental teratoma? Teratology 40:1-10.
16. Ruysch F. 1705. Thesaurus anatomicus sextus, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
17. Ruysch F. 1717. Adversariorum anatomico-medico-chirurgicorum decas prima, in qua varia notatu digna recensentur, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
18. Ruysch F. 1723a. Adversariorum anatomico-medico-chirurgicorum decas tertia, in qua varia notatu digna recensentur, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
19. Ruysch F. 1724. Curae posteriores seu thesaurus anatomicus omnium praecedentium maximus, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
20. Dutz W, Sadeghee S. 1968. A teratoid finger in the sigmoid colon. J Pathol Bacteriol. 95:289-291.

21. Damjanov I, Knowles BB, Solter D. 1983. The human teratomas. Experimental and clinical biology. Clifton NJ, Humana Press.
22. Ruysch F. 1702. Thesaurus anatomicus secundus, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
23. Washburne JF, Magann EF, Clauhan SP, Fratkin JD, Morrison JC. 1995. Massive congenital intracranial teratoma with skull rupture at delivery. Am J Obstet Gynecol. 173:126-128.
24. Bolat F, Kayaselcuk F, Tarim E, Kilicdag E, Bal N. 2008. Congenital intracranial teratoma with massive macrocephaly and skull rupture. Fetal Diagn Ther. 23:1-4.
25. Ruysch F. 1691a. Observationum anatomico-chirurgicarum centuria, accredit catalogus rariorum quae in museo Ruyschiano asservantur. Amsterdam: Hendrik & Theodoor Boom.
26. Ruysch F. 1691b. Musaeum anatomicum Ruyschianum, sive Catalogus rariorum, quae in authoris aedibus asservantur. Amsterdam: Hendrik & Theodoor Boom.
27. Ruysch F. 1703. Thesaurus anatomicus tertius, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
28. Kubosawa H, Iwasaki H, Kuzuta N, Suzuki H, Iura H. 2006. Adenocarcinoma with peritoneal dissemination secondary to multiple mature teratomas of the omentum. Gynecol Oncol. 101:534–536.
29. Ruysch F. 1701b. Thesaurus anatomicus primus, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
30. Iyoob SD, Tsai A, Ruchelli ED, Coleman BG, Wilson RD. 2006. Large umbilical cord hemangioma: sonographic features with surgical pathologic correlation. J Ultrasound Med. 25:1495-1498.
31. Ruysch F. 1707. Thesaurus anatomicus septimus, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
32. Ruysch F. 1728. Curae renovatae seu thesaurus anatomicus, post curas posteriores, novus, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
33. Ruysch F. 1716. Thesaurus magnus et regius qui est decimus thesaurum anatomicorum in quo praecipuaecorporis humani partes, ceu in statu vivo, nitidissime praeparatae, reservatur, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
34. Ruysch F. 1723b. Tractatio anatomica de musculo in fundo uteri observato, ante hac a nemine detecto cui, accedit depulsionis secundinarum, parturientium foeminarum, instruction. Amsterdam: Janssoons-van Waesberge.
35. Ruysch F. 1709. Thesaurus anatomicus octavus, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
36. Kerckring TT. 1670. Spicilegium Anatomicum, Continens Observationum Anatomicarum Rariorum Centuriam Unam, Nec Non Osteogeniam Foetuum. Amsterdam: Andreas Frisii.
37. Tulp N. 1641. Observationes medicae. Amsterdam:Lodewijk Elsevier.
38. Koppang HS, Boman H, Hoel PS. 1983. Oral abnormalities in the Saldino-Noonan syndrome. Virchows Arch. 398:247–262.
39. Ruysch F. 1701a. Epistola anatomica quarta & decima. Auctore Mauritio á Reverhorst, med. doct et anat. professore. Ad virum clarissimum Fredericum Ruyschium de nova artuum decurtandorum method. Amsterdam: Janssoons-van Waesberge.
40. Ruysch F. 1704. Thesaurus anatomicus quartus, cum figuris aeneis. Amsterdam: Janssoons-van Waesberge.
41. Goto K, Yoshinari H, Tajima K, Kotsuji F. 2006. Microperforate hymen in a primigravida in active labor: a case report. J Reprod Med. 51:584-586.

42. Ruysch F. 1665. Dilucidatio valvularum in vasis lymphaticis et lacteis cum figuris aeneis, accesserunt quaedam observations anatomicae rariores. The Hague:Herman Gael.
43. Schumacher R, Mai A, Gutjahr P. 1992. Association of rib anomalies and malignancy in childhood. Eur J Pediatr. 151:432-4.
44. Merks JH, Smets AM, Van Rijn RR, Kobes J, Caron HN, Maas M, Hennekam RC. 2005. Prevalence of rib anomalies in normal Caucasian children and childhood cancer patients. Eur J Med Genet. 48:113-129.
45. Loder RT, Huffman G, Toney E, Wurtz LD, Fallon R. 2007. Abnormal rib number in childhood malignancy: implications for the scoliosis surgeon. Spine (Phila Pa 1976). 32:904-910.
46. Anonymous. 1742. Musei Imperialis Petropolitani. Vol.I. Pars prima qua continentur res naturales ex regno animali. St. Petersburg: Academiae scientiarum.
47. Hyrtl J. 1860. Handbuch der praktischen Zergliederungskunst: als Anleitung zu den Sectionsübungen und zur Ausarbeitung anatomischer Präparate. Wien: Wilhelm Braumüller.
48. Hyrtl J. 1869. Vergangenheit und Gegenwart des Museums für menschliche Anatomie an der Wiener Universität. Wien: Wilhelm Braumüller.
49. Kimmel DL, Moyer EK, Peale AR, Winborne LW, Gotwals JE. 1950. A cerebral tumor containing five human fetuses: A case of fetus in fetu. Anat Rec. 106:141-65.
50. Naudin ten Cate L, Vermeij-Keers C, Smit DA, Cohen-Overbeek TE, Gerssen-Schoorl KB, Dijkhuizen T. 1995. Intracranial teratoma with multiple fetuses: pre- and post-natal appearance. Hum Pathol. 26:804-807.
51. Cairo MS, Grosfeld JL, Weetman RM. 1981. Gastric teratoma: Unusual cause for bleeding of the upper gastrointestinal tract in the newborn. Pediatrics. 67:721–724.
52. Ukiyama E, Endo M, Yoshida F, Tezuka T, Kudo K, Sato S, Akatsuka S, Hata J. 2005. Recurrent yolk sac tumor following resection of a neonatal immature gastric teratoma. Pediatr Surg Int. 21:585-588.
53. Gray SW, Johnson HC Jr, Skandalakis JE. 1964. Gastric teratoma in an adult: with a review of the literature. South Med J. 57:1346-1351.
54. Liu L, Zhuang W, Chen Z, Zhou Y, Huang X-R. 2009. Primary gastric teratoma on the cardiac orifice in an adult. World J Gastroenterol. 15:1782-1785.
55. Chen SS, Chen CP, Chien SC, Hsu CY.2008. Spontaneous rupture of omental teratoma mimicking a ruptured ovarian teratoma. Taiwan J Obstet Gynecol. 47:229-232.
56. Roellig K, Menzies BR, Hildebrandt TB, Goeritz F. 2011. The concept of superfetation: a critical review on a 'myth' in mammalian reproduction. Biol Rev. 86:77-95.
57. Gould GM, Pyle WL. 1896. Anomalies and Curiosities of Medicine. Philadelphia: W.B. Saunders.
58. Baijal N, Sahni M, Neeraj V, Kumar A, Parkhe N, Puliyel M. 2007. Discordant twins with the smaller baby appropriate for gestational age- unusual manifestation of superfoetation: A case report. BMC Pediatrics. 7:1-5.
59. Maso G, D'Ottavio G, De Seta F, Sartore A, Piccoli M, Mandruzzato G. 2005. First-trimester intrauterine hematoma and outcome of pregnancy. Obstet Gynecol. 105:339-344.
60. Pelizzari E, Valdez CM, Picetti Jdos S, Cunha AC, Dietrich C, Fell PR, Targa LV, Zen PR, Rosa RF. 2015. Characteristics of fetuses evaluated due to suspected anencephaly: a population-based cohort study in southern Brazil. Sao Paulo Med J. 133:101-108.
61. Visser GH, Laurini RN, de Vries JI, Bekedam DJ, Prechtl HF. 1985. Abnormal motor behaviour in anencephalic fetuses. Early Hum Dev. 12:173-182.

62. Ruysch F. 1697. Epistola anatomica, problematica decima. Auctore Bartholomaeo Keerwolff, med. doct. Ad virum clarissimum Fredericum Ruyschium de auricularum cordis, earumque fibrarum motricum structura. Amsterdam: Janssoons-van Waesberge.
63. Lebert H. 1852. Des kystes dermoides et de l'héterotopie plastique en général, communiqué de la société de biologie en août I852. Gazette médicale de Paris, 3me série 7:720-722, 793-796, 808-811.
64. Lannelongue OM, Achard C. 1886.Traité des kystes congénitaux. Paris: Asselin & Houzeau.
65. Mumey N. 1928. Dermoid cysts of the greater omentum. Am J Surg. 5:56-60.
66. Ewing J. 1919. Neoplastic diseases. A text book on tumors. London & Philadelphia: W.B. Saunders Company. p.953.
67. Eustermann GB, Sentry EG. 1922. Benign tumours of the stomach: report of 27 cases. Surg Gynecol Obstet. 34:372–378.
68. Geyl 1897. Betrachtungen über die Genese der sogenannten Ovarialdermoide mit Bezug auf ein Vaginalteratom. Sammlung klinischer Vorträge von Volkmann. Neue Folge No. 190.
69. Bandler SW. 1901. Dermoid and other cysts of the ovaries. Their origin from the Wolffian body. New York:William Wood & Company. p.42.
70. Müller J. 1838. Über die feineren Bau und die Formen der Krankhaften Geschwülste. Berlin: G. Reimer.
71. Johnson J, Johnsen HJ. 1840. The medico-surgical review and the Journal of physical medicine. London: S. Highley p.370.
72. Ollier LXEL. 1890. Exostoses multiples. Mémoires et comptes rendus de la Société des sciences médicales de Lyon 29:12.
73. Meckel JF. 1809. Über die Divertikel am Darmkanal. Archiv für die Physiologie Halle. 9:421-453.
74. Breslau RE. 1864. Geburtsgeschichte und Untersuchung eines Falles von foetus in foeto. Zurich. 30:406-17.
75. Jayle F. 1909. La dilatation congenitale idiopatique du colon observée au XVIIe siècle. Presse Med. 17:803-804.
76. Leenders E, Sieber WK. 1970. Congenital megacolon, observation by Frederici Ruysch 1691. J Pediatr Surg. 5:1-3.
77. Hirschsprung H. 1888. Stuhlträgheit Neugeborener in Folge von Dilatation und Hypertrophie des Colons. Jahrb Kinderheilk Phys Erzieh. 27:1–7.
78. Hutchinson J. 1893. A short-limbed polydactylous dwarf. In: Huchinson J. Archives of Surgery. Vol. IV. London:J. & E. Churchill.
79. McKusick VA, Egeland JA, Eldridge R, Krusen DE. 1964. Dwarfism in the Amish. I. The Ellis-Van Creveld syndrome. Bull Johns Hopkins Hosp. 115:306-336.
80. Nicholls AG. 1940. Theodore Kerckring and his "Spicilegium Anatomicum" Can Med Assoc J. 42:480-483.
81. Ellis RWB, Van Creveld S. 1940. A syndrome characterized by ectodermal dysplasia, polydactyly, chondro-dysplasia and congenital morbus cordis: report of three cases. Arch Dis Child. 15:65–84.
82. Majewski F, Pfeiffer RA, Lenz W, Müller R, Feil G, Seiler R. 1971. Polydaktylie, verkürzte Gliedmassen und Genitalfehlbildungen: Kennzeichen eines selbständigen Syndroms? Z Kinderheilkd. 111:118-138.
83. Spranger J, Grimm B, Weller M, Weissenbacher G, Herrmann J, Gilbert E, Krepler R. 1974. Short rib-polydactyly (SRP) syndromes, types Majewski and Saldino-Noonan. Z Kinderheilkd. 116:73–94.

84. Elçioglu NH, Hall CM. 2002. Diagnostic dilemmas in the short rib-polydactyly syndrome group. Am J Med Genet. 111:392-400.
85. Huber C, Cormier-Daire V. 2012. Ciliary disorder of the skeleton. Am J Med Genet C Semin Med Genet. 160C:165-74.
86. Schmidts M. 2014. Clinical genetics and pathobiology of ciliary chondrodysplasias. J Pediatr Genet. 3:46-94.
87. Haller A von. 1774. Bibliotheca Anatomica qua scripta ad anatomen et physiologiam facienta a rerum initiis recensentur. Zurich: Orell, Gessner & Fuessli. p.536.
88. Schwarz S. 1999. Die anatomische Privatsammlung der Anatomenfamilie Meckel unter besonderer Berücksichtigung ihres präparationstechnischen Profils. Thesis. Halle, Martin-Lüther-Universität Halle-Wittenberg.
89. Vater A. 1736. Regii in Academia ad Albim musei anatomici Augustei Catalogus universalis. Wittenberg: Henning.
90. Anonymous. 1751. Catalogus van de uitmuntende cabinetten […] van den professor Ruysch […] met veel moeite en kosten in een reeks van jaaren vergadert en nagelaten door den heere Albertus Seba. Amsterdam: Sluyter, Schut & Blinkvliet.
91. Anonymous. 1765. Catalogus van de alöm beroemde kabinetten, bestaande in sierlyke anatomische praeparata, onder welke van den prof. F. Ruysch […] Alles by één vergadert en aldus nagelaten door den heer Adrianus Deknatel. Amsterdam: Blinkvliet & Posthumus.
92. Anonymous. 1783. Musei Gaubiani pars sive catalogus partis supellectis qua usus est vir celeberrimus H.D. Gaubius […]. Amsterdam: Luchtmans.
93. Anonymous. 1787. Animalium musaei Houttuyniani index. Amsterdam: Posthumus & Blinkvliet.

.

3 History and Highlights of the Teratological Specimens in the *Museum Anatomicum* of the Leiden University, The Netherlands

This chapter has been published as:

Lucas L. Boer, Peter L. J. Boek, Andries J. van Dam, and Roelof-Jan Oostra. History and Highlights of the Teratological Specimens in the Museum Anatomicum of the Leiden University, The Netherlands. 2018. Am J Med Genet A. 176:618-37. doi: 10.1002/ajmg.a.38617.

Abstract

The anatomical collection of the Anatomical Museum of Leiden University Medical Center (historically referred to as *Museum Anatomicum Academiae Lugduno-Batavae*) houses and maintains more than 13,000 unique anatomical, pathological and zoological specimens, and include the oldest teratological specimens in The Netherlands. Throughout four centuries hundreds of teratological specimens were acquired by more than a dozen collectors. Due to the rich history of this vast collection, teratological specimens can be investigated in a unique retrospective sight going back almost four centuries. The entire 19th century collection was described in full detail by Eduard Sandifort (1742–1814) and his son Gerard Sandifort (1779–1848). Efforts were made to redescribe, rediagnose and recategorize all present human teratological specimens, and to match them with historical descriptions. In the extant collection a total of 642 human teratological specimens were identified, including exceptional conditions such as faciocranioschisis and conjoined twins discordant for cyclopia, and sirenomelia. Both father and son Sandifort differed in their opinion regarding the causative explanation of congenital anomalies. Whereas, their contemporaries Wouter Van Doeveren (1730–1783) and Andreas Bonn (1738–1817) both presented an interesting view on how congenital anomalies were perceived and explained during the 18th and 19th centuries; the golden age of descriptive teratology. Although this enormous collection is almost 400 years old, it still impresses scientists, (bio)medical students, and laymen visiting and exploring the collections of the *Museum Anatomicum* in Leiden, The Netherlands.

Introduction

The anatomical collection of the Anatomical Museum of the Leiden University Medical Center (historically referred to as *Museum Anatomicum Academiae Lugduno-Batavae*) currently maintains and houses the oldest Dutch collection of both dried and embalmed, anatomical, pathological, embryological, and teratological human specimens. Founded in 1575, the University of Leiden is the oldest university in The Netherlands. [1] Over 350 years, many thousands of specimens were brought together and were either purchased from or donated by multiple private and several institutionalized collections. [2] Due to several obtained private collections of different scientists, all with their own specific interests, the anatomical museum of Leiden can be seen as a treasure-trove for both historical and contemporary (dys)morphological research. [3]

The first publicly performed dissections at the University of Leiden are assigned to Geraert de Bondt (Gerardius Bontius, 1538–1599) who was professor of medicine, mathematics and astronomy. However, it was anatomy professor Pieter Pauw (Petrus Pavius, 1564–1617), an apprentice of Andries van Wesel (Andreas Vesalius, 1514–1564), who initiated anatomical education in Leiden between 1589 and 1617. [4] Pauw was one of the first Leiden professors who dissected human cadavers in order to use the thereby obtained anatomical specimens to educate anatomy on a grand scale; he publicly dissected more than 60 cadavers. [5] Some of these dissections were described in his posthumously published *Anatomiae Observationes Selectiores*, including that of a newborn child with an intracranial tumor. [6] Before the practical approach of using a *subjectum anatomicum*, anatomical education was mainly a theoretical exercise. [7] After several dissections, Pauw commanded the build of a *Theatrum Anatomicum* which subsequently opened its doors in 1594 as the first anatomical theater in The Netherlands. Allegorically arranged skeletons of both humans and animals were displayed on the balustrades and surrounded the anatomical theater in a circular manner. They were used for Pauw's lectures on osteology, although they probably also resembled emblems of *vanitas;* the skeletons were holding banners with Latin phrases about life, being indicative for mortality and fragility of (human) existence. The centerpiece consisted of the symbolized skeletons of Adam and Eve with the tree of knowledge in the foreground (Fig. 1).

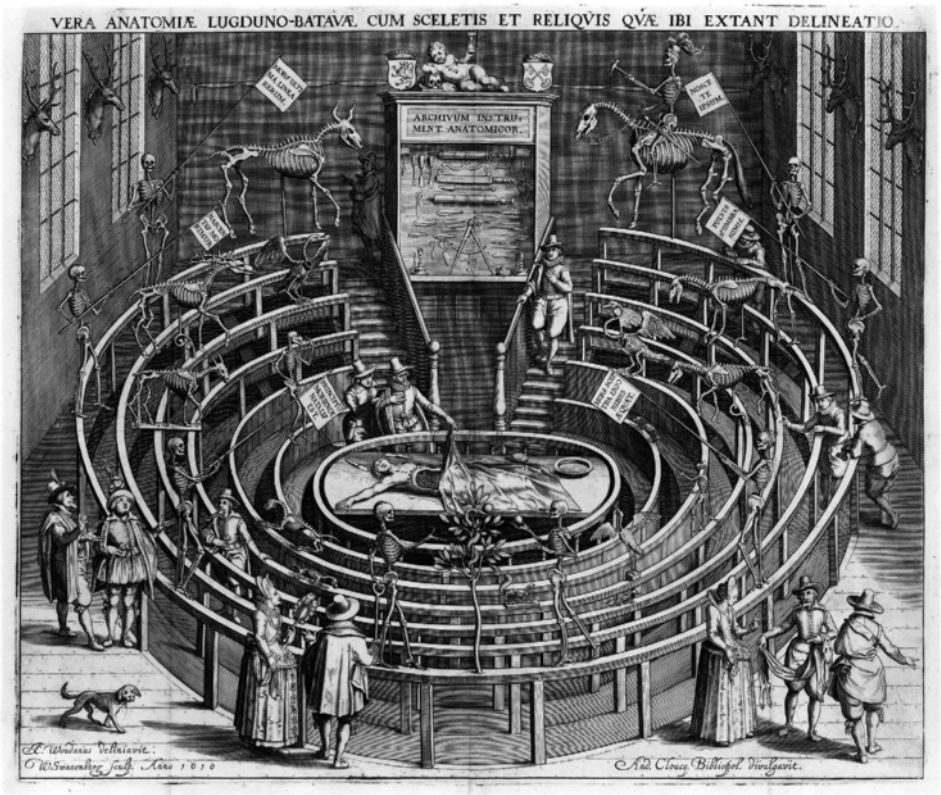


Fig. 1. The *Theatrum Anatomicum* of Leiden University in the early 17th century, copperplate. Willem van Swanenburg, 1610

The first anatomical specimens were collected shortly after the establishment of this theater. The newly built theater, located in the Faliede Bagijnenchurch (Church of the Faille-Mantled Beguines) on the banks of the main canal (the *Rapenburg*) in downtown Leiden (The Netherlands), additionally housed the anatomical cabinet, the university library, the cabinet of natural curiosities and the botanical gardens—serving as an inspiring intertwined working place for both students and scientists around the turn of the 16th century. [7] Additionally, the church served as a tourist destination for it was publicly accessible and open seven days a week. Public dissections attracted people with diverse backgrounds and became frequent and well-visited events. [8] After Pauw's demise in 1617, professor in medicine Otto van Heurn (Otto Heurnius, 1577–1652) was the patriarch in the establishment of the anatomical collection which would subsequently become the *Museum Anatomicum*. [4] In 1721 Bernard Siegfried Albinus (1697–1770) was appointed as professor of anatomy. Due to illness of his predecessor, Johannes Jacobus

Rau (1668–1719), the collection had deteriorated. Albinus was instructed to restore and make an inventory of Rau's collection. In 1725, Albinus published a catalogue entitled "*Index supellectilis anatomicae: quam academiae Batavae quae Leidae est legavit*" in which the collections of Rau are described. [9] Bernard Siegfried Albinus enriched the collection with a great number of red injected alcohol based specimens; some of these magnificent specimens are still present in the extant collection and show the refined technique of vascular injections from the 18th century. After Albinus passed away, his specimens were purchased by the Leiden University for 6,000 guilders in 1776. This collection, which consisted of 334 alcohol-based specimens and 418 dried specimens, was catalogued by his brother Frederik Bernard Albinus (1715–1778) and published as "*Supellex Anatomica Bernardi Siegfriedi Albini.*" [10] Around 1772, the church of the Faille-Mantled Beguines was further extended to house its growing anatomical collections. In 1784, the anatomical collection expanded with the collection of Wouter van Doeveren (1730–1783) which contained a large number of teratological specimens. [2]

On the 12th of January 1807 a disastrous event in the history of the collection's integrity occurred when a gunpowder ship, carrying 18.5 tons of gun powder, exploded when floating on the main canal of Leiden. When this enormous explosion occurred, over 200 buildings were swept and blown away, including the church which housed the anatomical collections. This dramatic scene was the inspiration for a painting by Johannes Jelgerhuis (1770–1836) which shows the enormous ravage the explosion caused (Fig. 2).

In subsequent years the damaged and decimated anatomical collection was re-extended by the purchase of the collection of Sebald Justinius Brugmans (1763–1819) in 1819, which consisted of 4,081 specimens, and parts of the collection of Andreas Bonn (1738–1817) in 1822. The now voluminous collection was described by Eduard Sandifort (1742–1814) and his son Gerard Sandifort (1779–1848) and resulted in an illustrated fourfold masterpiece entitled: *Museum Anatomicum Lugduno-Batavae Descriptum*. [5,11,12] With this work both father and son became internationally renowned. In these catalogues the Leiden collection of the 19th century is described in full detail including the collections of contemporaries Johan Jacobus Rau, Bernard Siegfried Albinus, Wouter van Doeveren, Andreas Bonn, and Sebald Justinus Brugmans and indexes almost 7,500 specimens. Strangely, both Eduard's and Gerard's own collections were not indexed in these catalogues. During the 19th century the collection was further expanded by subsequently acquired specimen collections of several medical professors at the Leiden University including Jacobus Rocquette, Adrianus Marinus Ledeboer, Meinardus Simon du Pui, Jacobus Cornelis Broers, Gerardus Suringar, and Cornelis Swaving. [2,3,8,13]

Fig. 2. The Rapenburg, Leiden, three days after the explosion of the gunpowder ship on 12 January 1807. Johannes Jelgerhuis, 1807. Rijksmuseum, Amsterdam

After consecutive movements and threats throughout more than four decades, the anatomical museum is now situated inside the medical educational building of the University of Leiden and comprises more than 13,000 unique items. Predominantly, the collection functions as an inspiring place to educate medical students and is publicly accessible a few days throughout the year. The Anatomical Museum presently comprises specimens of the old anatomical collections, the collections of the Department of Pathology (PA) and collections of the Department of Obstetrics & Gynecology (OG). We here report on the contemporary legacy of teratological specimens and descriptions of the *Museum Anatomicum* in Leiden and on the diagnoses that we made. Additionally, we discuss the scientific opinions of father and son Sandifort together with those of their contemporaries towards congenital malformations and we reflect on the present day value of this legacy.

Materials and methods

Our primary aim was to draw an inventory of the extant collection of human teratological specimens, to match them with (historical) descriptions from the *Museum Anatomicum* in Leiden, and to rediagnose the conditions they presented with.

We first investigated all, currently present, teratological specimens by means of external inspection. Second, based on the available information and using contemporary pathognomonic insights and dysmorphological terminology, we tried for each specimen to determine whether the presented anomalies met the criteria for diagnosing monogenic and chromosomal syndromes, complex non-syndromic conditions, neural tube defects, conjoined, parasitic and acardiac twinning, primary and isolated congenital organ, and skeletal anomalies. If no diagnostic classification was applicable, we diagnosed the presenting sequence of anomalies as "isolated," bearing in mind the restrictions of the investigation technique, that is, external inspection only. This restriction was due to the historic value of the specimens and therefore the impossibility for additional diagnostics.

Third, we explored the four Latin published *Museum Anatomicum Lugduno-Batavae* catalogues to find matching descriptions, pictures and annotations of the specimens. [5, 11,12] In these four catalogues the 19th century Leiden anatomical cabinets are described in full detail. The catalogues have been published as e-books on Google Books and can be inspected and downloaded for free. All inventoried existing teratological specimens were, when possible, assigned to a specific collector and were matched, where possible, to (historical) literate. As previously described all specimens and descriptions were reduced to "unique cases." [14] Finally, the most remarkable specimens were selected for a detailed description (See case 1–11) and the most influential collectors were described in detail (See Biographies). Due to the magnitude of the found specimens and matching with historical descriptions, it is beyond the borders and scope of this paper to include all matching results. Details regarding the matched specimens can be obtained from the corresponding author.

Results

In the existing collection present at Leiden University, we identified 642 specimens that showed isolated or combined congenital anomalies, 556 cases comprised singleton related anomalies and 86 cases concerned conjoined twins, or twin-related anomalies. In 59 cases a monogenic or chromosomal

syndrome was diagnosed or reasonably suspected. Isolated and complex non-syndromic conditions were diagnosed in 276 cases. 161 cases concerned neural tube malformations (See Tables I–IV). Additionally, we found 60 specimens that were out of anatomical context or insufficiently accessible which we therefore excluded from the four defined tables. Moreover, 116 specimens could be assigned to a certaincollector and 87 specimens to a certain institution, or department, whereas, 439 specimens could not be assigned (See Table V). Due to the magnitude of found specimens we decided to describe only 11 interesting cases in more detail (See cases 1–11).

Table I. Monogenic and chromosomal syndromes*

Achondroplasia	7
Acrofacial dysostosis	1
Apert syndrome	3
Craniodiaphyseal dysplasia	1 (plaster cast)
Craniosynostoses	12
Enchondromatosis	**1 (Case 1)**
Hypophosphatasia	**1 (Case 2)**
Ichthyosis congenita gravis	2
Incontinentia pigmenti	1
Lysosomal storage disorder (Hurler)	1
Majewski syndrome	1
Meckel syndrome	**3 (Case 3)**
Osteogenesis imperfecta	6
Oral-facial-digital syndrome NOS	**1 (Case 4)**
Skeletal dysplasia NOS	2
Thanatophoric dysplasia	2
Tetra-amelia syndrome	1
Treacher Collins	1
Trisomy 13	2
Trisomy 18	10
Total	59

*Monogenic and chromosomal syndromes are based on their external dysmorphological appearance. Unfortunately, the DNA quality of formalin- or alcohol-fixated specimens is insufficient for additional genetic testing. Most diagnoses are therefore tentative. **Bold** diagnoses are specimens which are described in more detail.

Table II. Isolated and complex non-syndromic conditions

craniocervical	
Arnold-Chiari malformation	1
Congenital struma	1
Hemifacial microsomia	1
Isolated cleft lip with or without cleft palate	19
Orbital tumor	**1 (Case 5)**
Pierre Robin sequence	1
cardiovascular	
Coarctatio aortae	4
Congenital lymphedema	1
Cor uniloculare biatriatum	1
Cor triloculare biatriatum	3
Ectopia cordis	1
Marfan syndrome (heart)	1
Persisting ductus Botalli	5
Septal defects heart	17
Tetralogy of Fallot	**4 (Case 6)**
Transposition of the great arteries	8
gastrointestinal	
Appendix aplasia	1
Diaphragmatic hernia	7
Duodenal atresia / stenosis	3
Esophageal atresia / tracheal fistula	4
Gallbladder agenesis	1
Hirschsprung disease	4
Imperforated anus	7
Intestinal stenosis NOS	3
Meckel diverticulum	3
Umbilical hernia and omphalocele	14
urogenital	
Ambiguous genitalia	2 (incl. 1 wax model)
Bladder – and cloacal exstrophy	14 (incl. 3 plaster casts)
Bladder diverticulum	1
Congenital hydronephrosis	4
Congenital megalourethra	1
Horseshoe kidney	5
Hypospadias	5
Persistent cloaca	1
Prune belly sequence	2
Sirenomelia sequence	8
(Unilateral) kidney agenesis	3

Ureteral duplication	8
Uterus bicornis	6
Uterus didelphys	3
Uterus unicornis	2
musculoskeletal	
Congenital dislocation of the hip	24
Intercalary limb deficiencies NOS	2
Longitundinal limb deficiencies NOS	8
Pre- and/or postaxial polydactyly/syndactyly	10
cystic organ conditions	
Congenital lung cysts	1
Congenital liver cysts	1
Congenital ovarian cysts	1
Congenital renal cysts	4
disruptions	
Amniotic band sequence/vascular disruption	13
Isolated gastroschisis	2
generalized conditions (incl. infections)	
Cowpox embropathy	1
Congenital syphilis	6
Hydrops fetalis / Cystic hygroma	11
Lithopaedion	2
Oligohydramnios sequence	2
schisis association	
Encephalocele and omphalocele	1
Encephalocele, rachischis and omphalocele	1
Holoacrania, rachischisis and omphalocele	3
Holoacrania, hypospadias and micropenis	1
Holoacrania and urinary tract malformations	1
Total	276

Bold diagnoses are specimens which are described in more detail.

Table III. Neural tube malformations

closure defects	
Craniorachischisis	15
Craniorachischisis totalis	8
Craniorachischisis with iniencephaly	11
Craniorachischisis posterior	1
Faciocranioschisis	**1 (Case 7)**
Holoacrania	20
Holoacrania with partial rachischisis	16
Iniencephaly	2
Iniencephaly with encephalocele	3
Lumbosacral spina bifida	23
Meroacrania	10
Occipital encephalocele	7
Occipito-cervical encephalocele	3
Occipital encephalocele with rachischsis	5
Parietal encephalocele	3
Spinal dysraphism	3
Thoracolumbar spina bifida	5
holoprosencephaly	
Cyclopia	11
Ethmocebocephaly	3
Otocephaly	3
combinations	
Closure defect + holoprosencephaly	8
Total	161

Bold diagnoses are specimens which are described in more detail.

Table IV. Pathological twins

*Symmetrical conjoined twins**	
Cephalothoracoileopagus	5
Dicephalus	5
Dicephalus discordant for cyclopia	**1** (Case 8)
Diprosopus	7
Ileoischiopagus	1
Ischiopagus	1
Pygopagus	1
Thoracoileopagus	19
Thoracoileoischiopagus	1
Thoracoilieopagus discordant for sirenomelia	**1** (Case 9)
Parasitic conjoined twins and teratomas	
Epignathus / perioral- nasopharyngeal teratoma	**6** (case 10)
Epigastrius / ventral teratoma	**1** (case 11)
Pygopagus parasiticus / sacral teratoma	10
Acardiac twins	
Acardius anceps	4
Acardius acephalus	13
Acardius NOS **	2
Other twin related conditions	
Foetus papyracaeus	8
Total	86

* The terminology we used to describe the various types of conjoined twinning is based on what is generally accepted. The *thoracoileopagus* category also comprised cases of *ileopagus* and *xiphopagus*, since we diagnosed the conditions on external criteria only. The infix -*ileo*- was used whenever the site of conjunction was continuous with a single umbilical insertion. Structures involved in the site of conjuction were considered as duplicated if they showed clear signs of complete or partial duplication. For instance, dicephalus conjoined twins, having a third median upper extremity with more than five digits, were specified with the suffix *tetrabrachius*. Discordant anomalies, when present, were mentioned separately.

**Acardius NOS = Not otherwise specified, consisted of intestinal specimens originating form an acardiac twin.

Bold diagnoses are specimens which are described in more deta

Table V. Identified teratological specimens from different collectors and institutions in the extant collection

*Collector**	*Found teratological specimens*
Johannes Jacobus Rau (1668-1719)	2
Bernhardus Siegfried Albinus (1697-1770)	1
Wouter van Doeveren (1730-1783)	**2**
Andreas Bonn (1738-1817)	**39**
Eduard Sandifort (1742-1814)	**8**
Jacobus Rocquette (1744-1809)	4
Sebald Justinus Brugmans (1763-1819)	27
Gerard Sandifort (1779-1848)	**2**
Jacobus Cornelis Broers (1795-1847)	2
Adrianus Marinus Ledeboer (1797-1887)	3
Willem Vrolik (1801-1863)	1
Gerardus Conradus Bernardus Suringar (1802-1874)	19
Hidde Halbertsma (1820-1865)	1
Teunis Zaaijer (1837-1902)	1
Johannes Antonius James Barge (1884-1952)	4
Institutions	
Department of Obstetrics & Gynecology university of Leiden	54
Westeinde hospital, The Hague	33
Unknown	439
Total	642

*Collectors are categorized by their date of birth followed by several institutions and the specimens of unknown collector. Names in **bold** refer to collectors that are described in more detail (see Discussion / Biographies).

Enchondromatosis

Case 1. Specimen Pa0260 concerns a left skeletonized hand with multiple tumors arising from the metacarpals and both proximal and distal phalanges of the first, third, and fourth finger (Fig. 3A) kept in fluid. Originally, this case was described by Andreas Bonn, as part of the *Hovius collection* most of which is presently situated in Museum *Vrolik* in Amsterdam (*Descriptio thesauri ossium morbosorum Hoviani.* p. 96, nr. CCCXXXV). [15] Subsequently, the same case was (re)described and depicted (Fig. 3B and C) by Gerard Sandifort (Mus. Anat. Vol. III. p. 349, nr. CCCI-CCCIX and p. 391, nr. DCXXXIX-DCXLV and Mus. Anat. Vol. IV. p. 81 and tabula CLXXXV and CLXXXVI). [11,12] This case originally concerned 15 skeletal elements from a 27-year-old man who suffered, according to Bonn, from congenital rickets, leading to bony deposits and aberrant growth of the long bones. The men's posture, hands and legs were deformed, severely bent and swollen. According to the original description the malformed man fell from a great height which led to excessive bruises, edema, fever, and limping which eventually led to death. Autopsy revealed a retroperitoneal tumor which was attached to the lower lumbar vertebrae, sacrum and pelvic bones. At the site where the tumor was attached, the normal bone was destroyed. Together with the overall clinical report, the engravings and the extant specimen this case can be diagnosed as multiple enchondromatosis (MIM:166000).

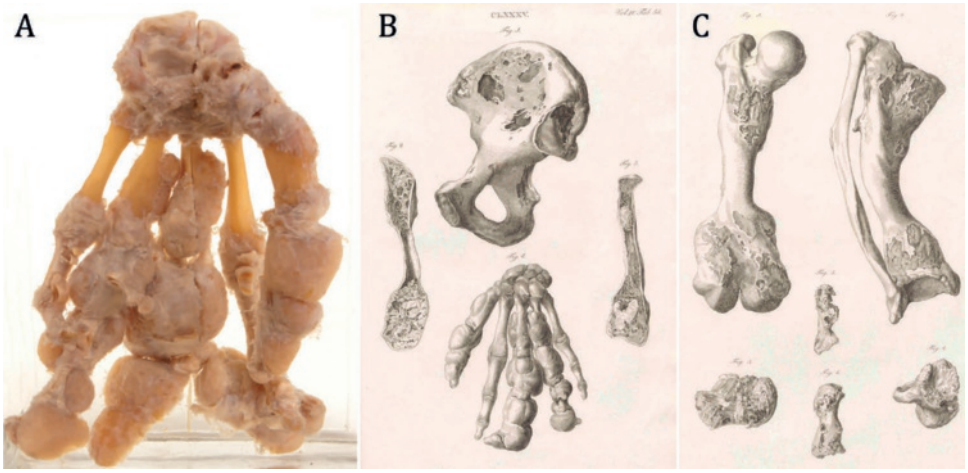


Fig. 3. Case 1. Enchondromatosis. [A] Specimen Pa0260, hand affected by multiple enchondromata. From the collection of the *Museum Anatomicum* Leiden; The Netherlands. [B/C] Copperplate Sandifort (1835). [12]

Hypophosphatasia

Case 2. Specimens Pa0268 (Fig. 4A) and Pb0260 (Fig. 4B) concern a neonatal skeleton and skull originally collected by Andreas Bonn and described by Gerard Sandifort (Mus. Anat. III. p. 353. nr. CCCXV (skeleton), p. 389. nr. DCXXVIII, p. 390 nr. DCXXXIII/DCXXXV and Mus. Anat. IV. p. 67–68 and tabula CLXXIII and p.91–92 tabula CXCII). [11,12] According to the description this child suffered from congenital rickets. During preparation of the skeleton (Fig. 4C) the striking appearance of "soft" and "flexible" bones was mentioned. Moreover, it was mentioned that the periosteum could not be peeled off as easily as in other cases. The skull was described as being affected by hydrocephaly and consisted of multiple fragmented bones (Fig. 4D). The ribs showed multiple bony bulges; Sandifort described that these protruding deposits originated from healed fractures. Moreover, the ischial bones were located in close proximity and the acetabula were situated more ventrally than normal. The bones of all extremities, including the clavicles and shoulder blades were severely malformed and consisted of multiple bulges of bone deposits. According to Sandifort, the bones of the hands and feet were unaffected. Taking the soft and flexible bones in mind, we are inclined to diagnose this condition as infantile hypophosphatasia (MIM:241500), although the general appearance of the skeleton resembles osteogenesis imperfecta type 2. Interestingly, hypophosphatasia can produce rickets-like deformities as described here. Moreover, misshapen skulls, beading of costochondral junctions, enlarged joints from metaphyseal flaring and premature bony fusion of sutures can occur, [16,17] as well as, fractures and bone deformities. [18]

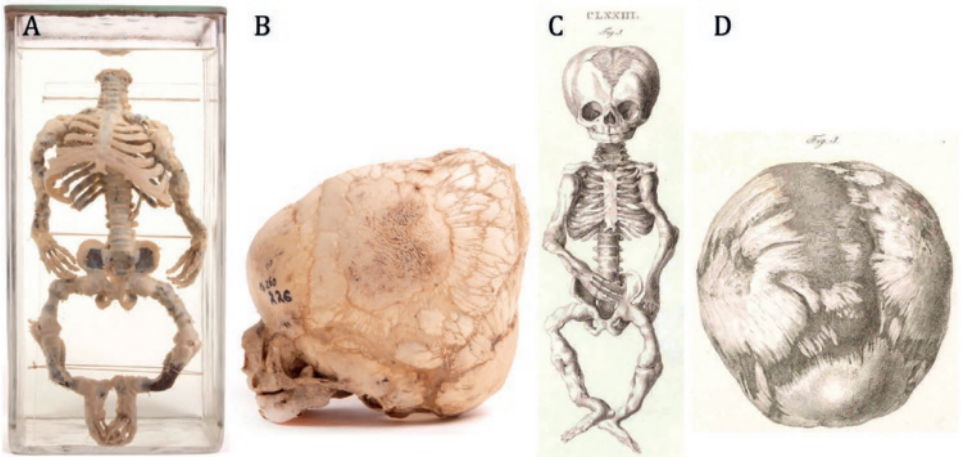


Fig. 4. Case 2. Hypophosphatasia [A] Specimen Pa0268, skeleton affected by hypophosphatasia, and [B] specimen Pb0260, skull with multiple fragmented bones. From the collection of the *Museum Anatomicum* Leiden; The Netherlands. [C/D] Copperplate Sandifort (1835). [12]

Meckel syndrome

Case 3. Specimen Eb0220 (Fig. 5) is a female neonate of unknown collector. On examination the head was very small, with a strongly sloping forehead, and a sac-like occipital encephalocele. The markedly distended abdomen was similar to a "prune belly;" presumably due to enlarged and cystic kidneys. The hands, as well as, the clubbed feet showed symmetrical postaxial hexadactyly. We diagnosed this condition as Meckel syndrome, which was also diagnosed in two other similar fetuses.

Fig. 5. Case 3. Specimen Eb0220, Meckel syndrome. From the collection of the *Museum Anatomicum* Leiden; The Netherlands.

Orofaciodigital syndrome

Case 4. Specimen Eb007 (Fig. 6) concerns a term female neonate of unknown collector with hypertelorism, broad nasal bridge, bilateral cleft of the upper lip and cleft palate, lobulated tongue, both pre and post axial polydactily on all four extremities and bilateral club foot. Based on the characteristic external characteristics we are inclined to diagnose this condition as orofaciodigital syndrome of unknown type.

Fig. 6. Case 4. Specimen Eb007, orofaciodigital syndrome. From the collection of the *Museum Anatomicum* Leiden; The Netherlands.

Orbital tumor

Case 5. Specimen Pb0207 concerns the macerated skull of a young child, aged only a couple of months (Fig. 7A). The child suffered from a left sided intra-orbital tumor which pushed the left nostril closed and caused a depression of the left corner of the mouth. This is one of the very few 19th century, both pre-and postmortem annotated cases of a child with a complicated orbital tumor. This specimen was originally collected by Andreas Bonn and described and depicted (Mus. Anat. Vol. III. p. 379, nr. DLXIX and Mus. Anat. Vol. IV. p. 6 and tabula CXXVIII) [11,12] in full detail by Gerard Sandifort (Fig. 7B). According to the comprehensive description, the tumor was in coherence with the ocular muscles and the periosteum of the zygomatic bone. In the left canthus of the affected eye a misshapen lacrimal caruncle was noticeable, the conjunctiva were sebaceous, and the cornea indistinct. Moreover, the eye showed microphthalmia and proptosis. Shortly before the child died, the eye and surrounding tissue showed extensive putrefaction. The tumor, which mainly consisted of fat, pushed the left eye out of its socket making the eyelids unable to close. The upper eyelid was more affected than the lower eyelid due to a tubercular swelling. After the child died, the skull was dissected and depicted (Fig. 7C). Unfortunately, the description did not mention if the tumor was present from birth. On exploration, the skull showed extensive orbital enlargement with deformities of the zygomatic bone and maxilla. The orbital bones were smooth, indicating the absence of any degenerative or infiltrative bone disease. The optic foramen and the superior orbital fissure were unaffected, the inferior orbital fissure was broadened and widened, the cranial vault was secondarily deformed. Although no other abnormalities regarding the autopsy were described the diagnoses orbital neuroblastoma, retinoblastoma, rhabdomyosarcoma, encephalocraniocutaneous lipomatosis, or oculocerebrocutaneous (Delleman) syndrome are the most obvious candidates. Unfortunately, there were no statements on brain and skin (accessory periocular cystic appendages) malformations which could make the diagnose of Delleman syndrome either more or less plausible.

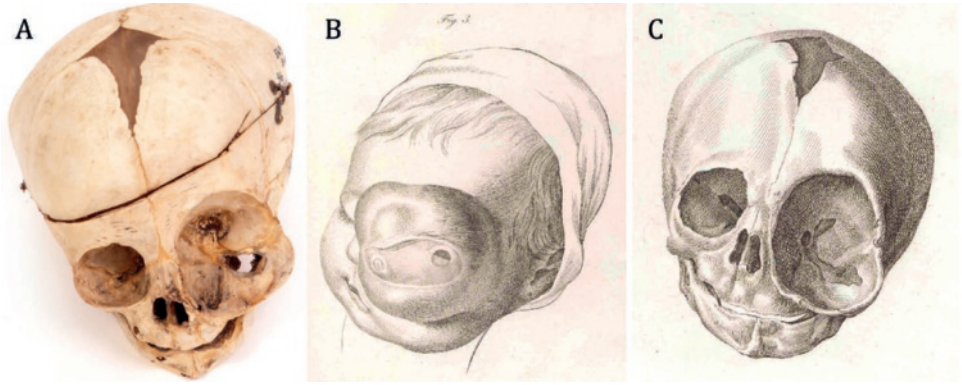


Fig. 7. Case 5. Orbital tumor. [A] Specimen Pb0207, neonatal skull with an affected left orbit. From the collection of the *Museum Anatomicum* Leiden; The Netherlands. [B/C] Copperplate Sandifort (1835). [12]

Tetralogy of Fallot

Case 6. Specimen Ag0046 (Fig. 8A) concerns a malformed skull of a neonate which was thoroughly described by Eduard Sandifort. The skull was part of a larger case report described in detail on page 1 till 41 in Part III of his *Observationes Anatomico-Pathologicae*. [19] Additionally, Eduard depicted this case in several engravings (tabula I–VI starting on p. 171). [19] These particular specimens and this case report were not mentioned in any of the *Museum Anatomicum* catalogues. However, the skull in this description is the only surviving specimen of this case. On examination the skull shows excessively large parietal bones and a horizontal occipital bone. The engraving of the child from which this skull originated showed a thoracolumbar spina bifida, large omphalocele and distinct head abnormalities (Fig. 8B). Moreover, the child suffered from multiple organ deformities including: cystic kidneys with distension of both ureters and tetralogy of Fallot. The combination of anomalies is not specific for any particular diagnosis but could match with an aneuploidic condition, that is, trisomy 18. Prior to the above attested case Eduard described, on page 1–38 of Part I of his *Observationes Anatomico-Pathologicae*, [20] four characteristics (pulmonary stenosis, dextroposition of the aorta, interventricular septal defect, and hypertrophy of the right ventricle) in a heart of a cyanotic 12-year-old boy who complained of fatigue, headaches, fainting and edema, which fits perfectly with tetralogy of Fallot. [21] Both case reports predated the one by Arthur Fallot (1850–1911) in Marseille Médical in 1888, [22] although the condition was already described by Niels (Steno) Stensen (1638–1686) in 1671. [23]

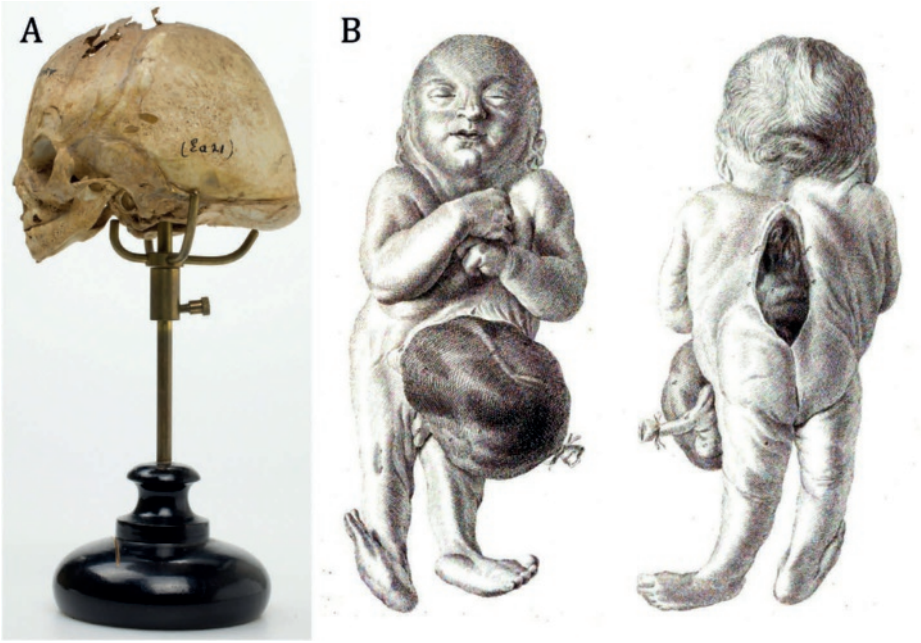


Fig. 8. Case 6. Tetralogy of Fallot [A] Specimen Ag0046, skull of the child. From the collection of the *Museum Anatomicum* Leiden; The Netherlands. [B] Copperplate of the child with multiple congenital anomalies and tetralogy of Fallot. Copperplate Sandifort (1779). [19]

Faciocranioschisis

Case 7. Specimen Eb0231 is one of the two specimens which could be assigned to Van Doeveren's original collection and concerns a male neonate with an exceptionally rare neural tube defect (Fig. 9A). This case was described by Van Doeveren on page 46 of his *Specimen observationum* and depicted this child in full detail (Figure 9B). [24] In this description it was stated that the entire child was normally developed with exception of the severely malformed head with a cleft of the soft and hard palate. Subsequently, Eduard Sandifort described and depicted this specimen (Mus. Anat. I. p. 300–301, nr. V and Mus. Anat. II. P. 119 and tabula CXXII). [5] On examination, the microcephalic head showed a severe closure defect that extended mid-craniofacial from the upper lip trough the entire face. We diagnosed this case as (isolated) faciocranioschisis.

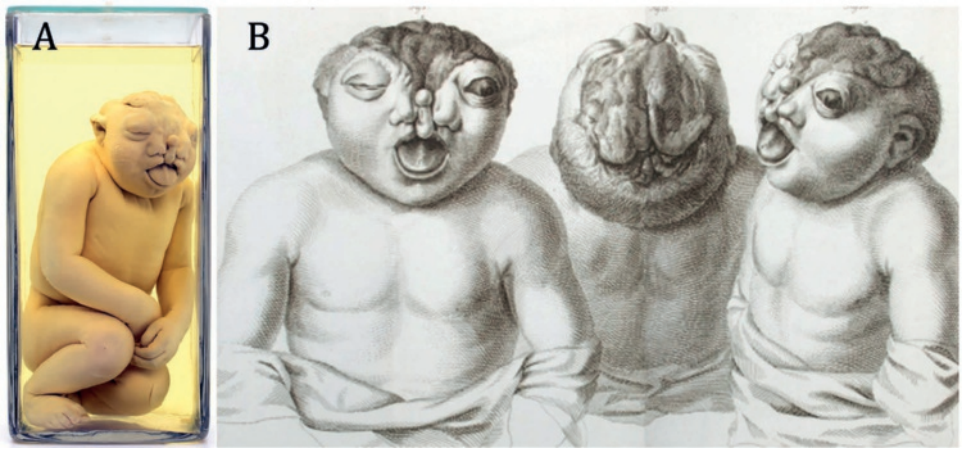


Fig. 9. Case 7. Faciocranioschisis. [A] Specimen Eb0231 male neonate with faciocranioschisis. From the collection of the *Museum Anatomicum* Leiden; The Netherlands. [B] Copperplate Sandifort (1793). [5]

Conjoined twins

Case 8. Specimen Ac0107 (Fig. 10) collected by Andreas Bonn concerns a female dicephalic conjoined twin originally described by Gerard Sandifort as "*Infans biceps. In capite uno oculi conjuncti sunt*" (Mus. Anat. Vol. III. p. 370–372. Nr. CDXC). [11] This specific case was also described by Willem Vrolik (1801–1863) in 1836. [25] Unfortunately, this case was not depicted in the *Museum Anatomicum* catalogues. On external examination a rudimentary extremity in the medio-sacral area and an indeterminable malformation of the external genitalia is seen. Moreover, the right microcephalic head shows cyclopia (holoprosencephaly or aprosencephaly). We diagnosed this case as parapagus dicephalus dibrachius tripus discordant for cyclopia. This rare association is known to occur, albeit only sporadically described. [26]

Fig. 10. Case 8. Specimen Ac0107, parapagus dicephalus dibrachius tripus discordant for cyclopia. From the collection of the *Museum Anatomicum* Leiden; The Netherlands.

Case 9. Specimen Eb0020 (Fig. 11) concerns a full-term female thoracoileopagus tetrabrachius tripus with a unilateral concomitant but discordant sirenomelia (sympus monopus) sequence of unknown collector. This specific discordance for thoracoileopagus tetrabrachius tripus was not found in the current available literature. The only found publication on this topic is from Tannuri *et al* (2013) who described a craniopagus conjoined twin discordant for sirenomelia. [27]

Fig. 11. Case 9. Specimen Eb0020, thoracoileopagus tetrabrachius tripus discordant for sympus monopus. From the collection of the *Museum Anatomicum* Leiden; The Netherlands.

Nasopharyngeal teratoma

Case 10. Specimen Eb0081 is one of the 39 existing teratological specimens assigned to Andreas Bonn and concerns a full-term female neonate which on external examination shows an intra-orally and intra-nasally located, non necrotizing, protruding mass (Fig. 12A). According to Bonn the mouth was completely filled with the "polyp" which after progressive growth protruded

from the mouth. Moreover, the tumor appeared to protrude from both nostrils. This case was only scantily described (Mus. Anat. Vol. III. p. 377–378, nr. DLVIII and Mus. Anat. Vol. IV. p. 98 and tabula CXCV) but was depicted in detail by Gerard Sandifort (Fig. 12B). [11,12] Based on the engravings and the specimen this case is diagnosed as an epignathus or nasopharyngeal teratoma. However, an oropharyngeal rhabdomyosarcoma cannot be ruled out without performing any further diagnostics.

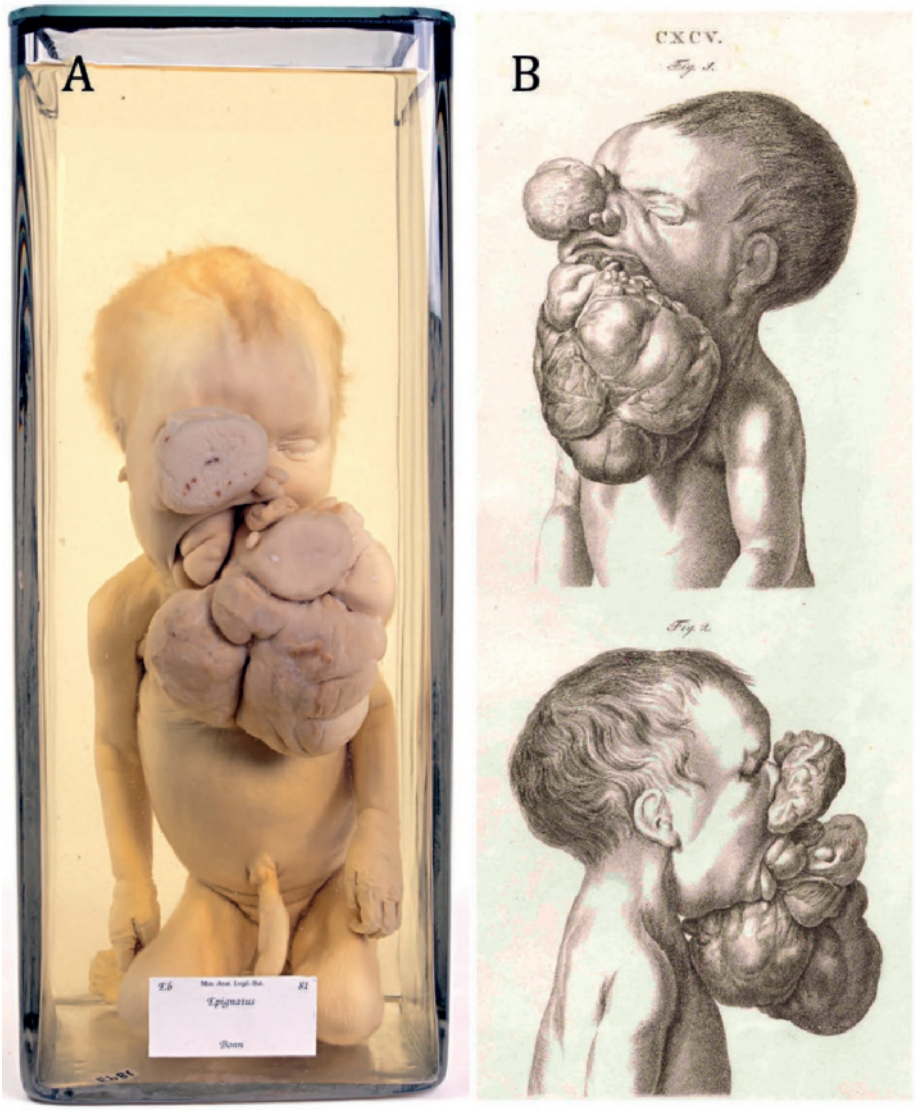


Fig. 12. Case 10. Nasopharyngeal teratoma. [A] Specimen Eb0081 female neonate with a nasopharyngeal teratoma. From the collection of the *Museum Anatomicum* Leiden; The Netherlands. [B] Copperplate Sandifort (1835). [12]

Epigastric heteropagus conjoined twin

Case 11. Specimen Eb0011 is the second of the two existing teratological specimens originating from Wouter van Doeveren. It was described by Eduard Sandifort as "*Infans monstrosus*" [5] and subsequently by the inventory of Elshout as "*Thoracopagus parasiticus masculinus.*" [2] This case concerns a term male neonate with an incomplete smaller body attached to its thoraco-abdominal transition (Fig. 13A). This case was described (Mus. Anat. I. p. 302–303, nr. XIII and Mus. Anat. II. P. 121 and tabula CXXV) and depicted (Fig. 13B) by Eduard Sandifort. [5] In this description he stated that a healthy 40-year-old woman, who delivered five healthy children previously, now delivered this unusual child which lived for three successive days before it died. We diagnosed this specimen as an epigastric heteropagus conjoined twin.

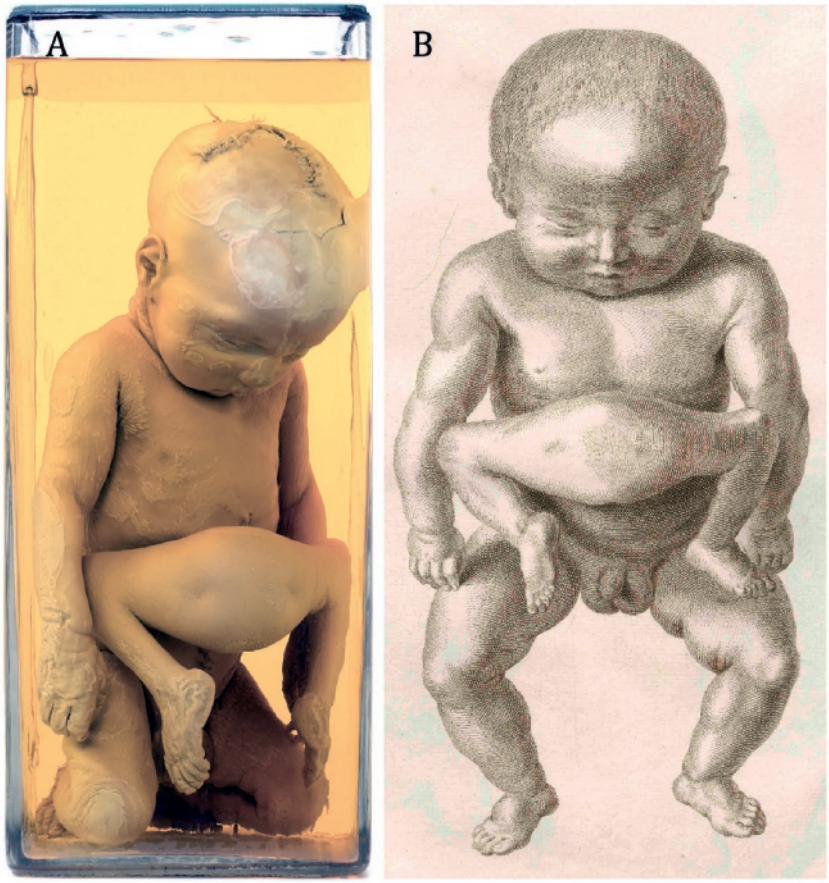


Fig. 13. Case 11. Epigastric heteropagus conjoined twin. [A] Specimen Eb0011: male neonate with asymmetric twin attached to the epigastric region. From the collection of the *Museum Anatomicum* Leiden; The Netherlands. [B] Copperplate Sandifort (1793). [5]

Discussion

Throughout the 17th and 18th centuries, teratological specimens were prominent parts of privately owned cabinets of curiosities; these specimens were first and foremost unique rarities. However, in the course of the 18th and 19th century, teratological specimens became more than singular cases of intriguing curiosities. Teratological specimens and collections became part of natural classifications and taxonomy. During the mid-18th century many privately owned collections were institutionalized after their collectors died. These institutionalized collections were often systematically arranged, in contrast to the mostly anecdotally collected morphology specimens many anatomists collected privately. The *Museum Anatomicum* of the Leiden University is a shining example of a vast and versatile collection which finds its origin in multiple privately owned collections and include teratological specimens which were collected over multiple centuries; collections were either purchased by the university or donated by their original collectors or their heirs. Due to the present day paucity of full-term fetuses with congenital anomalies, institutionalized teratological collections become more valuable over time. Nevertheless, these collections are prone to neglect and at risk for decline of its contents. This makes old teratological collections increasingly rare and often underrepresented in medical curricula. On the other hand, several teratological collections in medical museums worldwide are still operational up to present day and are accessible for the general public and the medical student. These residual collections can be seen as "time capsules" full of nature's creations waiting to be explored with, for example, radiological or genetic techniques in order to exploit their excellent educational and scientific potentials. [28-30] The collection of the *Museum Anatomicum* can be used to retrospectively study how congenital anomalies were perceived during the heydays of collecting teratological specimens. Historical perspectives regarding the original collectors and their contemporaries can be studied and matched with their original specimens.

Although large parts of the extant collection is described in four Latin published catalogues, by father and son Sandifort, it was merely impossible to match all existing specimens with these Latin descriptions. Many specimens showed certain characteristics that were not mentioned in the specimen descriptions. It is conceivable that in the course of time specimens or specimen-numbers got changed, that new specimens were added and other specimens were discarded, making it impossible to assign them all to specific collectors or descriptions. Moreover, it is imaginable that specific specimens or preparation techniques were copied by other collectors and subsequently

incorporated in the collection; again making it difficult to match all specimens to a specific collector. As it turns out we found 642 teratological specimens during re-examination, rediagnosing and redescribing of the extant collection with some exceedingly rare conditions such as faciocranioschisis and conjoined twins discordant for holoprosencephaly and sirenomelia. Although the collection of Sebald Justinus Brugmans consisted of 4,081 specimens, [11] we only found 27 teratological specimens of his original collection. His collection was mainly characterized by comparative anatomy, pathological bones and fossils and included only 154 human specimens. Although, Brugmans did collect some teratological specimens, there was no additional literature found concerning this topic. The teratological specimens found in the extant collection consisted of congenital dislocations of the hip, hydrocephaly, anencephaly, cleft lip and palate, skeletal dysplasias, bladder exstrophy, sacralization, anal atresia, and some minor skeletal anomalies. The collection of Gerardus Suringar, donated to the museum in 1866, originally comprised more than 800 anatomical specimens, but we retrieved only 19 specimens which could be reasonably assigned to him. These included congenital luxations of the hip, hydrocephaly, neural tube defects (anencephaly and spina bifida), conjoined twins, cleft lip and palate, syndromes and some organ anomalies, none of which was described in more detail at the time.

Biographies

The following section contains the biographies of Wouter van Doeveren, Andreas Bonn, Eduard Sandifort, and Gerard Sandifort. These four collectors were chosen because of their contributions to the teratological collection or their significant role in describing the specimens.

Wouter Van Doeveren (1730–1783)

Wouter van Doeveren studied medicine in Leiden and obtained his doctor degree in the same city. In 1754, Van Doeveren was appointed professor in anatomy, surgery, and obstetrics in Groningen and became professor of medicine in Leiden in 1770. [31] During his career in Groningen, Van Doeveren published his most admired work: "*Specimen observationum academicarum ad monstrorum historiam, anatomen, pathologiam, et artem obstetriciam, praecipue spectantium.*" [24] In this masterpiece he described anomalies in both animals and humans. Teratology was a subject in which Van Doeveren was very interested and well ahead of his contemporaries; he was one of the first who attempted to build a systematic collection of teratological specimens,

moving them from the "sphere of wonder and curiosity" into the world of naturalization; as such, teratology became part of natural classifications and taxonomy. [32] Additionally, this systematic approach led to a paradigm shift that placed the "monstrous births" from a negative into a positive point of view. In the first chapter of his "*Specimen observationum*" Van Doeveren described the generally accepted opinions regarding the origin of congenital anomalies of his time. Two theories were described: 1) The "*monstra primigenia*," a theory that explains anomalies as being present from the first conception without any involvement of exogenous factors; and 2) the "*monstra accidentalis*," a theory stating that anomalies arise during development in utero; meaning that there was initially a normally developed embryo which became deformed under exogenous factors. In his description of a lamb with two heads (Fig. 14) Van Doeveren stated that this beautifully formed Siamese lamb proved that "monsters" are meant to be the way they are and have a wonderful, symmetrical and purposefully built body. Moreover, he postulated that the lamb could not possibly be the result of two separate developed embryos which were fused secondarily, as the putative fusion should have been perfect and seamless. According to Van Doeveren this lamb was a perfect example of "*monstra primigenia.*"

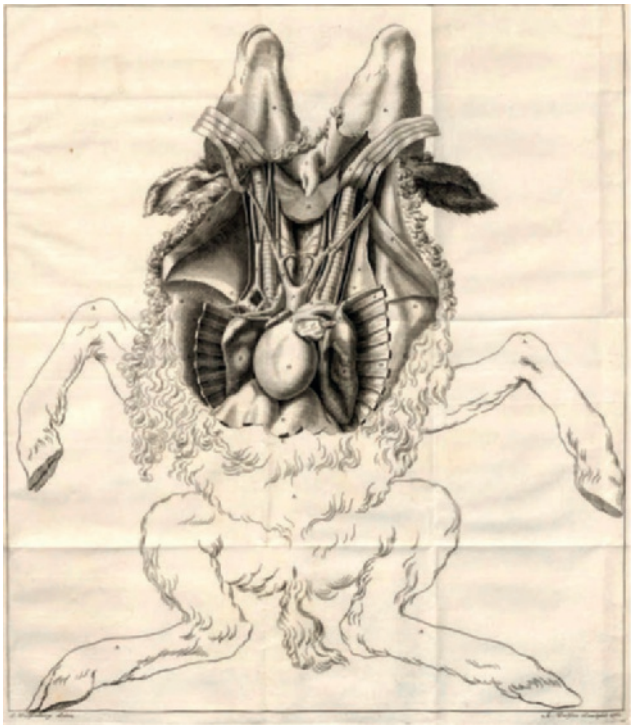


Fig. 14. Siamese lamb, copperplate. Van Doeveren (1765). [24]

Conversely, on page 47 of the second chapter, he described a case of an anencephalic fetus with clefts of the nose, upper jaw and palate which he considered to result from an unknown accidental cause when the skull was still weak and soft during development, thereby confirming the "*monstra accidentalis*" concept. [24] However, he stated that one should refrain from any opinion or verdict about the cause of an anomaly when one is in doubt. Moreover, Van Doeveren confirmed that dissecting and describing congenital anomalies is useful and will eventually lead to more knowledge about how they arise. Van Doeveren believed that inside the anatomy of the "*monstra*," the "*semina veri*" (seeds of truth) are located which are not unveiled in the normal anatomical situation of the human body. Although he admits that the exact cause is unknown, Van Doeveren prefers the theory of "*monstra primigenia.*" [13] Many contemporaries who believed in the "*monstra accidentalis*" theory, often had vague and abstruse ideas about the origin of anomalies, for example, "*imaginatio materna*" (*maternal imagination*). Van Doeveren's aversion of these vaguely described factors were maybe due to the impossibility to place them into an exact and rational framework; an important factor for systematic research. According to Van Doeveren, maternal imagination was no satisfying explanation for anomalies found in humans, as plants and animals—which, after all, have no reason, morals or imagination—could also produce a monstrous progeny. In Van Doeveren's opinion, this argument refutes maternal imagination as a cause of congenital anomalies. Looking throughout the work of Van Doeveren it is clear that he raised two different theories (*monstra primigenia* or *monstra accidentalis*) as the cause of different congenital anomalies. It looks like Van Doeveren thought he had to choose one of the two theories to explain all congenital anomalies. It is conceivable that he saw anomalies which he could not explain and subsequently was indecisive in his conclusion that both theories could be applicable. The time spirit did not yet allow the awareness of the fact that both malformations and/or deformations can occur individually or in the same affected child. Malformations are homologous to *monstra primigenia* and deformations are homologous to *monstra accidentalis.* After Van Doeveren' dead in 1783, his private collection was publically auctioned on the 18th of April 1785 and consisted over 3,000 items including zoological specimens, fossils, minerals, instruments and various scientific objects. [33] Part of the auctioned items were bought by the faculty board members for 4,300 guilders. Eduard Sandifort listed 441 specimens belonging to Van Doeveren's collection in his *Museum Anatomicum* catalogue, which included 23 human anomalies and 15 animal anomalies. He depicted many of his specimens in the second part of the *Museum Anatomicum* catalogues. [5] Unfortunately, the Van Doeveren collection

was seriously damaged during the explosion of the gunpowder ship; only 32 specimens remained. [2] In the extant collection we found only two teratological specimens which could be reasonably assigned to Van Doeveren's original collection (See cases 7 and 11). One of them is an exceptionally rare case of faciocranioschisis (Case 7). Only two descriptions of faciocranioschisis are described in the modern literature. [34,35] Oostra *et al.,* (1998) who reviewed the teratological collection residing in Museum *Vrolik*, described a 19th century case of faciocranioschisis in a female neonate. [35] Our case dates back to 1765 a case description of faciocranioschisis published more than 100 years earlier, being the first historical description and the third attested case in modern literature so far.

Andreas Bonn (1738–1817)

Andreas Bonn studied medicine in Amsterdam and Leiden. He obtained his doctor degree in Leiden with his dissertation named "*Specimen anatomico-medicum inaugurale, de continuationibus membranarum.*" [36] In this work Bonn described the morphology of the skin, joint capsules, periosteum and the membranes of the body cavities and the meninges; subjects in which Bonn was well ahead of his contemporaries. [13] In 1764 Bonn continued his study in Paris and became a well-known physician in Amsterdam in the same year. In 1771 he was appointed professor of anatomy and surgery at the *Atheneum Illustre* in Amsterdam. [2,11] In his younger years Bonn was engaged in describing the pathological bone collection of Amsterdam's physician and anatomist Jacobus Hovius (1710–1786). Bonn published this meticulously described collection in his "*Descriptio thesauri ossium morbosorum Hoviani.*" [15] Currently, the Hovius cabinet represents the oldest anatomical collection in Amsterdam and is still on display in Museum *Vrolik* (Fig. 15). Bonn's personal collection contained beautiful and elegant red injected specimens of general and comparative anatomy and pathology. To make up for the losses that resulted from the exploded gunpowder ship the Bonn collection, amended with several Hovius specimens, was donated to the Leiden anatomical museum in 1822 with the specific clause that specimens of structures and conditions that already were present in the anatomical museum would be sent to other universities. [8] Gerard Sandifort assessed Bonn's specimens and selected 737 preparations which could be added to the anatomical museum. Sandifort stated that he was particularly pleased with the "monstra" and pathological bones since these were often underrepresented in anatomical collections. [11] The remaining specimens were sent to the University of Ghent (Belgium), albeit that the present whereabouts of these specimens remains unknown.

Fig. 15. The Hovius cabinet from 1773 in the *Vrolik* Museum. Photo: Hans van den Bogaard (2009), Museum *Vrolik*, Academic Medical Centre, Amsterdam, The Netherlands.

Bonn's interest in congenital anomalies can be seen back throughout his career. He not only collected teratological specimens of human and zoological origin, but also specimens of deviant fruits. According to Bonn these dysmorphic fruits were also to be ascribed as "monstra." Bonn published papers on congenital hip dysplasia's [37] and on urogenital anomalies in both sexes. [38-41] Bonn explained that the cause of congenital abnormalities (including spina bifida, bladder exstrophy, hypospadias and cleft lips/palates) all resulted from a mechanical injury during embryonic development. He thought that hypospadias was caused by a rupture of the urethral orifice and that the cause of an ectopic bladder was due to a rupture of the ventral body wall during birth. However, he admitted that these statements were inadequate and he confessed that the field of teratology was difficult to understand for himself and most of his contemporaries. However, in his paper about an acardiac twin he acknowledged that the cause of the acardiac was an "abnormal process" during embryological development rather than a mechanical injury. [42] Bonn was, as was Van Doeveren, indecisive in explaining all observed anomalies with the "*monstra accidentalis*" or the "*monstra primigenia*" theory. Moreover, he assumed that the cause of the acardiac twin was situated "inside" the embryo itself, although he was not familiar with what the exact "internal genesis" in the embryo could be. He described the absence of the heart and head as a "lack of human factors" and the partial absence of the abdominal

organs—according to Bonn the "seat of desire and lust"—as a "lack of animal factors," thus taking a more or less mystical and vague point of view in the origin of malformations. [2] Additionally, Bonn quoted that the cause of the acardiac twin could be the result of a disturbed "*nisus formativus*, *vormdrift*, *or Bildungstrieb*;" again posing a different theory on the cause of an anomaly. He also stated that a woman cannot be designated to be the cause of the malformed child she gave birth to, confirming that the "*imaginatio materna*" theory was an unsatisfactory explanation. Looking at the many different theories Bonn described, he did not seem to be convinced himself that any of these explanations were true. The donated parts of the Bonn and Hovius collections are described by Gerard Sandifort in Vol. III and depicted in Vol. IV of the *Museum Anatomicum* catalogues. [11, 12] In the extant collection we found a total of 39 teratological specimens which could reasonably be assigned to Bonn's original collection.

One of Bonn's specimens concerned a rare type of conjoined twinning: a parapagus dicephalus dibrachius tripus that was discordant for cyclopia (See case 8). It is known that concomitant anomalies have a much higher incidence in monozygotic twins compared to singletons and are evenmore frequent in conjoined twins. These anomalies can be designated as early structural defects with frequent discordance and mainly concerning midline structures as is the case in holoprosencephaly. [43] Unfortunately, due to the historic value of this specimen and therefore the absence of additional diagnostics, we were not able to make any statements about the internal morphology of the non-holoprosencephalic head in this specimen. Oostra *et al*., (1998) [35] described a case of a diprosopus tetraophthalmus distomia diotis with cebocephaly that was not apparent on external examination but was only noticeable after radiological imaging. [44] One of the earliest known reports of a conjoined twin with concomitant holoprosencephaly is from Dutch professor h.c. Louis (Lodewijk) de Bils (1624–1669). De Bils was an autodidact without formal medical education who became renowned for his embalming and dissection techniques, for which he received the title "professor *honorarius anatomiae*" by the Illustrious School of 's Hertogenbosch. [45] De Bils described and depicted a dicephalus in which one head clearly showed a proboscis and synophthalmia (Fig. 16). [46] Rating (1933) reported a case of diprosopus which showed two separate eyes and a normal nose in the right face and synophthalmia and a proboscis in the left face. [47] However, as mentioned some reports exist that describe holoprosencephaly in concomitance with conjoined twining, although these associations remain exceedingly rare.

Fig. 16. Copperplate of the conjoined twin with concomitant holoprosencephaly De Bils (1661). [46]

Another set of specimens of Bonn's collection concerned a case of multiple enchondromatosis wherein Bonn described the entire skeleton. [15] These specimens, either dried or fixated in ethanol were initially part of the Hovius collection. Part of this set moved with the Bonn collection to Leiden in 1822, whereas the remainder stayed in Amsterdam, where it still resides. Gerard Sandifort described 15 of these specimens (nr. CCCI-CCCIX were dried bones, specimens DCXXXIX-DCXLV were fixed and kept in alcohol). As it turns out the left femur, left tibia, left radius/ulna, left scapula, left clavicle, a sacrum with three lumbar vertebrae, a right finger and a left sided hip bone were identified in the extant Hovius collection in Museum *Vrolik*. The right-sided skeletal elements but also the left hand and both fibular bones were described in the catalogue of the Leiden collection. [11,12] The only specimen of this case which was identified in the extant Leiden collection is that of the left hand

(See case 1). Bonn posed quite an interesting opinion regarding the cause of this rare bone dysplasia. He presumed that the cause of the tumors would have been present from birth or presented itself within the first year, because of their size. He considered the child to have suffered from congenital rickets (a default diagnose in the 18th and 19th century for many bone diseases which involved bending of tubular bones) which caused the distal ends of the bones to cease growing and to become malformed and replaced by nodular proliferations. Bonn was right in that the tumors were, most probably, present around birth or in the first year as enchondromatosis is a rare primary bone dysplasia appearing in childhood. [48]

Eduard Sandifort (1742–1814)

Eduard Sandifort studied medicine in Leiden and obtained his doctor degree in the same city with his dissertation entitled: "*Dissertatio anatomico-obstreticia de pelvi, ejusque in partu dilatation*." [50] In this work he described the pelvic dilatation during parturition. In subsequent years Sandifort worked as a physician in The Hague (The Netherlands) and was engaged in the variolation against cattle-plague and smallpox. [3] In 1765, at the age of twenty-three, Eduard began to publish his Nature- and Medical Library (Natuur– en Geneeskundige Bibliotheek) which appeared every year for ten successive years. In this compiled work hundreds of observations concerning botany, physics and medicine are described. In 1770 he became *Praelector Anatomiae et Chirurgiae* and only one year later he was appointed *Professor Anatomiae et Chirurgiae* at the University of Leiden. Between 1777 and 1781, Eduard Sandifort wrote four well illustrated Latin catalogues, entitled "*Observationes Anatomico-Pathologicae.*" [19,20,50,51] In this work Sandifort included 47 descriptions of anatomical and pathological findings including some congenital anomalies. The aim of this work was twofold: 1) to register his pathological findings during dissections; and 2) to propagate his theories about pathology and congenital defects to a broad audience. Among others, he described a hydatiform mole, complex malformations, a horseshoe kidney, an acardiac twin, a duplicated ureter and bladder malformations. Noteworthy is the case on page 29 of the fourth book, entitled "*De labio leporino, congenito, duplici et complicate.*" [51] This description is one of the earliest extensively described cases, both pre- and postmortem, of a child with a complicated bilateral cleft lip, palate and nose (Fig. 17A). Eduard Sandifort stated that this complex anomaly would be very difficult to operate and impossible to cure; his only intention was to describe the child's anomaly in full detail. Unfortunately, the child died of malnourishment when it was only 22 weeks old. Sandifort

convinced the parents to donate the child's body for further research; he additionally gave a detailed description of the child's skull after he carefully dissected it (Fig. 17B).

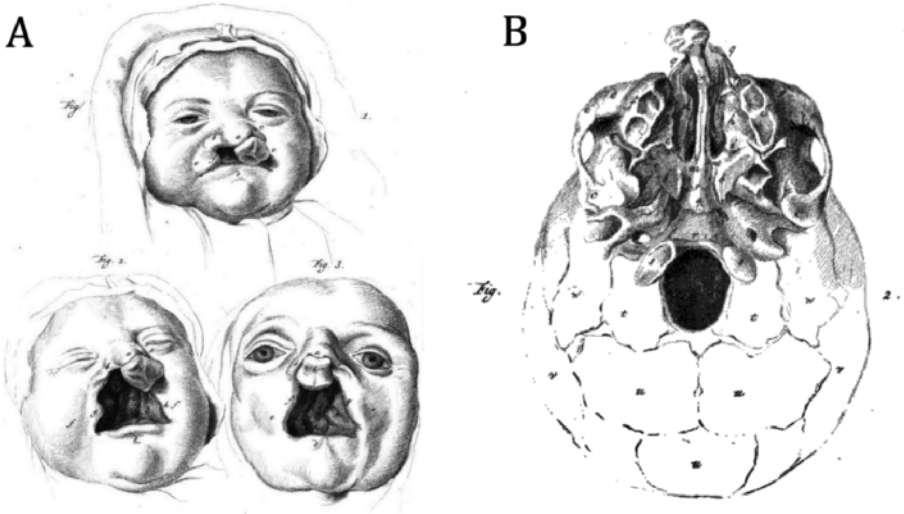


Fig. 17. [A] Copperplate of the child with a complicated bilateral cleft lip, palate and nose. [B] Copperplate of the skull of the same child after Eduard Sandifort cleaned the skull after the child deceased. [51]

It was in 1793, that he became internationally renowned when he published the first two parts of the *Museum Anatomicum Lugduno-Batavae* catalogues. [5] In these two volumes, written on request of the board of Leiden's University curators, Sandifort described the 17th and 18th century inherited anatomical collections of the *Museum Anatomicum* in their then present state and described the collections of contemporaries Johannes Jacobus Rau (1668–1719), Bernhardus Siegfried Albinus (1697–1770) and Wouter van Doeveren (1730–1783). In 1802 his private collection was bought by the anatomical museum. [3] The collection of Eduard Sandifort is recognized by its exceptionally beautiful and elegant, with mercury and red-colored wax injected, specimens of the lymphatic system. [2] Another noteworthy script about congenital anomalies is his book entitled "*Icones herniae inguinalis congenitae.*" In this book, he gave detailed information about congenital inguinal hernias accompanied by some fine engravings. [52] Furthermore, Eduard wrote a book entitled "*Anatome infantis cerebro destituti.*" In this impressive work he described the morphology of the head, brain, skull, and vertebrae of the anencephalic child in great detail. [53] Although Eduard primarily dedicated himself to careful descriptions rather than speculative interpretations about the cause of congenital anomalies, he stated that the cause of anencephaly is

a primary hydrocephalus. This hydrocephaly eventually degraded the brain and bones of the skull, resulting in an absent brain and hence confirming the "*monstra accidentalis*" theory. This in contrast to the indecisive opinions of his contemporaries Wouter van Doeveren and Andreas Bonn. He was one of the first to give a detailed description of a 12-year-old cyanotic boy who suffered from a cardiac malformation (See case 6), presently known as tetralogy of Fallot. [20,21] Furthermore, Eduard described many anomalies of the blood vessels, [13] again affirming his interest in congenital anomalies. In the extant collection we found a total of eight teratological specimens which could be reasonably assigned to Eduard's original collection.

Gerard Sandifort (1779–1848)

Gerard Sandifort, son of Eduard Sandifort, was aged only thirteen when he began to help his father with dissecting anatomical specimens. He studied medicine and obtained his doctor's degree in Leiden with his dissertation called "*Dissertatio medica inauguralis*, *de pleuritide.*" [54] In 1801 he was appointed professor of anatomy and subsequently became professor of anatomy, surgery and medicine in 1802. Gerard followed his father's footsteps and published part three and part four of the *Museum Anatomicum Lugduno-Batavae* catalogues. [11,12] In these two works the collections of contemporaries Sebald Justinius Brugmans (1763–1819) and Andreas Bonn (1738–1817) are described. In the preface of part four Gerard noted that it was his main goal to describe the most important pathological specimens and included some exceptionally well-illustrated cases of congenital anomalies. Like his father, Gerard was renowned for his excellent observations and meticulous descriptions of both anatomical, pathological and teratological specimens. His expertise and opinion about the cause of congenital anomalies can be analyzed throughout his many treatises and observations which were published between 1817 and 1848 for the "Royal Dutch Institute" (Koninklijk Nederlandsch Instituut). He wrote 46 treatises; however, only 15 concerned congenital defects in animals or humans. Gerard meticulously described and depicted the morphology of acardiac twins and anencephalic fetuses. [55,56] In 7 of the 15 treatises concerning congenital anomalies, he gave his conception about the cause of an anomaly. In the early descriptions of Gerard (around 1820), he was convinced that an excess of nerves was the main cause of an anomaly. Around 1824, he changed his opinion and stated that the cause of an anomaly was the result of a disturbed "*nisus formatives.*" [31] This opinion shift could possibly have been triggered by a dissertation of the Leiden professor in pharmacy Gerardus Suringar (1802–1874) entitled: "*Dissertatio medica*

inauguralis de nisu formativo ejusque erroribus" (An inaugural dissertation on the impulse of nature's formation and its mistakes). [57] The overarching term "*nisus formativus*" can be seen as the source of all propagation, growth, and nourishment. The concept of "*nisus formativus*" was initially presented in 1781 by Johann Friedrich Blumenbach (1752–1840), who stated that this theory could explain the impulse of nature to create forms and how those forms were managed; every living organism was pre-formed and contained a kind of "potency" that only had to develop to its future shape. [58] For decades, this theory was the starting point to explain the formation of both normal and abnormal morphogenesis, as well as, the regeneration and conservation of all structures and included the origin and nature of congenital anomalies. This "*nisus formativus*" theory was sanctioned by Gerard until approximately 1839, when he stated in his treatise on rare malformations of the head in quadrupeds that the real cause of the anomalies was rather doubtful and still unknown. [59] However, as late as 1847, he sometimes referred to an excess of nerves as the cause of certain anomalies. [60] Comparable to his father, Gerard had a private collection of specimens that were not described in the *Museum Anatomicum* catalogues. After he died in 1849, these 432 anatomical specimens were publically auctioned; the present status and whereabouts of these specimens are unknown. [61] Nevertheless, in the extant collection we found two specimens, both concerning the skeleton of an acardiac twin, that could be reasonably assigned to Gerard's original collection albeit not further elaborated, indicating that at least some of the auctioned specimens were purchased by the museum, at the time.

Conclusion

The cause of congenital anomalies during the 18th and 19th centuries heydays of collecting teratological specimens was still debated and differently envisioned by several collectors. Subjects such as heredity and modern "concepts of developmental biology" were completely absent during the time in which these specimens were collected. Moreover, due to the absence of additional diagnostics such as genetics and radiology and the shortage of concrete causative theories it is rather astonishing that these old collectors were already able to describe, and in many cases, diagnose a congenital anomaly. Many historically made diagnoses could not be changed after rediagnosing the specimens with contemporary dysmorphological knowledge, actually confirming that these old collectors were perhaps the first dysmorphologists and can be seen as true pioneers in the field of teratology. The external descriptions these old collectors gave to these specimens were equivalent to

concepts such as malformations and deformations we now abundantly use to describe congenital anomalies. Apparently, no collector was able to recognize that both expressions do not exclude each other and can be applicable individually to different conditions. Up to the present day teratology is still an elusive field of science with many open questions. However, historical theories about the cause of congenital anomalies can be used for further explorations into contemporary theories. Finally, exploiting old teratological collections can yield rare discordant associations, can give more insights in very rarely occurring birth defects and can be used to expand the clinical spectra of certain conditions. Therefore, old teratological collections have to be treasured for the future scientists in teratological research.

Acknowledgements

The authors gratefully acknowledge the help of Prof. dr. Marco C de Ruiter for his cooperation and providing all the possibilities to investigate the extant collection in the *Museum Anatomicum* in Leiden, The Netherlands. Furthermore, we thank Dr. Laurens de Rooy for his help in providing information and photographs from the *Vrolik* museum in Amsterdam, The Netherlands and thank dr. Willemijn Klein for her comments on the final manuscript.

References

1. Otterspeer W. Groepsportret met Dame I, Het bolwerk van de vrijheid. De Leidse Universiteit.
   Amsterdam: Bert Bakker, pp: 1575–1672.
2. Elshout AM. 1952. Het Leidse kabinet der anatomie uit de achttiende eeuw. De betekenis van een wetenschappelijke collectie als cultuurhistorisch monument. Leiden: Leiden University Press.
3. Smit P. 1986. Hendrik Engel's alphabetical list of Dutch zoological cabinets and menageries. Amsterdam: Rodopi.
4. Muntendam P. 1923. Prof. Dr. J. A. J. Barge, De Leidsche anatomie herdacht. Leiden: S. C. van Doesburgh.
5. Sandifort E. 1793. Museum Anatomicum Academiae Lugduno-Batavae descriptum. Volume I and II. Leiden: S. & J Luchtmans.
6. Pauw P. 1657. Petri Pawij Observationes anatomicæ selectiores, jam primum editæ, curante Th. Bartholino. Ex typographia Adriani Vlacq. Hafniæ: Literis Petri MorsingI, Reg. & Ac. Typ.
7. Huisman T. 2009. The Finger of God. Anatomical Practice in 17th Century. Leiden: Primavera Pers.
8. Huistra H. 2013. Preparations on the move: The Leiden Anatomical Collections in the Nineteenth Century. Doctoral Thesis. Leiden: Leiden University.
9. Albinus BS. 1725. Index supellectilis anatomicae: quam academiae Batavae quae Leidae est legavit. Leiden: Apud Henricum Mulhovium.
10. Albinus BS. 1775. Supellex Anatomica Bernardi Siegfriedi Albini. Leiden: Apud Petrum Delfos.
11. Sandifort G. 1827. Museum anatomicum Academiae Lugduno-Batavae descriptum. Volume III. Leiden: S. & J Luchtmans.
12. Sandifort G. 1835. Museum anatomicum Academiae Lugduno-Batavae descriptum. Volume IV. Leiden: S. & J Luchtmans Johannes.
13. Van der Boon A. 1851. Geschiedenis der ontdekkingen in de ontleedkunde van den mensch, gedaan in de noordelijke Nederlanden, tot aan het begin der negentiende eeuw. Utrecht: C. van der Post, Provinciaal Utrechtsch genootschap.
14. Boer LL, Radziun AB, Oostra RJ. 2017. Frederik Ruysch (1638-731) Historical perspective and contemporary analysis of his teratological legacy. American Journal of Medical Genetics Part A. 173:16–41.
15. Bonn A. 1783. Descriptio thesauri ossium morbosorum Hoviani. Amsterdam: apud J. C Sepp.
16. Collmann H, Mornet E, Gattenlohner S, Beck C, Girschick H. 2009. Neurosurgical aspects of childhood hypophosphatasia. Childs Nervous System. 25:217–223.
17. Whyte MP. 2016. Hypophosphatasia—aetiology, nosology, pathogenesis, diagnosis and treatment. Nature Reviews Endocrinology. 12:233–246.
18. Whyte MP, Greenberg CR, Salman NJ, Bober MB. et al. 2012. Enzyme-replacement therapy in life threatening hypophosphatasia. The New England Journal of Medicine. 366:904–913.
19. Sandifort E. 1779. Observationes Anatomico-Pathologicae vol. III. Leiden: Apud Eyk P. V. d., Vygh, D.

20. Sandifort E. 1777. Observationes Anatomico-Pathologicae vol. I. Leiden: Apud Eyk P. V. d., Vygh, D.
21. Bennett LR. 1946. Sandifort's "Observationes," chapter I, concerning a very rare disease of the heart. Bulletin of the History of Medicine. 20:539–570.
22. Fallot ELA. 1888. Contribution a l'anatomie pathologique de la maladie bleu (cyanose cardiaque) (138–158, 207–223, 270–286, 341–354, 403–420). Marseille Med, 25, 77–93.
23. Stensen, N. (1671). Embryo monsto affinis Parisiis dissectur. Acta Medica and Philosophica Hafniensia, 1, 202–203.
24. Van Doeveren W. 1765. Specimen observationum academicarum ad monstrorum historiam, anatomen, pathologiam, et artem obstetriciam, praecipue spectantium. Groningen/Leiden: J.Bolt and SJ. Luchtmans.
25. Vrolik W. 1836. Over den aard en oorsprong der cyclopie. Nieuwe verhandelingen der eerste klasse van het koninklijk Nederlands Instituut. 5:25–112.
26. Blaas HG, Eriksson AG, Salvesen KA, Isaksen CV, Christensen B, Møllerløkken G, Eik-Nes SH. 2002. Brains and faces in holoprosencephaly: Pre- and postnatal description of 30 cases. Ultrasound in Obstetrics and Gynecology. 19:24–38.
27. Tannuri ACA, Batatinha JAP, Velhote MCP, Tannuri U. 2013. Conjoined twins—twenty years' experience at a reference center in Brazil. Clinics. 68:371–377.
28. Boer LL, Morava E, Klein WM, Schepens-Franke AN, Oostra RJ. 2017. Sirenomelia: A multi-systemic polytopic field defect with ongoing controversies. Birth Defects Research, 109:791–804.
29. Boer LL, Naue J, de Rooy L, Oostra RJ. 2017. Detection of G1138A mutation of the FGFR3 gene in tooth material from a 180-year-old museological achondroplastic skeleton. Genes (Basel). 28:8. pii: E214.
30. Boer LL, Schepens-Franke AN, van Asten JJA, Bosboom DGH et al. 2017. Radiological imaging of teratological fetuses: What can we learn? Insights Imaging. 3:301–310.
31. Van der Zwaag P. 1970. Leven en werken van een 18e-eeuws hoogleraar in de geneeskunde. Wouter Van Doeveren. medicus, 1730-1783. Assen: van Gorcum & comp.
32. Hagner M. 1999. Enlightened monsters. The sciences in enlightened europe. Chicago: University of Chicago Press.
33. Wijnpersse D, Brugmans SJ. 1785. Musei Doevereniani catalogus. Leiden: S. & J. Luchtmans Johannes.
34. Urioste M, Rosa A. 1998. Anencephaly and faciocranioschisis: Evidence of complete failure of closure 3 of the neural tube in humans. American Journal of Medical Genetics. 75:4–6.
35. Oostra RJ, Baljet B, Hennekam RC. 1998. Congenital anomalies in the teratological collection of museum vrolik in Amsterdam, The Netherlands. IV: Closure defects of the neural tube. American Journal of Medical Genetics. 80:60–73.
36. Bonn A. 1763. Specimen anatomico-medicum inaugurale, de continuationibus membranarum. Leiden: Apud Georgium Wishoff.
37. Bonn A. 1782. Commentatio de humero luxato. Leiden: Apud S. et J. Luchtmans.
38. Bonn A. 1778. Verhandelingen over eenige voorname oorzaken van onvruchtbaarheid in vrouwen waargenomen. Verh Zeeuws Genootsch Wetensch Vlissingen. 4:330–353.
39. Bonn A. 1782. Verhandelingen over eene bijzondere wanschapenheid der pisblaas en teedelen. Geneesk Natuur Huish Kabinet. 3:1–28.
40. Bonn A. 1791. Ontleed- en heelkundige aanmerkingen eene algemeene oorzaak van verschillende wanstaltigheden der roede en pisblaas. Verh Genootsch Bevord Heelkd Amsterdam. 1:147–204.

41. Bonn A. 1818. Het aangeboren blaasgebrek, en dat der roede eens bejaarden mans. Amsterdam: R. J. Berntrop.
42. Bonn A. 1794. Ontleedkundige beschrijving en aanmerkingen over het maaksel en de voeding ener zeldzame en wanstaltige menschelijke vrucht. Verh Genootsch Bevord Heelkd Amsterdam. 3:123–194.
43. Schinzel AA, Smith DW, Miller JR. 1979. Monozygotic twinning and structural defects. The Journal of Pediatrics. 95:921–930.
44. Oostra RJ, Baljet B, verbeeten B, Hennekam RC. 1998. Congenital anomalies in the teratological collection of museum vrolik in Amsterdam, The Netherlands. V: Conjoined and acardiac twins. American Journal of Medical Genetics. 80:74–89.
45. Jansma JR. 1919. Louis de Bils en de anatomie van zijn tijd. Doctoral thesis. Hoogeveen: C. Pet.
46. De Bils L. 1661. Specimina anatomica, cum clariss. Doctissimorumque virorum epistolis aliquot & testimoniis. Rotterdam: Interprete G. Buenio, Ex officinâ, Arnoldi Leers.
47. Rating B. 1933. Ueber eine ungewoehnliche gesichtsmissbildung bei anencephalie. Virchows Archiv Fur Pathologische Anatomie Und Physiologie Und Fur Klinische Medizin. 288:223–242.
48. Kadar A, Kleinstern G, Morsy M, Soreide E, Moran SL. 2016. Multiple enchondromas of the hand in children: Long-term follow-up of mean 15.4 years. J Pediatr Orthop. 38:543-548.
49. Sandifort E. 1763. Dissertatio anatomico-obstreticia de pelvi, ejusque in partu dilatation. Leiden: Apud P.v.d. Eyk et D. Vygh.
50. Sandifort E. 1778. Observationes Anatomico-Pathologicae vol. II. Leiden: Apud Eyk P. V. d., Vygh, D.
51. Sandifort E. 1781a. Observationes Anatomico-Pathologicae vol. IV. Leiden: Apud Eyk P. V. d., Vygh, D.
52. Sandifort E. 1781b. Eduardi Sandifort Icones herniae inguinalis congenitae. Leiden: Apud Eyk P. V. d., Vygh, D.
53. Sandifort E. 1784. Eduardi Sandifort Anatome infantis cerebro destituti. Leiden: Apud, S., Luchtmans, J., Eyk P. V. d., Vygh, D.
54. Sandifort G. 1824. Dissertatio medica inauguralis, de pleuritide. Lugduni Batavorum: apud viduam M Cyfveer J.
55. Sandifort G. 1820. Ontleedkundige beschrijving en beschouwing van twee hoofdeloze misgeboorten. Rapporten, VII, nr.17.
56. Sandifort G. 1823. Verhandeling omtrent twee hersenlooze misgeboorten. Rapporten, VII, nr.32.
57. Suringar G. 1824. Dissertatio medica inauguralis, de nisu formativo ejusque erroribus. Leiden: Apud H. W. Hazenberg juniorem.
58. Blumenbach J. 1781. Über den Bildungstrieb und das Zeugungsgeschäfte. Göttingen: Joh. Christian Dieterich.
59. Sandifort G. 1840. Beschouwing van eenige zeldzaam voorkomende misvormingen aan het hoofd van viervoetige dieren. Rapporten, XIII, nr.36.
60. Numan A. 1847. Bijdrage tot de ontleedkundige kennis der horens van het rund en hunne physiologische betrekking tot de geslachtswerkingen van dit dier. Rapporten, XV, Nr. 37.
61. Anonymous. 1849. Catalogus librorum cum medicorum, anatom., chirurg., artis obstetr., materiei medicae et chemiae, tum historiae natural., geogr., itin., litteraturae, historiae, item effigierum. Leiden: Per E.J. Brill, Academia Typographum.

The Present of Dutch Teratological Collections

4 Paleodysmorphology and Paleoteratology: Diagnosing and Interpreting Congenital Conditions of the Skeleton in Anthropological Contexts

This chapter has been published as:

Roelof-Jan Oostra, Lucas L. Boer and Alie E. van der Merwe.
Paleodysmorphology and Paleoteratology:
Diagnosing and Interpreting Congenital Conditions of the Skeleton
in Anthropological Contexts. 2016.
Clin Anat. 29:878-91. doi: 10.1002/ca.22769.

Abstract

Most congenital conditions have low prevalence, but collectively they occur in a few percent of all live births. Congenital conditions are rarely encountered in anthropological studies, not least because many of them have no obvious effect on the skeleton. Here, we discuss two groups of congenital conditions that specifically affect the skeleton, either qualitatively or quantitatively. Skeletal dysplasias (osteochondrodysplasias) interfere with the histological formation, growth and maturation of skeletal tissues leading to diminished postural length, but the building plan of the body is unaffected. Well-known skeletal dysplasias represented in the archeological record include osteogenesis imperfecta and achondroplasia. Dysostoses, in contrast, interfere with the building plan of the body, leading to e.g. missing or extra skeletal elements, but the histology of the skeletal tissues is unaffected. Dysostoses can concern the extremities (e.g., oligodactyly and polydactyly), the vertebral column (e.g., homeotic and meristic anomalies), or the craniofacial region. Conditions pertaining to the cranial sutures, i.e., craniosynostoses, can be either skeletal dysplasias or dysostoses. Congenital conditions that are not harmful to the individual are known as anatomical variations, several of which have a high and population-specific prevalence that could potentially make them useful for determining ethnic origins. In individual cases, specific congenital conditions could be determinative in establishing identity, provided that ante-mortem registration of those conditions was ensured.

Introduction

Congenital anomalies have intrigued mankind since the earliest times. Initially considered to result from divine intervention or maternal imagination, their true nature has been progressively unraveled since the late 17th century. Physical conditions are considered "congenital" if they result from a prenatally present cause. This does not necessarily imply that the conditions themselves are always apparent before or at birth. Conditions with an insidious onset can show their first symptoms in childhood, adolescence, or even adulthood. The collectively recorded prevalence of all congenital conditions is around 2.4% of live births. [1] However, there is a transitional region rather than a sharp boundary between normal morphology and congenital anomalies, which encompasses the so-called anatomical variations. Although they deviate from the default building plan of the human body, these variations are not significantly disadvantageous to the affected subject.

Congenital conditions in humans are studied by two overlapping disciplines: dysmorphology and teratology. Dysmorphology is a medical, mostly pediatric, discipline that focuses on the clinical diagnosis and symptomatology of physically apparent patterns of congenital anomalies, whereas teratology, a biological discipline, deals with the epidemiology and pathogenesis of congenital conditions. This review surveys what these two disciplines can offer to physical and forensic anthropologists in assessing skeletonized human remains presenting with congenital anomalies.

Congenital conditions: causes, distributions and archeological representation

The cause of a congenital condition can be endogenous (i.e., fetal), exogenous (i.e., maternal) or a combination of both, although in many cases no exact cause can be established. Endogenous causes include more than 5,000 presently known genetic conditions and many chromosomal anomalies (aneuploidies) and sporadic conditions with no clear-cut genetic involvement. Exogenous causes mainly comprise placental transmittable infections, intoxications and metabolic conditions that render the fetus either deprived of or overexposed to certain metabolites. Well-known examples of exogenously induced conditions are congenital syphilis and fetal alcohol syndrome.

Congenital conditions are not distributed equally over the global population. Depending on the causes, their prevalences can differ profoundly among population groups. For instance, certain genetic conditions are significantly more (or less) prevalent in geographically and/or culturally

isolated, hence inbred, communities in which ancestral mutations are preserved in subsequent generations and become part of a stagnant and increasingly homogeneous gene pool. This phenomenon is known as the "founder effect". Exogenous causes, especially maternal infections and intoxications, can also show community-specific prevalences in relation to health care provision and socioeconomic stratification.

Apart from the fact that pathology, whether acquired or congenital, can be difficult or even impossible to assess in skeletal remains, there are several reasons for the paucity of congenital conditions in the anthropological record. First, most congenital and genetic conditions are rare, with incidences well below 1 in 50,000. Moreover, a substantial portion of these, e.g., cardiovascular and genitourinary defects, cause no skeletal lesions. Secondly, most individuals with congenital anomalies, in particular the more severely affected ones, die in infancy or while they are juvenile. These age groups are underrepresented in the archaeological record. [2] Thirdly, several skeletal conditions such as skeletal dysplasias affect the histological architecture of bones, rendering them vulnerable to diagenetic processes. Finally, in some instances, seemingly congenital conditions can in fact have postnatal causes.

Several excellent papers have been published on the archeological presentation of certain congenital conditions, including neural tube closure defects, [3] Down syndrome [4] and orofacial clefts. [5] Leaving aside conditions with a predominantly extraskeletal focus, the remainder of this review will focus on the morphological characteristics of congenital conditions that directly and specifically concern the skeleton, qualitatively and/or quantitatively. These conditions are known, respectively, as skeletal dysplasias and dysostoses, of which more than 400 are presently known and categorized in accordance with their radiographic, biochemical and genetic characteristics. [6] Although most of these conditions are quite rare, their overall prevalence is around 2.3-7.6 per 10,000. [7] However, since mildly affected individuals often remain undiagnosed, the actual prevalence could be higher.

Skeletal dysplasias

Skeletal dysplasias (osteochondrodysplasias) mostly originate from genetic defects resulting in abnormal histological formation, growth and maturation of cartilaginous and/or osseous tissues. They usually affect most skeletal elements equally, leading to diminished postural length (dwarfism). Skeletal dysplasias are therefore a generalized qualitative disorder of the skeleton, but the building plan of the body, including four extremities and pentadactylous hands and feet, is unaffected.

Before the 1860s, children born with neonatally apparent skeletal dysplasias were considered to suffer from a congenital form of rickets, a common disease in those days, because of their shortened and often curved extremities. However, true congenital rickets is rare, with only 25 cases reported to date, [8] and is one of the very few skeletal dysplasias that may result from an exogenous cause (i.e., maternal vitamin D deficiency). The Dutch anatomist Willem Vrolik (1801–1863) was one of the first to consider an alternative diagnosis in a stillborn child with numerous congenital fractures (Fig. 1), which he considered to result from imperfect bone formation rather than rickets. [9] He named the condition "osteogenesis imperfecta." [10] Still known as such today, osteogenesis imperfecta (OI) is a genetic disease mainly caused by dominant mutations in genes encoding subunits of collagen type I, a structural protein crucial for the architecture of bone and various other mesenchymal tissues. Mutations in other genes involved in collagen metabolism are increasingly being recognized as causal in different OI types. [11] Occurring in 1 in 15–20,000 births, [12] OI is rare, yet it is one of the most common skeletal dysplasias. Its most important feature is the fragility of bones, leading to an increased tendency to fracture and to bow after healing. Shortening of the long bones, which is the main characteristic of most other skeletal dysplasias, occurs only secondarily in OI, i.e., when fractures fragment the diaphyses or destroy the growth plates. Depending on the severity of the condition and the mutations responsible, at least four different types are recognized, type II being lethal within the first two years of life while type I can have up to normal life expectancies. Several archaeological cases of what seems to be OI are known. These include mainly young adults with a mild phenotype, and a two year old child and a near-term fetus with presumably OI type II or III. [13] The latter is exceptional considering the frailty of fetal bones in general, and especially when affected by OI. Nevertheless, in none of the described cases could the diagnosis of OI be established with certainty, leaving room for differential diagnoses that include other skeletal dysplasias.

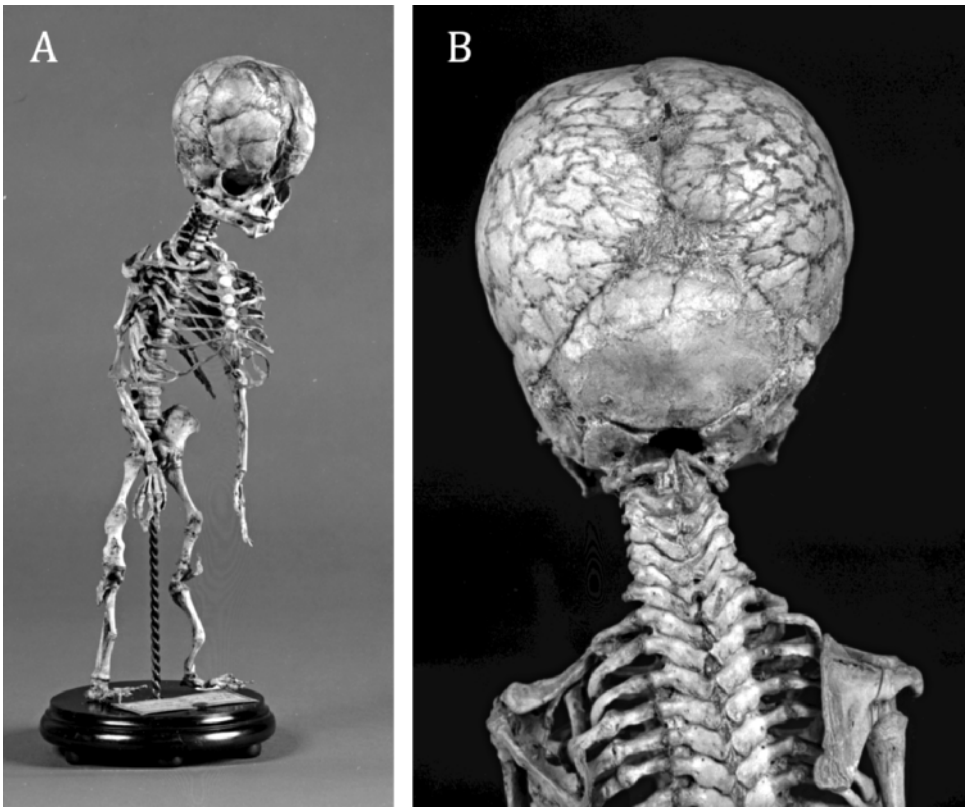


Fig. 1. Osteogenesis imperfecta type II. [A] Complete macerated skeleton, showing numerous fractures in all tubular bones and ribs. [B] Detail of the occipital part of the skull, showing many Wormian bones. Museum *Vrolik*, Amsterdam, The Netherlands. [9]

Similarly prevalent as OI is a condition known as achondroplasia, which occurs in 1 in 10,000 to 1 in 30,000 births. [14] Initially described by Parrot (1876), [15] it inappropriately became a generic name for any short-limbed skeletal dysplasia, even well after the advent of radiology, leading to much misdiagnosis. Achondroplasia is characterized by an average adult stature of 120–130 cm, rhizomelic shortening of the limbs (the upper arms and thighs being more affected than the forearms and legs), which is disproportionate to the shortening of the trunk, and macrocephaly with bulging forehead (Fig. 2). [16] Intellectual development and lifespan are usually within normal ranges. Other features include midfacial retrusion, exaggerated lumbar lordosis, limitation of elbow extension, genu varum, brachydactyly, and trident appearance of the hands. [17] The short stature results not only from the diminished length of the tubular bones in the lower extremities but also from the reduced height of the vertebral bodies, which is known as platyspondyly. This is found in most skeletal dysplasias that are characterized by short stature. Radiographically, the short and relatively thick tubular bones in achondroplasia show

metaphyseal flaring and cupping. [18] Typically, there is overgrowth of the fibula, which correlates strongly with the degree of genu varum. [19] Like many skeletal dysplasias, achondroplasia mainly affects enchondrally rather than intramembranously ossifying skeletal elements, hence the bulging forehead and midfacial retrusion. Achondroplasia is caused by mutations in a gene encoding a receptor of fibroblast growth factors (*FGFR3*), [20] the same dominant mutation being found in nearly all affected individuals. [21] However, since most of them are born to healthy, noncarrying parents, especially with increased paternal age, [22] it has long been assumed that this gene locus is a highly mutable "hotspot" in the human genome. Nevertheless, it appears that spermatogonial stem cells carrying the mutation have a proliferative advantage over non-mutated cells, thereby selectively increasing the number of mutated sperm cells. [23] As a result of the mutation, *FGFR3* can be activated without binding to fibroblast growth factors, [24] and since *FGFR3* normally inhibits cartilage proliferation, diminished diaphyseal growth ensues. [25]

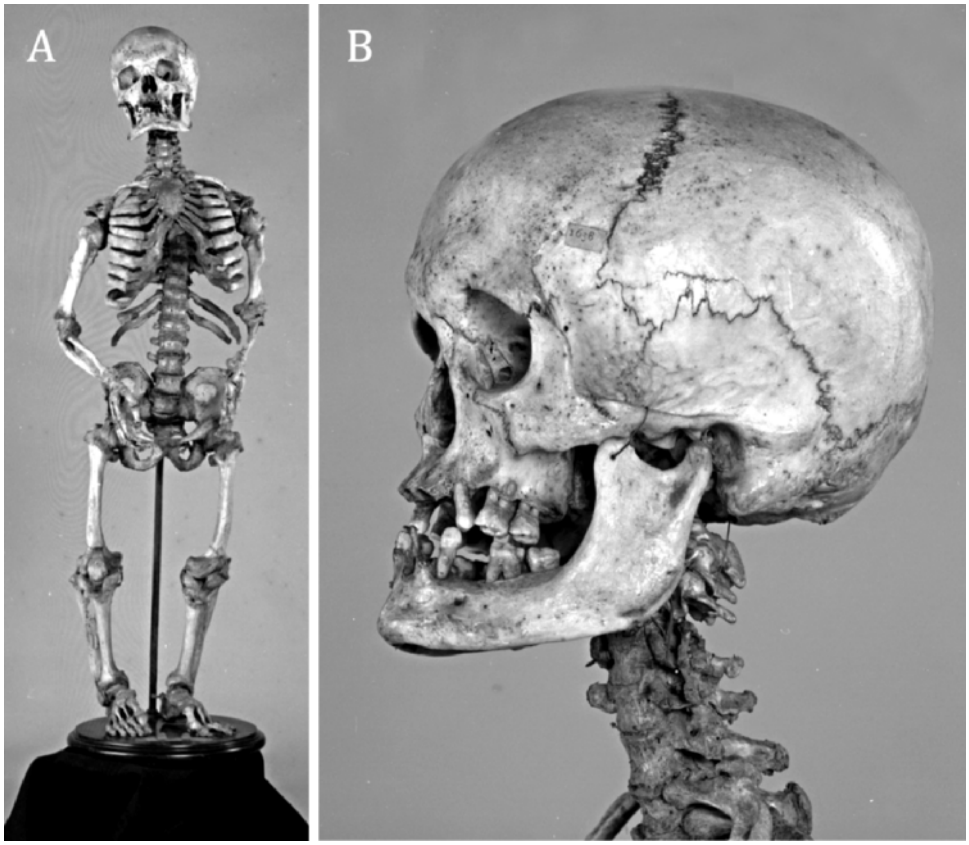


Fig. 2. Achondroplasia. [A] Complete macerated skeleton, showing rhizomelic shortening of the extremities. [B] Detail of the skull, showing relative macrocephaly and retracted cranial base. Museum *Vrolik*, Amsterdam, The Netherlands. [9]

Some other skeletal dysplasias are also caused by dominant germline mutations in *FGFR3*. They all resemble achondroplasia in their clinical and radiographic characteristics but they differ in severity. Intriguingly, the same mutations that repress cartilage proliferation can stimulate (malignant) proliferation of other tissues when they occur somatically, indicating that *FGFR* signaling dynamics are much more complex than originally assumed. [26-28] Hypochondroplasia is the mildest *FGFR3*-related skeletal dysplasia. Whereas achondroplasia is usually recognized at birth, hypochondroplasia can go unnoticed until early childhood and is one of the conditions that mainly accounts for initially undiagnosed skeletal dysplasias in idiopathic short stature. [29] As a result, the actual incidence and prevalence of hypochondroplasia are unknown, [30] but it is generally assumed that these numbers approach those of achondroplasia. Although its symptomatology is similar to but mostly milder than achondroplasia, it typically lacks the cranial dysmorphology, which was the key feature in differential diagnosis prior to the discovery of their molecular causes. [31,32]

The most severe, neonatally lethal skeletal dysplasias associated with *FGFR3* mutations are thanatophoric dysplasia (TD) types I and II. The incidence of TD has been investigated in different populations and seems to be equal to or somewhat less than that of achondroplasia, though it is likely that other lethal skeletal dysplasias are sometimes misdiagnosed as TD. [33-35] As with hypochondroplasia, TD resembles achondroplasia but the symptoms are much more severe. The lethality results from the ribs being extremely short—as are all other enchondrally ossifying bones—and this is accompanied by pulmonary hypoplasia, which leads to perinatal suffocation. A comparable clinical phenotype can occur in patients born to parents who are both affected with achondroplasia or hypochondroplasia, from whom they have inherited two mutated alleles. The two types of TD, each caused by specific *FGFR3* mutations, differ especially in the morphology of the femur, which is profoundly curved in TD type I and straight in type II (Fig. 3). Also, type II is often accompanied by premature closure of all calvarial sutures (cloverleaf skull). This indicates that *FGF* signaling is also involved in intramembranous ossification, which will be discussed further.

The occurrence of achondroplasia is relatively frequent in all times and all places, as reflected by its abundant representation in archaeological artifacts, especially in cultures with a positive appreciation of dwarfing conditions. [36-38] The oldest skeletal remains that have been diagnosed with achondroplasia are of two Egyptian adults—a 45- to 50-year-old male and a 25- to 30-year-old female—dated to the third millennium BCE. [39] Several other osteoarcheological cases of achondroplasia have been reported [40] but, to the best of our knowledge, none of hypochondroplasia or TD, which is surprising considering their equally frequent occurrence.

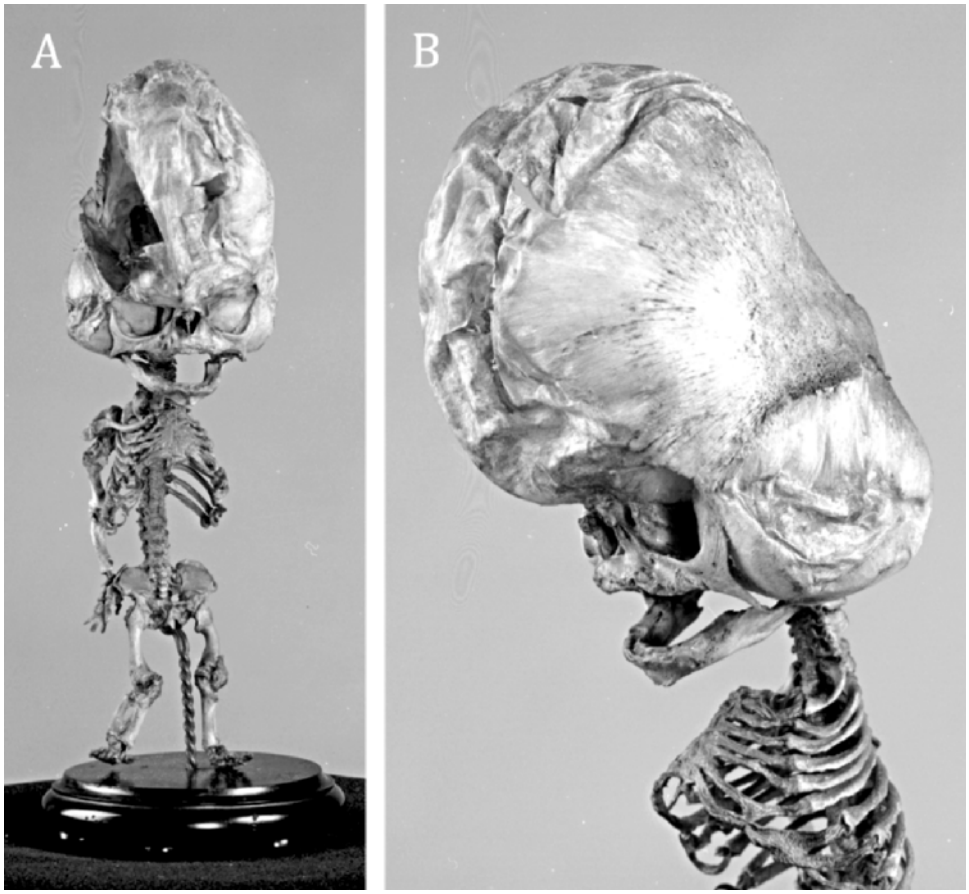


Fig. 3. Thanatophoric dysplasia type II. [A] Complete macerated skeleton, showing severe rhizomelic shortening of the extremities. [B] Detail of the skull, showing pansynostosis and bulging of the brain at the sites of the anterior and mastoid fontanelles. Museum Vrolik, Amsterdam, The Netherlands. [9]

Dysostoses

In contrast to skeletal dysplasias, dysostoses result from localized quantitative developmental disorders, with exogenous and endogenous causes that affect the building plan of the body, leading to e.g. missing or extra skeletal elements. However, the histology of skeletal tissues is unaffected. Dysostoses are usually categorized in terms of their effect on the building plan, which can involve an excess of elements (e.g. polydactyly), a shortage of elements (e.g. oligodactyly, phocomelia and peromelia) or a persistence of embryonic morphology (e.g. syndactyly). They can occur as solitary entities or as parts of complex conditions such as malformation syndromes and disruptions. They can also

co-occur with skeletal dysplasias. A well-known example of this is Ellis Van Creveld syndrome, a skeletal dysplasia characterized by short ribs, mesomelic shortening of the limbs, polydactyly, cleft palate and several other anomalies. Like most other recessively inherited conditions it is rare but its occurrence differs among populations as a result of the founder effect (see previously). Ellis Van Creveld syndrome is particularly prevalent among the Old Order Amish in Pennsylvania. [41]

Polydactyly—an excess of fingers and/or toes—is a relatively common dysostosis, occurring in 19 per 10,000 births. [42,43] They range from a barely visible pedunculated skin tag or a partially duplicated distal phalanx to multiple completely developed and articulated extra digits, which can occur unilaterally or bilaterally on the radial/tibial (preaxial) and/or ulnar/fibular (postaxial) side of the hands and feet or in their center (mesoaxial). Many categories can be recognized depending on the location of the extra elements, their extent, and the co-occurrence of other anomalies such as syndactyly. [42,44,45] More and more genes involved in hand and foot development are being recognized and mutations in them could cause a whole range of (syndromic) forms of polydactyly. [46] Many of these genes are implicated in the embryonic development of the anteroposterior (radio-ulnar) axis of the hand and foot. By far the most frequent type, either isolated, monogenetic or part of a syndrome, is postaxial polydactyly, which occurs in 6–15 per 10,000 births. [42] Remarkably, the incidence of this type is ten times higher in Negroid populations than Caucasoids, although this only concerns the pedunculated type. [47,48] Archaeological reports of polydactyly are scarce, despite the rather high incidence of polydactylous conditions. [49] This is no surprise with respect to the pedunculated type, which usually lacks osseous elements. However, completely formed extra digits can also go unnoticed if the investigated remains are disarticulated or arise from more than one individual. In fact, the most consistently recognizable types are those involving a bifurcated phalanx or an osseous branch attached to a metacarpal or metatarsal.

Oligodactyly, the lack of (parts of) fingers and/or toes, also is common dysostotic condition, ranging from shortness to complete absence of one or more digits, involving either the forearms or legs or both. The preaxial, postaxial and/or mesoaxial regions of the extremities can be affected. Conrad and Ezaki (2002) [50] reviewed the condition and recognized four categories, with incidences ranging from 1 in 10,000 to 1 in 100,000. Unless they are bilateral, most cases of oligodactyly occur sporadically and can result when an initially normal developmental process is disrupted. Such disruptions are considered to arise from e.g. necrosis caused by vascular malformations. Heritable forms of oligodactyly can show population-specific prevalences.

Explicit examples include the various reports on (large) kindreds presenting with "split hand-foot" syndrome (mesoaxial oligodactyly), including an African village inhabited by an "ostrich-footed" tribe. [51] As with polydactyly, and for similar reasons, oligodactyly is scarcely represented in the archeological record. Additionally, it can be difficult if not impossible to differentiate between congenital reductions of the digits and postnatal traumatic amputations. It is claimed that the pharaoh Tutankhamun (14th century BCE), who suffered from several, mostly acquired conditions, had a mild form of oligodactyly, manifest in the absence of the middle phalanx of his left second toe. [52]

The vertebral column can also be involved in dysostotic conditions that result in either an excess or a shortage of vertebrae—together known as meristic or numerical anomalies (see below)—or in a persisting embryonic morphology such as butterfly vertebrae, which results from notochordal remnants that interfere with the positions of ossification centers. [53] Other conditions result from an aberrant segmentation of the mesoderm that gives rise to the alternating pattern of vertebrae and intervertebral discs, leading to hemivertebrae, block vertebrae and other dyssegmentations. These conditions usually occur sporadically but can also be components of more complex (syndromic) conditions. A particular group of vertebral dysostoses result from aberrant expression of homeotic selector (*Hox*) genes along the anteroposterior body axis of the early developing embryo. On each transverse level a specific set of *Hox* genes is expressed that determines the phenotypic identity of the vertebra formed at that level. Alterations in the expression of *Hox* genes—resulting either from functional mutations in those genes or from longitudinal shifts in their expression patterns—will therefore cause phenotypic changes in the vertebrae affected. Typically, the phenotypic characteristics of these vertebrae resemble those of adjacent vertebrae. These changes, known as homeotic transformations, are best recognized at the level of regional transitions (i.e., occipitocervical, cervicothoracic, thoracolumbar, lumbosacral and sacrococcygeal) (Fig. 4). In anterior homeotic transformations (AHT), the affected level phenotypically resembles the level above it. An example of this is lumbar ribs, in which the first lumbar vertebra resembles the twelfth thoracic and hence features true ribs (thoracalization) (Figs. 5A and 5B). Comparably, cervical ribs are an example of a posterior homeotic transformation (PHT), because the seventh cervical vertebra resembles the first thoracic (Figs. 5C and 5D). Although the number of vertebrae does not change in homeotic transformations, it can be difficult or even impossible to differentiate them from meristic anomalies, [54] especially if the vertebral column cannot be completely retrieved. An isolated sacral bone consisting of six vertebrae, for example, could have resulted from

sacralization of either the first coccygeal (AHT) or the fifth lumbar (PHT) vertebra or from an extra vertebra. A block vertebra of the second and third cervical vertebrae, although generally resulting from either segmentation defects or from degenerative (hence acquired) conditions, can result from an anterior homeotic transformation that causes the third cervical vertebra to resemble an axis with the body of the second cervical vertebra as its dens. [54]

Homeotic and meristic anomalies of the vertebral column are remarkably common, with a reported prevalence as high as 17%. [55] Although they result from aberrations early in embryonic development, their direct clinical implications seem quite limited, except for cervical ribs, which can occasionally lead to thoracic outlet syndrome. However, they appear to be associated with both malignancies [56] and several congenital malformations, and have a prevalence of 75–80% in stillborn and therapeutically aborted fetuses. [57,58] This implies that either the *Hox* genes themselves—and their targets—or their upstream enhancers are more intricately involved in tissue and organ development and proliferation than has yet been established. In accordance with their high prevalence, homeotic and meristic anomalies are frequently encountered in archeological settings, often in combination with other vertebral anomalies. Barclay-Smith (1911) [59] described an AHT ranging from the atlanto-occipital down to the lumbosacral junction, together with several cleft neural arches, in the skeleton of a young Egyptian female dating from 500–600 BCE. An unusual case of meristic anomalies was described by Usher and Christensen (2000), [60] who found no fewer than three additional vertebrae in the skeleton of a 12th century Danish female.

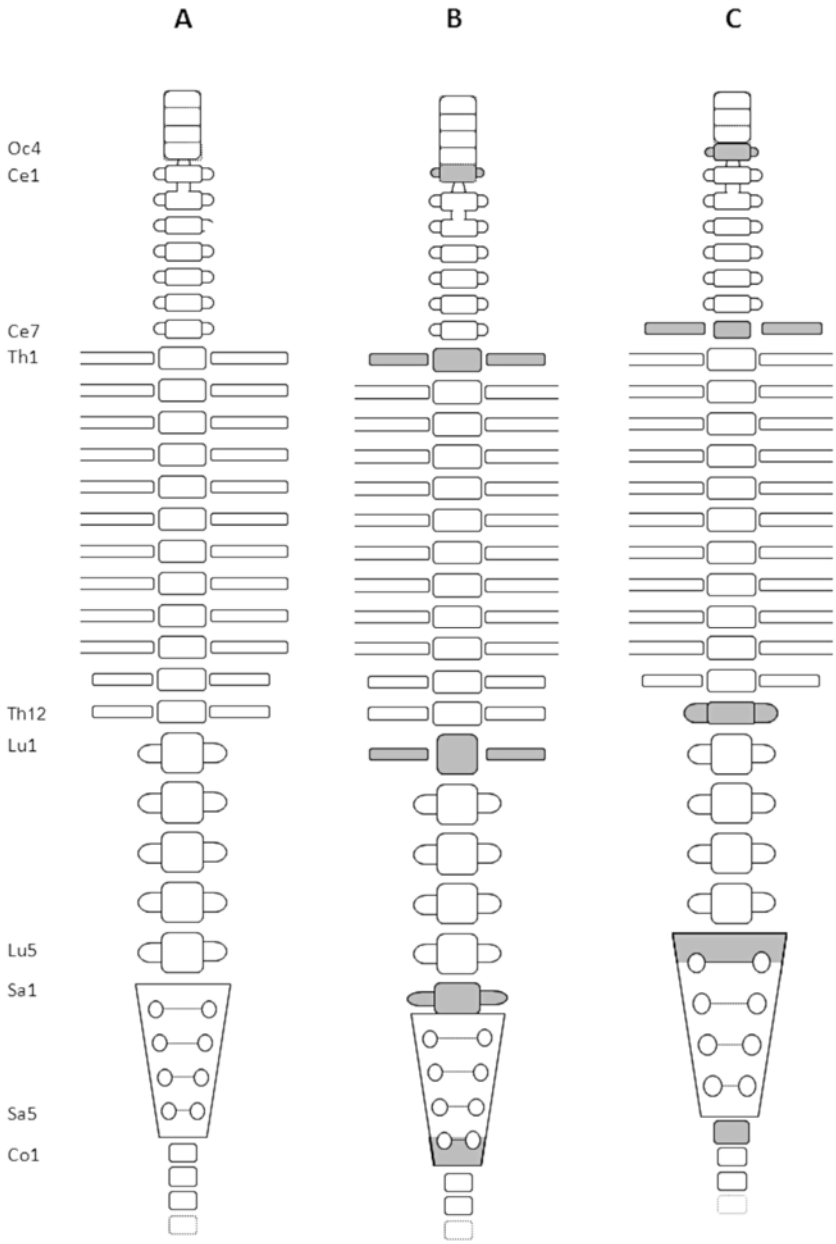


Fig. 4. Schematic representation of the interregional effects of homeotic transformations (in gray). [A] Normal situation. [B] Anterior transformations include: occipitalization of the first cervical vertebra (atlas assimilation), cervicalization of the first thoracic vertebra (hypoplastic first ribs), thoracalization of the first lumbar vertebra (lumbar ribs), lumbarization of the first sacral vertebra, and sacralization of the first coccygeal vertebra. [C] posterior transformations include: cervicalization of the last occipital segment (occipital vertebra), thoracalization of the seventh cervical vertebra (cervical ribs), lumbarization of the twelfth thoracic vertebra, sacralization of the fifth lumbar vertebra, and coccygealization of the fifth sacral vertebra. [54]

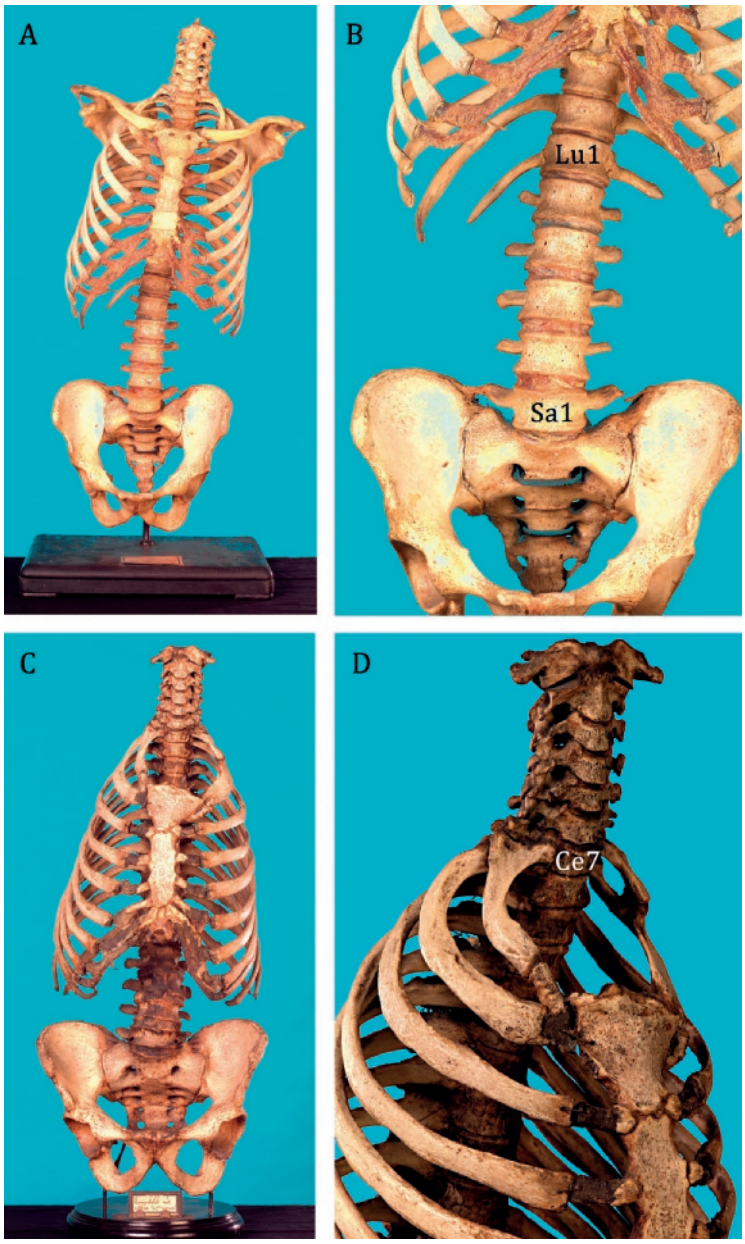


Fig. 5. Homeotic transformations. [A] Macerated trunk skeleton with multi-level anterior homeotic transformations. [B] Detail of the lower part of the vertebral column, showing thoracalization of the first lumbar vertebra (Lu1) and lumbarization of the first sacral vertebra (Sa1). [C] Macerated trunk skeleton with multi-level posterior homeotic transformations. [D] Detail of the upper part of the vertebral column, showing thoracalization of the seventh cervical vertebra (Ce7). Museum *Vrolik*, Amsterdam, The Netherlands. [54]

Cranial sutures in dysplasia and dysostoses

It is often less straightforward to recognize and distinguish between dysplasias and dysostoses in the craniofacial region than in the postcranial skeleton. For instance, facial and palatal clefts could be considered as dysostoses though they are generally not categorized as such, whereas hypoplasias such as mandibulofacial dysostosis usually are. Sutural disorders of the cranial vault, with or without concomitant macro- or microcephaly, can result from both dysplasias and dysostoses.

Unlike the postcranial skeleton, the bones of the cranial vault develop without a cartilaginous intermediate, i.e., intramembranously instead of enchondrally, and are therefore among the first osseous elements to be formed during late embryonic and early fetal life. The appearance of ossification centers is followed by the radial expansion of bone formation. Where the ossification fronts of adjacent calvaria meet, a type of fibrous joint called a cranial suture is formed. During subsequent pre- and post-natal development the proliferating mesenchyme of which these sutures consist serves as a source of new bone tissue, thereby allowing the cranial vault to expand in directions perpendicular to the sutures. This process ends as soon as the mesenchyme of (parts of) a suture ceases to proliferate and consequently ossifies. In most cranial sutures this will occur when the cranium has reached its final size. Physiological obliteration of cranial sutures is therefore age-related, but because the time-course of initiation, progression and completion of closure is variable, its value for estimating age is rather limited. By contrast, sutures between the bones of the facial cranium remain open throughout life. While most calvarial sutures start closing after the 3rd decade of life, some close much earlier, including the metopic suture between the two halves of the frontal bone, which normally closes during the first 3–9 postnatal months. [61] The various ossification centers in the squamous part of the occipital bone even coalesce during early fetal life. [62]

Abnormalities of sutural biology can be divided into premature closure, prolonged persistence and supernumerary ossification centers. In contrast to the latter two conditions, premature closure—known as craniosynostosis—leads to skull shape anomalies, especially in the cranial vault, that result from abolished growth across the closing suture and compensatory growth across the sutures that are still open (Fig. 6). Earlier onset generally results in more severe shape anomalies. Sagittal synostosis, the most common type of single-suture craniosynostosis, leads to a narrow but elongated skull (dolicho-, scapho- or clinocephaly, Figs. 6A–6C), whereas bicoronal synostosis results in a short but broadened skull (brachycephaly, Figs. 6D–6F).

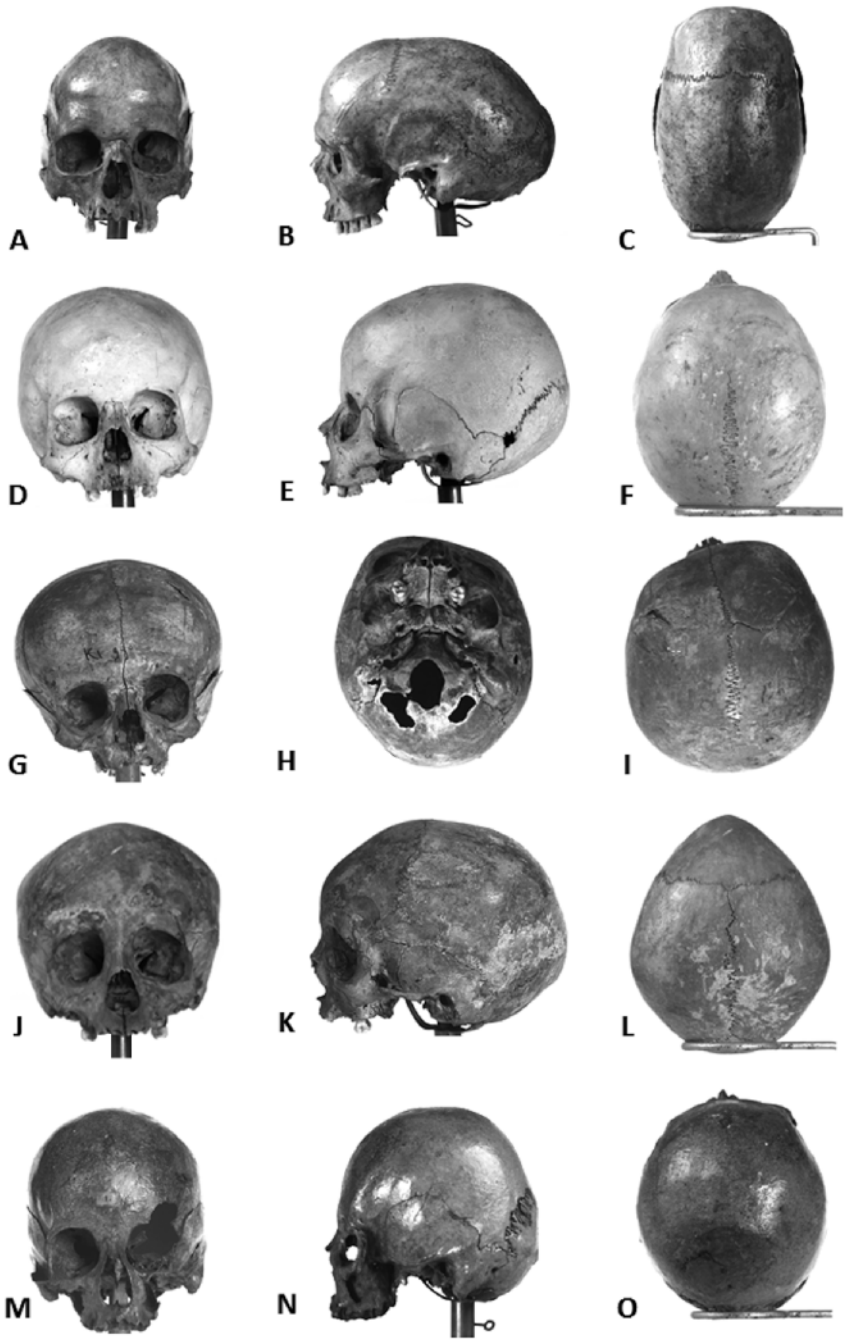


Fig. 6. Craniosynostoses. [A-C] sagittal synostosis (dolichocephaly); [D-F] bicoronal synostosis (brachycephaly); [G-I] unicoronal synostosis (plagiocephaly); [J-L] metopic synostosis (trigonocephaly); [M-O] sagittal and bicoronal synostosis (acrocephaly). Museum *Vrolik*, Amsterdam, The Netherlands. [68]

Asymmetry of the cranial vault can result from synostosis of one of the coronal or one of the lambdoid sutures, with compensatory growth across the still-open contralateral suture (plagiocephaly, Figs. 6G–6I). Closure of the metopic suture prior to birth results in a narrow, pointy forehead and compensatory widening across the sagittal suture, giving the cranial vault a triangular contour when seen from above. This condition is known as trigonocephaly (Figs. 6J–6L). If two or more sutures are involved, e.g. the coronal together with the sagittal or lambdoid sutures, a complex, more or less tower-shaped deformation results known as acro-, oxy-, turri- or hyps-encephaly (Figs. 6M–6O). This sometimes co occurs with closure of the squamosal suture, [63] which as an isolated condition is exceedingly rare. [64] If all calvarial sutures close prenatally, the growing brain can only expand at the sites of the anterior and mastoid fontanelles, resulting in a bizarre shape anomaly known as cloverleaf skull, with reference to its trilobed appearance (Fig. 3B). It should be noted that most shape anomalies described here can also result from other, non-suture-related conditions, most of which have a postnatal, exogenous cause. Well-known examples are the intentional skull deformations practiced by numerous cultures throughout history. Moreover, premature closure should be differentiated from early but adequate closure in response to cerebral growth arrest, in which case closure occurs secondarily and should not be considered a congenital or genetic condition per se.

Craniosynostoses involving one or more calvarial sutures can occur in isolation or as part of more complex congenital conditions (syndromes). Isolated craniosynostosis has an overall incidence of 1 in 2,000 and about half of all cases involve the sagittal suture. [65] For unknown reasons, sagittal synostosis is three times more frequent in males, [66] whereas coronal synostosis is two to three times more frequent in females. [67]

Nonsyndromic, single-suture conditions rarely have a genetic cause or familial occurrence, in contrast to bi- and multi-sutural and syndromic conditions, in which an increasing number of genes appear to be causally involved. [65] Conditions in the latter group, which have much lower incidences, include some dysplasias as well as dysostoses. In TD type II (see above) a severe metaphyseal dysplasia occurs together with pansynostosis, whereas in dysostotic syndromes such as Apert, Pfeiffer, Carpenter and Greig, multiple sutural synostoses are combined with mild to severe (poly)syndactylies. Intriguingly, there is not only a phenotypic but also a genetic overlap between dysplasias and dysostoses that feature craniosynostosis. Pathogenic mutations in e.g. *FGFR* genes are found in dysplasias, both with (e.g., TD type II) and without (e.g., achondroplasia) craniosynostosis, in dysostoses with craniosynostosis (e.g., Apert and Pfeiffer), and in craniosynostotic syndromes

without other skeletal involvement (e.g., Crouzon and Beare-Stevenson). The effect of craniosynostosis on the neurocognitive development of the patient depends on several factors, including the age at onset and the number of sutures involved. Intracranial pressure is significantly elevated even when only one suture is involved. [69]

In accordance with their relatively high incidences, isolated craniosynostoses are well represented in the archeological record, [63] in particular isolated sagittal synostosis. Pankowska *et al.* (2010) [70] described this condition in a more than 4,500 year old adult female skeleton. The oldest known case of craniosynostosis is that of a 500,000 years old *Homo heidelbergensis* child with a unilateral lambdoid synostosis, found at Atapuerca, Spain. [71] Syndromic craniosynostosis, which is rarely encountered in an archeological context, was described in a young adult 16th-19th century female from Siena, Italy, who was diagnosed with Crouzon syndrome. [72]

Rather than closing prematurely, sutures that should close at a certain age can remain open for longer or even throughout life. Partial or complete persistence of the metopic suture beyond the first year of life—known as metopism—is a well-known phenomenon that has been extensively studied in numerous dry bone collections, making this one of the very few congenital conditions that is better known from the archeological record and anatomical collections than from living individuals. Its prevalence ranges from 1% to 10% depending on the population investigated and the definitions applied, without consistent gender differences. [73] The condition itself is harmless but it can co-occur with other more serious affections including hydrocephalus, [74] craniosynostosis, [72] and basilar impression, [68] in which cases metopic persistence could allow compensatory expansion. It was argued in the past that metopism is accompanied by agenesis of the frontal sinuses, although several subsequent studies failed to confirm this. [75] The oldest known case of metopism, although diagnostically disputed, [76] concerns the 2.5 million year old skull of an *Australopithecus africanus* child aged 3–4 years and found near Taung, South-Africa. [77] This ignited the idea that persistence of the metopic suture well beyond birth, as in modern humans and apparently in earlier hominids but not in chimpanzees or gorillas (our closest living relatives), reflects prolonged growth of the frontal lobes and hence advanced cognitive development. [78]

Most variations in sutural morphology are found at the back of the skull, for example the occipital bone, which develops from several enchondral and intramembranous ossification centers. Four centers surrounding the foramen magnum give rise to the enchondrally ossifying basioccipital, left and right exoccipital, and squamous supraoccipital bones. [79] The remainder

of the squamous part—the interparietal bone—develops from ten paired intramembranous ossification centers, [62,80] although their number seems to vary. [81] Around the beginning of the fetal period the first two ossification centers, one on either side of the midline, appear above the superior margin of the supra-occipital bone, giving rise to the intermediate segment between the future superior and highest nuchal lines. [62] Subsequently, four sets of two centers each arise above the intermediate segment, giving rise to a lateral and a medial plate on either side of the midline. Normally, the borders between these centers and plates have disappeared by the end of the first trimester to form the interparietal bone *sensu stricto*, which in its lateral aspects is still separated from the intermediate segment. [62] These lateral fissures are usually obliterated by the end of the second year [79] but remnants can persist into adulthood. [82] This pattern varies when borders between ossification centers persist and subsequently give rise to additional sutures. A well-known example is the mendosal suture, which results from persistence of the fissure between the intermediate segment and the lateral plates. When the latter are normally fused with the medial plates a separate interparietal bone is formed. However, since any of the eight centers in the medial and lateral plates can either fuse or remain separate from the rest and/or from the intermediate segment, a vast number of bone and suture patterns results, as vividly illustrated by e.g., Srivastava (1992), [62] Hanihara and Ishida (2001), [83] and Thanapaisal *et al.* (2013). [80] Collectively, these variations are known as Inca bones (ossa incae), since they were first described in skulls originating from the indigenous population of Peru. [84] Indeed, the prevalence of ossification variations of the occipital bone differs markedly among populations worldwide, ranging from <1% to >10%, [80,83] but the incidence among South American natives has been reported as high as 27%. [85] Apart from the demographic differences, interparietal bones seem to be more frequent in patients with craniosynostoses. [86]

The variations in ossification patterns as described above, resulting in aberrant calvarial partitions, should be differentiated from Wormian bones, which are intrasutural (rather than intersutural) bones resulting from supernumerary (rather than generic) ossification centers. The presence of one or more Wormian bones, ranging in size from less than a millimeter up to several centimeters, is so common, with prevalences ranging from 55% to 80%, [87] that it seems inappropriate to consider their presence rather than their absence as an anatomical variation. Wormian bones occur even more often in combination with sutural conditions such as craniosynostosis [88] and metopism. [89] Since they are mostly found within the lambdoid suture, co-occurrence with Inca bones can lead to very complex patterns, especially

when the Wormian bones are formed not only in the lambdoid but also in the additional sutures. The fact that Wormian bones are associated with many other conditions, including skeletal dysplasias such as Osteogenesis imperfecta (Fig. 1B) and Hajdu-Cheney syndrome, and also with postural and intentional skull deformities, has led to the idea that their occurrence is triggered by mechanical stress on the forming bone tissue within the sutures in combination with an (epi)- genetic predisposition. [90] This suggests that in certain cases, Wormian bones touch the limits of what can still be called a congenital and/or inherited condition rather than an acquired one.

Conclusions and perspective

Most congenital conditions, especially those that are deleterious to the owner, have a low prevalence in extant populations and even more so in the anthropological record. Nevertheless, as a group they occur in a few percent of all live births and their occurrence should be anticipated. Although it is therefore important to recognize anomalies as deviant from normal development, detailed knowledge of all these conditions is of limited value in archeological and forensic practice, considering their rarity. Specific diagnoses can sometimes be made in collaboration with pathologists, radiologists, and geneticists who have specialized expertise in developmental osteology.

On the other hand, some anatomical variations pertaining to the skeleton have a high and population specific prevalence which, in selected situations, could make them useful for determining ethnic origins. In this respect, although outside the scope of this article, creating a database of anatomical variations and their specific occurrence and prevalence in populations and geographical areas would be helpful for physical anthropologists. In individual cases the presence of specific congenital conditions could be determinative in establishing identity, provided that ante mortem registration of these conditions was ensured.

Acknowledgments

We thank Dr. Laurens de Rooy for his help in providing the pictures of the specimens from Museum *Vrolik* in Amsterdam (The Netherlands).

References

1. Dolk H, Loane M, Garne E. 2010. The prevalence of congenital anomalies in Europe. Adv Exp Med Biol. 686:349–364.
2. Pinhasi R, Bourbou C. 2008. How representative are human skeletal assemblages for population analysis? In: Pinhasi R, Mays S, editors. Advances in Human Paleopathology. Chichester: Wiley. p 33–34.
3. Kumar A, Tubbs RS. 2011. Spina bifida: A diagnostic dilemma in paleopathology. Clin Anat. 24:19–33. Lajeunie E, Le Merrer M, Bonaiti-Pellie C, et al. 1995. Genetic study of nonsyndromic coronal craniosynostosis. Am J Med Genet. 55:500–504.
4. Rivollat M, Castex D, Hauret L, Tillier AM. 2014. Ancient Down syndrome: An osteological case from Saint-Jean des-Vignes, northeastern France, from the 5–6th century AD. Int J Paleopathol. 7:8–14.
5. Tur SS, Syvatko SV, Nechvaloda AI. 2016. Cleft lip case in a Middle Bronze Age young man from Altai, Russia. Int J Osteoarchaeol. 27:276-287.
6. Warman ML, Cormier-Daire V, Hall C, Krakow D, Lachman R, LeMerrer M, Mortier G, Mundlos S, Nishimura G, Rimoin DL, Robertson S, Savarirayan R, Sillence D, Spranger J, Unger S, Zabel B, Superti-Furga A. 2011. Nosology and classification of genetic skeletal disorders: 2010 revision. Am J Med Genet A. 155:943–968.
7. Panda A, Gamanagatti S, Jana M, Gupta AK. 2014. Skeletal dysplasias: A radiographic approach and review of common non-lethal skeletal dysplasias. World J Radiol. 6:808–825.
8. Paterson CR, Ayoub D. 2015. Congenital rickets due to vitamin D deficiency in the mothers. Clin Nutr. 34:793–798.
9. Oostra RJ, Baljet B, Dijkstra PF, Hennekam RC. 1998. Congenital anomalies in the teratological collection of Museum Vrolik in Amsterdam, The Netherlands. II. Skeletal dysplasias. Am J Med Genet. 77:116–134.
10. Vrolik W. 1849. Tabulae ad Illustrandam Embryogenesin Hominis et Mammalium tam Naturalem Quam Abnormem. Amsterdam: Londonck.
11. Shaker JL, Albert C, Fritz J, Harris G. 2015. Recent developments in osteogenesis imperfecta. 4:681.
12. Forlino A, Marini JC. 2016. Osteogenesis imperfecta. Lancet. 387:1657–1671.
13. Cope DJ and Dupras TL. 2011. Osteogenesis imperfecta in the archeological record: An example from the Dakhleh Oasis, Egypt. Int J Paleopathol. 1:188-199.
14. Horton WA, Hall JG, Hecht JT. 2007. Achondroplasia. Lancet. 370:162–172.
15. Parrot MJ. 1876. L'achondroplasie et les l_esions osseusses de la syphilis hereditaire et du rachitisme. Arch Physiol. 2:133–139.
16. Jones KL, Jones MC, Del Campo MD. 2013. Smith's Recognizable Patterns of Human Malformation, 7th ed. Philadelphia: WB Saunders.
17. Pauli RM, Legare JM. Achondroplasia. 1998. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2019. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1152/
18. Cheema JI, Grissom LE, Harcke HT. 2003. Radiographic characteristics of lower-extremity bowing in children. Radiographics. 23:871–880.
19. Lee ST, Song HR, Mahajan R, Makwana V, Suh SW, Lee SH. 2007. Development of genu varum in achondroplasia: Relation to fibular overgrowth. J Bone Joint Surg Br. 89:57–61.

20. Shiang R, Thompson LM, Zhu YZ, Church DM, Fielder TJ, Bocian M, Winokur ST, Wasmuth JJ. 1994. Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, a chondroplasia. Cell. 78:335–342.
21. Bellus G, Hefferon T, Ortiz de Luna R, Hecht J, Horton W, Machado M, Kaitila I, McIntosh I, Francomano C. 1995. Achondroplasia is defined by recurrent G380R mutations in FGFR3. Am J Hum Genet. 56:368–373.
22. Crow JF. 2000. The origins, patterns and implications of human spontaneous mutation. Nat Rev Gene. 1:40–47.
23. Shinde DN, Elmer DP, Calabrese P, Boulanger J, Arnheim N, Tiemann-Boege I. 2013. New evidence for positive selection helps explain the paternal age effect observed in achondroplasia. Hum Mol Genet. 22:4117–4126.
24. Webster MK, Donoghue DJ. 1996. Constitutive activation of fibroblast growth factor receptor 3 by the transmembrane domain point mutation found in achondroplasia. EMBO J. 15:520-7.
25. Deng C, Wynshaw-Boris A, Zhou F, Kuo A, Leder P. 1996. Fibroblast growth factor receptor 3 is a negative regulator of bone growth. Cell. 84:911–921.
26. Goriely A, Hansen RM, Taylor IB, Olesen IA, Jacobsen GK, McGowan SJ, Pfeifer SP, McVean GA, Rajpert-De Meyts E, Wilkie AO. 2009. Activating mutations in FGFR3 and HRAS reveal a shared genetic origin for congenital disorders and testicular tumors. Nat Genet. 41:1247–1252.
27. Foldynova-Trantirkova S, Wilcox WR, Krejci P. 2012. Sixteen years and counting: The current understanding of fibroblast growth factor receptor 3 (FGFR3) signaling in skeletal dysplasias. Hum Mutat. 33:29–41.
28. Krejci P. 2014. The paradox of FGFR3 signaling in skeletal dysplasia: Why chondrocytes growth arrest while other cells over proliferate. Mutat Res Rev Mutat Res. 759:40–48.
29. Flechtner I, Lambot-Juhan K, Teissier R, Colmenares A, Baujat G, Beltrand J, Ajaltouni Z, Pauwels C, Pinto G, Samara-Boustani D, Simon A, Thalassinos C, Le Merrer M, Cormier-Daire V, Polak M. 2014. Unexpected high frequency of skeletal dysplasia in idiopathic short stature and small for gestational age patients. Eur J Endocrinol. 170:677–684.
30. Wynne-Davies R, Walsh WK, Gormley J. 1981. Achondroplasia and hypochondroplasia. Clinical variation and spinal stenosis. J Bone Joint Surg Br B. 63:508–515.
31. Specht EE, Daentl DL. 1975. Hypochondroplasia. Clin Orthop Relat Res. 110:249–255.
32. Oberklaid F, Danks DM, Jensen F, Stace L, Rosshandler S. 1979. Achondroplasia and hypochondroplasia. Comments on frequency, mutation rate, and radiological features in skull and spine. J Med Genet. 16:140–146.
33. Donnelly DE, McConnell V, Paterson A, Morrison PJ. 2010. The prevalence of thanatophoric dysplasia and lethal osteogenesis imperfecta type II in Northern Ireland—A complete population study. Ulster Med J. 79:114–118.
34. Moffitt KB, Abiri OO, Scheuerle AE, Langlois PH. 2011. Descriptive epidemiology of selected heritable birth defects in Texas. Birth Defects Res a Clin Mol Teratol. 91:990–994.
35. Stevenson DA, Carey JC, Byrne JL, Srisukhumbowornchai S, Feldkamp ML. 2012. Analysis of skeletal dysplasias in the Utah population. Am J Med Genet A. 158:1046–1054.
36. Kozma C. 2006. Dwarfs in ancient Egypt. Am J Med Genet A. 140: 303–311.
37. Kozma C. 2008. Skeletal dysplasia in ancient Egypt. Am J Med Genet A. 146:3104–3112.
38. Rodriguez CA, Isaza C, Pachajoa H. 2012. Achondroplasia among ancient populations of Mesoamerica and South America: iconographic and archaeological evidence. Colomb Med (Cali). 43:212– 215.

39. Kozma C, Sarry El Din AM, El Shafy El Banna RA, El Samie Kandeel WA, Lachman R. 2011. Genetic drift. The ancient Egyptian dwarfs of the pyramids: The high official and the female worker. Am J Med Genet A. 155:1817-1824.
40. Woo EJ, Lee WJ, Hu KS, Hwang JJ. 2015. Paleopathological study of dwarfism-related skeletal dysplasia in a late Joseon dynasty (South Korean) population. PLoS One 10:e0140901.
41. McKusick VA, Egeland JA, Eldridge R, Krusen DE. 1964. Dwarfism in the Amish. I. The Ellis-van Creveld syndrome. Bull. Johns Hopkins Hosp. 115:307-336.
42. Castilla EE, Lugarinho da Fonseca R, da Graça Dutra M, Bermejo E, Cuevas L, Martínez Frías ML. 1996. Epidemiological analysis of rare polydactylies. Am J Med Genet. 65:295–303.
43. Castilla EE, Lugarinho R, da Graça Dutra M, Salgado LJ. 1998. Associatedanomalies in individuals with polydactyly. Am J Med Genet. 80:459–465.
44. Temtamy S, McKusick VA. 1969. Synopsis of hand malformations with particular emphasis on genetic factors. Birth Defects Orig Artic Ser. 5:125–184.
45. Lösch GM, Buck-Gramcko D, Cihak R, Schrader M, Seichert V. 1984. An attempt to Classify the malformations of the hand based on morphogenetic criteria. Chir Plastica. 8:1-18.
46. Biesecker LG. 2002. Polydactyly: How many disorders and how many genes? Am J Med Genet. 112:279–283.
47. Woolf CM, Myrianthopoulos NC. 1973. Polydactyly in American Negroes and Whites. Am J Hum Genet. 25:397-404.
48. Buck-Gramcko D. 1998. Congenital Malformations of the Hand and Forearm. London: Churchill-Livingstone.
49. Wrobel GD, Helmke C, Nash L, Awe JJ. 2012. Polydactyly and the Maya: A review and a case from the site of Peligroso, upper Macal river valley, Belize. Ancient Mesoamerica. 23:131–142.
50. Conrad M, Ezaki M. 2002. Fewer than 10: Oligodactyly-diagnoses and patterns of malformation. J Am Soc Surg Hand. J Hand Surg. 2:110–120.
51. Viljoen DL, Beighton P. 1984. The split-hand and split-foot anomaly in a central African Negro population. Am J Med Genet. 19:545–552.
52. Hawass Z, Gad YZ, Ismail S, Khairat R, Fathalla D, Hasan N, Ahmed A, Elleithy H, Ball M, Gaballah F, Wasef S, Fateen M, Amer H, Gostner P, Selim A, Zink A, Pusch CM. 2010. Ancestry and pathology in King Tutankhamun's family. JAMA. 303:638–647.
53. Postma AV, Alders M, Sylva M, Bilardo CM, Pajkrt E, van Rijn RR, Schulte-Merker S, Bulk S, Stefanovic S, Ilgun A, Barnett P, Mannens MM, Moorman AF, Oostra RJ, van Maarle MC. 2014. Mutations in the T (brachyury) gene cause a novel syndrome consisting of sacral agenesis, abnormal ossification of the vertebral bodies and a persistent notochordal canal. J Med Genet. 51:90–97.
54. Oostra RJ, Hennekam RC, de Rooij L, Moorman AF. 2005a. Malformations of the axial skeleton in Museum Vrolik I: homeotic transformations and numerical anomalies. Am J Med Genet A. 134:268–281.
55. Bornstein PE, Peterson RR. 1966. Numerical variation of the presacral vertebral column in three population groups in North America. Am J Phys Anthropol. 25:139–146
56. Merks JH, Smets AM, Van Rijn RR, Kobes J, Caron HN, Maas M Hennekam RC. 2005. Prevalence of rib anomalies in normal Caucasian children and childhood cancer patients. Eur J Med Genet. 48:113–129.
57. Ten Broek CM, Bakker AJ, Varela-Lasheras I, Bugiani M, Van Dongen S, Galis F. 2012. Evo-Devo of the human vertebral column: On homeotic transformations, pathologies and prenatal selection. Evol Biol. 39:456–471.

58. Castori M, Servadei F, Laino L, Pascolini G, Fabbri R, Cifani AE, Sforzolini GS, Silvestri E, Grammatico P. 2016. Axial skeletogenesis in human autosomal aneuploidies: A radiographic study of 145 second trimester fetuses. Am J Med Genet Part A. 170:676–687.
59. Barclay-Smith E. 1911. Multiple anomaly in a vertebral column. J Anat. 45:144–171.
60. Usher BM, Christensen MN. 2000. A sequential developmental field defect of the vertebrae, ribs, and sternum, in a young woman of the 12th century AD. Am J Phys Anthropol. 111:355–367.
61. Vu HL, Panchal J, Parker EE, Levine NS, Francel P. 2001. The timing of physiologic closure of the metopic suture: A review of 159 patients using reconstructed 3D CT scans of the craniofacial region. J Craniofac Surg. 12:527–532.
62. Srivastava HC. 1992. Ossification of the membranous portion of the squamous part of the occipital bone in man. J Anat. 180:219-224.
63. Duncan WN, Stojanowski CM. 2008. A case of squamosal craniosynostosis from the 16th century southeastern United States. Int J Osteoarcheol. 18:407–420.
64. Tandon YK, Rubin M, Kahlifa M, Doumit G, Naffaa L. 2014. Bilateral squamosal suture synostosis: A rare form of isolated craniosynostosis in Crouzon syndrome. World J Radiol. 6:507–510.
65. Kimonis V, Gold J-A, Hoffman TL, Panchal J, Boyadjiev SA. 2007. Genetics of craniosynostosis. Semin Pediatr Neurol. 14:150–161.
66. Hunter A, Rudd N. 1976. Craniosynostosis. I. Sagittal synostosis: Its genetics and associated clinical findings in 214 patients who lacked involvement of the coronal suture. Teratology. 14:185–193.
67. Lajeunie E, Le Merrer M, Bonaiti-Pellie C, et al. 1995. Genetic study of nonsyndromic coronal craniosynostosis. Am J Med Genet. 55: 500–504.
68. Oostra RJ, van der Wolk S, Maas M, Hennekam RC. 2005b. Malformations of the axial skeleton in the museum Vrolik. II. Craniosynostoses and suture-related conditions. Am J Med Genet A. 136:327–342.
69. Thompson DN, Malcolm GP, Jones BM, et al. 1995. Intracranial pressure in single-suture craniosynostosis. Pediatr Neurosurg. 22:235–240.
70. Pankowská A, Peška J, Vrána J. 2010. Congenital cranial defect in a female from a Funnel Beaker Culture settlement burial in Náměšť na Hané (Czech Republic)—A case report. IANSA. 1:77–89.
71. Gracia A, Martínez-Lage JF, Arsuaga JL, Martínez I, Lorenzo C, Pérez-Espejo MA. 2010. The earliest evidence of true lambdoid craniosynostosis: The case of "Benjamina", a Homo heidelbergensis child. Childs Nerv Syst. 26:723–727.
72. Giuffra V, Sbrana F, Caramella D, Giustini D, Tixier B, Fornaciari G. 2011. Syndromic craniosynostosis in a modern-age skeleton from Siena, Italy. J Craniofac Surg. 22:1743–1745.
73. Da Silva Ido N, Fernandes KJ, Ramalho AJ, Bispo RF, Rodrigues CF, Aragao JA. 2013. Occurrence of metopism in dry crania of adult brazilians. ISRN Anat. 158341.
74. Baaten PJ, Haddad M, Abi-Nader K, Abi-Ghosn A, Al-Kutoubi A, Jurjus AR. 2003. Incidence of metopism in the Lebanese population. Clin Anat. 16:148–151.
75. Marciniak R, Nizankowski C. 1959. Metopism and its correlation with the development of the frontal sinuses: A roentgen-anatomic study. Acta Radiol. 51:343–352.
76. Holloway RL, Broadfield DC, Carlson KJ. 2014. New high-resolution computed tomography data of the Taung partial cranium and endocast and their bearing on metopism and hominin brain evolution. Proc Natl Acad Sci USA. 111:13022–13027.
77. Hrdlicka A. 1925. The Taungs ape. Am J Phys Anthropol. 8:379–392.

78. Falk D, Zollikofer CP, Morimoto N, Ponce de Le_on MS. 2012. Metopic suture of Taung (Australopithecus africanus) and its implications for hominin brain evolution. Proc Natl Acad Sci USA. 109:8467–8470.
79. Shapiro R, Robinson F. 1976. Embryogenesis of the human occipital bone. Am J Roentgenol. 126:1063-8.
80. Thanapaisal C, Duangthongpon P, Kitkuandee A, Chaiciwamongkol K, Morthong V. 2013. Incidence and variation of interpretably bone (os incae) in northeastern Thailand. J Med Assoc Thai. 96:117–123.
81. Niida, S. Yamasaki, A. and Kodama, H. 1992. Interference with interparietal growth in the human skull by the tectum synoticum posterior. J. Anat., 180:197-200.
82. Tubbs RS, Salter EG, Oakes WJ. 2007. Does the mendosal suture exist in the adult? Clin Anat. 20:124–125.
83. Hanihara T, Ishida H. 2001. Os incae: Variation in frequency in major human population groups. J Anat.198:137–152.
84. Oetteking B. 1930. Craniology of the north pacific coast. Mem Am Mus Nat Hist: The Jessup North Pacific Expedition Vol. XI:1–391.
85. Garcia-Hernandez F, Murphy-Echeverria G. 2008. Frecuencia del hueso interparietal o incaico en craneos atacamenos (licanantai) prehispanicos del norte de chile. Int J Morphol.26:629–634.
86. Wu JK, Goodrich JT, Amadi CC, Miller T, Mulliken JB, Shanske AL. 2011. Interparietal bone (Os Incae) in craniosynostosis. Am J Med Genet A.155:287–294.
87. Cirpan S, Aksu F, Mas N. 2015. The incidence and topographic distribution of sutures including Wormian bones in human skulls. J Craniofac Surg. 26:1687–1690.
88. Sanchez-Lara PA, Graham JM Jr, Hing AV, Lee J, Cunningham M. 2007. The morphogenesis of wormian bones: A study of craniosynostosis and purposeful cranial deformation. Am J Med Genet A. 143:3243–3251.
89. Cirpan S, Aksu F, Mas N, Magden AO. 2016. Coexistence of Wormian bones with metopism, and vice versa, in adult skulls. J Craniofac Surg. 27:493–495.
90. Bellary SS, Steinberg A, Mirzayan N, Shirak M, Tubbs RS, Cohen-Gadol AA, Loukas M. 2013. Wormian bones: A review. Clin Anat. 26:922–927.

5 Sirenomelia: A Multi-Systemic Polytopic Field Defect with Ongoing Controversies

This chapter has been published as:

Lucas L. Boer, Eva Morava, Willemijn M. Klein,
Annelieke N. Schepens-Franke and Roelof-Jan Oostra.
Sirenomelia: a multi-systemic polytopic field defect with ongoing controversies. 2017.
Birth Defects Res. 109:791-804. doi: 10.1002/bdr2.1049.

Abstract

The most impressive phenotypical appearance of sirenomelia is the presence of a 180˚-rotated, axially positioned, single lower limb. Associated gastrointestinal and genitourinary anomalies are almost always present. This rare anomaly is still subject of ongoing controversies concerning its nosology, pathogenesis and possible genetic etiology. Sirenomelia can be part of a syndromic continuum, overlapping with other complex conditions including caudal dysgenesis and VATER/VACTERL/VACTERL-H associations, which could all be part of a heterogeneous spectrum, and originate from an early defect in blastogenesis. It is imaginable that different "primary field defects" whether or not genetically based, induce a spectrum of caudal malformations. In the current paper we review the contemporary hypotheses and conceptual approaches regarding the etiology and pathogenesis of sirenomelia, especially in the context of concomitant conditions. To expand on the latter we included the external and internal dysmorphology of one third trimester sirenomelic fetus from our anatomical museum collection, in which multiple concomitant but discordant anomalies were observed compared to classic sirenomelia and was diagnosed as VACTERL-H association with sirenomelia.

Introduction

The most obvious phenotypical characteristic in sirenomelia (also called: symmelia or mermaid syndrome) is the single axially positioned lower limb. However, sirenomelia can be seen as a (lethal) multi-system condition based on a polytopic field defect with a wide phenotypical variability and frequently observed co-occurring musculoskeletal, central nervous system, cardiopulmonary and other visceral anomalies in addition to the almost always present gastrointestinal and genitourinary anomalies. [1] Sirenomelia is still subject of debate due to its ongoing controversies regarding its etiopathogenesis. Sirenomelia shows a heterogeneous phenotypic variability and overlap with caudal regression syndrome/sacro-coccygeal dysgenesis, [2-4] small pelvic outlet syndrome, [5] VATER/VACTERL, [6-9] and VACTERL-H associations. [10] There have been several attempts to classify these receptacles of caudal malformations, unfortunately without satisfying results. Clearly, diagnostic overlap exists between these conditions and the interpretation of their etiopathogenetic origin. The purpose of this paper is to give an overview of multiple facets of this dysmorphological puzzle and review what is known about the co-occurrence of sirenomelia and other birth defects in particular those assigned to the VACTERL(-H) spectrum. The existing literature on these conditions is supplemented by one additional case of sirenomelia with a concomitant VACTERL-H association.

Sirenomelia

Sirenomelia can be seen as a rare polytopic and multi-systemic anomaly named in analogy of the single axial positioned lower limb present in the mythical creatures ‘sirens’ or ‘mermaids’ (Fig. 1). These hybrid creatures were depicted in Greek mythology as half woman / half fish and portrayed as enchanting singing creatures who lured sailors to death. [12] Although the mythical creature mermaid is more frequently depicted as a woman, the counterpart is the merman or Triton type sirenomelia, which is often depicted with a trident and a twisted con shell to calm or raise the waves (Fig. 2). That the mermaid is depicted more often than the merman is probably due to the more attractive nature of the female’s hair, eyes and bosom; thereby increasing its esthetic sensual attributes as the external genitalia were absent. [12] Sirenomelia intrigued mankind since the earliest times, as can be seen in a 2000-year-old found terracotta pottery (Fig. 3) of the pre-Colombian Tumaco-Tolita culture which clearly represented a case of sirenomelia. [13] In the mid-16th century, the first objective descriptions concerning sirenomelia appeared and were ascribed to Rocheus in 1542 and Palfyn in 1553. [14] In

the early 18th century sirenomelia was called Monopodia or Sympodia. [15] In the mid-19th century, Saint-Hilaire (1836) [16] and Forster (1865) [17] described cases of sirenomelia and named the three externally discernible variants of sirenomelia: 1) Symèles or Sympus dipus (presence of two feet), 2) Uromèles or Sympus monopus (presence of one foot) or 3) Syrénomèle or Sympus apus (no discernible foot). These essentially abandoned classifications focused on the degree of lower limb presence denoted by the presence of the feet. It can be stated that, in general, the degree of absent foot structures correlates with the severity of the anomaly. The currently used classification of sirenomelia is according to Stocker and Heifetz (1987), [3] who classified sirenomelia into 7 types based on the number of skeletal elements present in the lower limb. However, the classifications can be considered nothing more than discrete groups of a sirenomelic spectrum. [12] Additionally, reports exist in which sirenomelic fetuses do not fit in the Stocker and Heifetz classification. [15] This was due to the presence of dysmorphological ossified structures in the lower limb that did not correlated with one of the seven types. Moreover, efforts are reported to a proposed extended classification of sirenomelia that was based on measurements on the iliac-sacral distance, indicating the need for reviewing and elucidating the classification of sirenomelia. [18] An overview of the existing classifications of sirenomelia is given in Table I.

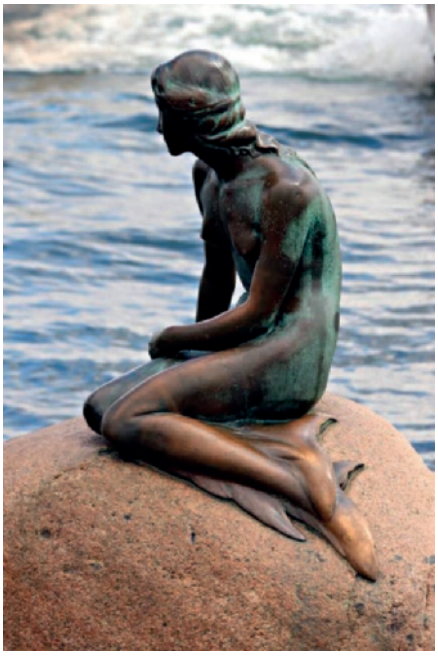


Fig. 1. The little mermaid in bronze by Edvard Erikson at the Langelinie promenade in Copenhagen, Denmark. From 1913 this statue is a Copenhagen icon and a major tourist attraction.

Fig. 2. Copperplate from the book: A General Treatise of the Dominion of the Sea: and a Compleat Body of the Sea-laws by T. Page, W. and F. Mount, 1724. Neptune wielding British flags at sea. In the right corner a triton or merman is depicted which is blowing on his conch to calm or raise the waves. [19]

Fig. 3. Terracotta pottery from the pre-Colombian Tumaco-Tolita culture which clearly represented a case of sirenomelia. [13]

Table I. Overview of the classifications of sirenomelia.

Saint-Hilaire (1837) and Forster (1865) classification based on the presence of the feet	
Type I	Symèles or Sympus dipus (presence of two feet)
Type II	Uromèles or Sympus monopus (presence of one foot)
Type III	Syrénomèle or Sympus apus (no discernible foot)
Stocker and Heifetz (1987) classification based on presence of ossified structures	
Type I	Presence of two separated femurs, two tibiae and two fibulae
Type II	Presence of two separated femurs, two tibiae and a medially fused fibula
Type III	Presence of two separated femurs, two tibiae and no fibula
Type IV	Presence of a partially fused femur, two tibiae and a medially fused fibula
Type V	Presence of a partially fused femur, two tibiae and no fibula
Type VI	Presence of a complete fused femur and a single fused tibia
Type VII	Presence of a complete fused femur
Kjaer *et al.* (2003) classification based on the iliac-sacral distance (ISD)	
Normal ISD	Separate ilia and femora
Mildly increased ISD	Fused ilia or femora
Greatly increased ISD	Single iliac and femur bones

* Note that the first two classifications are applied and propagated most widely and are often intertwined when describing sirenomelic fetuses. The 2003 classification is included for its completeness but is actually rarely used.

Familial recurrence and occurrence of sirenomelia

True recurrence of sirenomelia was reported in only one family with two out of five affected by sirenomelia. [20] These authors proposed evidence for a major dominant gene (with reduced penetrance) in familial caudal malformations. However, a comprehensive study from Orioli *et al.* (2011), [12] concerning 249 cases of sirenomelia reported no familial recurrence of sirenomelia. Familial occurrence of sirenomelia in one and urogenital and/or anorectal anomalies in other individuals has been described repeatedly. Selig *et al.* (1993) [21] reported a family where three siblings and a maternal aunt had renal anomalies consistent with renal dysplasia (renal agenesis, cystic dysplastic kidneys, and urethral valves). A fourth sibling had sirenomelia. Both parents had normal renal ultrasound examinations. Other reports concern monozygotic twins in which one of the twins was affected by sirenomelia and the other by imperforated anus. [22] Gerard *et al.* (2012) [23] described a mother who had undergone surgery for an imperforate anus and whose first pregnancy was terminated because of bilateral renal agenesis in the fetus. Her second pregnancy was complicated

by sirenomelia. In another family a firstborn child had imperforate anus, absence of S3-S5 and coccyx, abnormal pelvic floor and bifid bladder, whereas the second child was affected by sirenomelia. No diabetes was present during the pregnancies in either of these two families. Assimakopoulos *et al.* (2004) [24] described two cases of diabetic, dizygotic twin pregnancies, each with one normal and one affected fetus. In the first twin pregnancy the affected fetus had caudal regression syndrome, the other was normal. In the second twin pregnancy the affected fetus suffered from sirenomelia and the other twin showed no abnormalities. Additionally, Gabriele and Gianpaolo (2013) [1] reviewed the familial occurrence of sirenomelia and found 5 singleton related cases and nine twin related cases of caudal malformations. A paper from Rigon *et al.* (2013) [25] described sirenomelia followed by a caudal mesodermal defect in a sibling. These cases of familial occurrence of sirenomelia and other caudal dysgenesis-related conditions are suggestive of shared intrinsic (i.e. genetic) and/or extrinsic (i.e. teratogenic) etiopathogenetic elements.

Maternal diabetes and twinning in sirenomelia

The only maternal disease that is described to be associated with sirenomelia is diabetes mellitus. It is believed that one in five sirenomelic children are born to a diabetic mother. [26] However, Oriolli *et al.* (2011) [12] reported that it was impossible to confirm this relative high prevalence. Multiple reports are present that describe mothers with diabetes mellitus who gave birth to sirenomelic children. Both Gürakan *et al.* (1996) [27] and Lynch and Wright (1997) [28] reported sirenomelia in an infant born to a diabetic mother and stated that the association of sirenomelia and maternal diabetes is somewhat controversial and has not been firmly established. Moreover, Castori *et al.* (2010) [29] reported a diabetic mother who had three pregnancies, one sirenomelic child, one miscarriage, while the third was terminated for fetal malformations, diagnosed post-mortem as VACTERL association, indicating a possible causal relationship. Additionally, reports exist of caudal dysgenesis without maternal diabetes mellitus. [30] Gabriele and Gianpaolo (2013) [1] reported that sacro-coccygeal dysgenesis (caudal regression syndrome) is strongly associated with diabetes mellitus (21–28%) while imperforate anus, a constant finding in sirenomelia is a less common anomaly in caudal dysgenesis, even in its most severe form.

Multiple reports exist that correlate diabetes mellitus and caudal malformations and *vice versa*. Although the co-occurrence of maternal diabetes mellitus in sirenomelia and in other caudal malformations has not been firmly established it seems possible that at least cases of sirenomelia have an etiopathogenesis linked to maternal diabetes.

It is generally accepted that sirenomelia occurs more common in monozygotic twin pregnancies. [31] Escobar (1990) [32] reported that the frequency of sirenomelia is 8-10% higher in twinning and approximately 100-150 times higher in monozygotic twins in respect to dizygotic twins or singletons. Schinzel et al (1979) [33] reported that 7% of sirenomelic children were part of monozygotic twins and Thottungal *et al.* (2010) [34] reported an incidence of 11% of sirenomelia that were part of monozygotic twins. The possible correlation between sirenomelia and twinning has yet to be elucidated.

Prevalence, prenatal diagnosis and prognosis of sirenomelia

The birth prevalence of sirenomelia is estimated by Orioli *et al.* (2011) [12] to be 0.98 per 100.000 births. Due to the absence of external genitalia in sirenomelia and the often missing information on gonadal or chromosomal sex, data concerning the male-female ratio are very limited. Interestingly, Källén *et al.* (1992) [36] described an increased risk for sirenomelia under the maternal age of twenty and above the age of forty, however these finding were not statistically significant. A large epidemiologic study by Orioli *et al.* (2011) [12] noted an increased risk for sirenomelia with maternal ages less than 20. Strangely, the co-occurrence of maternal diabetes or twinning in sirenomelia are usually associated with higher maternal ages.

Sirenomelia can be diagnosed during prenatal screening by e.g. transvaginal or abdominal ultrasonography [36-38] and fetal MRI. [39] Often this (3D) prenatal ultrasonographical screening is hindered by the presence of severe oligohydramnios. [41] However, bladder and renal agenesis, lumbosacral defects and lower limb abnormalities are almost always present in sirenomelia and can be detected during screening. The earliest detection of sirenomelia was at 9 weeks (reviewed by Gabriele and Gianpaolo, 2013). [1] Thottungal (2010) [34] reported 5 out of 9 sirenomelic fetuses with normal amniotic fluid volumes due to the timing of prenatal screening and the dominance of maternal contribution to the amniotic fluid volume; a narrow window is present between gestational weeks 8 and 16 when the limb structures can be visualized with ultrasound and the amniotic fluid volume is still depending on maternal production. [12]

The overall prognosis of sirenomelia in terms of survival is very poor and mainly depends on the severity of the urogenital abnormalities and the resulting oligohydramnios and lung hypoplasia. [41] Only a few exceptional cases of surviving newborns have been reported. [42,31,43-46]

Teratogenically induced sirenomelia

Teratogenic drug induced sirenomelia in humans has only been scarcely reported. Sarpong and Headings (1992) [47] reported two cases of cocaine abuse during the first trimester of pregnancy, which supposedly led to vasoconstriction and sirenomelia. A study by Holmes (2002) [48] found no plausible teratogens which were associated with sirenomelia. However, Taghavi *et al.* (2009) [49] reported a case of sirenomelia after heavy use of snuff-tobacco. Orioli *et al.* (2011) [12] studied a large cohort of 249 sirenomelic fetuses and found only one case of possible teratogenic induced sirenomelia after misoprostol (an anti-peptic ulcer drug) use. Additionally, fever (>38° C) in the first trimester of pregnancy was reported in six cases of sirenomelia. Dosedla *et al.* (2012) [50] described sirenomelia after exposure of the antibiotic trimethoprim and Tica *et al.* (2013) [51] described a sirenomelic stillborn in a pregnant epileptic patient who used phenobarbital and carbamazepine during her pregnancy. A report by Cozzolino *et al.* (2016) [52] concluded that early administration of methylergonovine maleate, a drug to prevent and control of postpartum haemorrhage would cause sirenomelia. In addition to human studies, animal studies showed that sirenomelia could be induced by administration of a combination of cadmium and lead in the golden hamster, [53] ochratoxin A (a fungal toxin) in chickens, [54] and retinoic acid in rats. [55] Hence, multiple papers described teratogens causing sirenomelia. Nevertheless, teratogens are very discrete in their nature and determination of true teratogenic mechanisms remains complicated. Many potential mechanisms, pathogenetic routes and ultimate morphological outcomes are present which makes it very difficult to elucidate certain teratogens that induce congenital anomalies. [56,57] Although some studies apparently report a relationship between sirenomelia and certain drugs, these findings are statistically weak and can very well be coincidental findings.

Sirenomelia in chromosomal and monogenetic conditions

Multiple studies exist that report chromosomal and monogenetic alterations in sirenomelia. A study by Orr *et al.* (1982) [58] described craniofacial, caudal and also visceral anomalies in sirenomelic mice in which the *Srn* gene is considered to be responsible for causing sirenomelia. Abu-Abed *et al.* (2001) [59] reported one occurrence of sirenomelia in *Cyp26A1*-null mutant mice. Noteworthy is that the superfamily of *Cyp* genes are cytochrome oxidases regulating the metabolism of ingested chemicals and thus could be responsible for increased sensitivity to environmental toxins. *Cyp26A1* is the receptor of retinoic

acid. Malformations of the lower vertebral column have been reported in pregnancies exposed to high doses of retinoic acid at different gestational ages, linking sirenomelia and retinoic acid intoxication. [60] Moreover, Zakin *et al.* (2005) [61] found frequent occurrence of sirenomelia in *Tsg-/-; BMP7-/-* mice and frogs. This report also stated that *OTX2* expression, which is an anterior marker, was significantly increased in the forebrain of xenopus embryos that were inactivated for *Tsg* and *BMP7* by antisense MO oligonucleotides. Susuki *et al.* (2012) [63] reported a new mouse model with a *Isl1Cre;Bmp4(flox/flox)* condition that was associated with sirenomelia. Garrido-Allepuz *et al.* (2012) [63] reported that the removal of one or both functional alleles of the Sonic Hedgehog (*SHH*) gene leads to a sirenomelia phenotype in mice. Very few chromosomal conditions have been reported in human cases of sirenomelia. Kurosawa *et al.* (2012) [64] found a de novo balanced 46,X,t(X;16)(p11.23;p12.3) translocation. Gabriele and Gianpaola (2013) [1] described a triploidy mosaic sirenomelic fetus (69,XXX/46,XX) after karyotyping the chorionic villi. Evidently, more research is needed to correlate the reported genetic and chromosomal conditions with the aetiology and pathogenesis of sirenomelia.

Sirenomelia and its overlap with caudal dysgenesis

An often cited theory postulates that caudal dysgenesis and sirenomelia belong to the same heterogeneous spectrum. Caudal dysgenesis is hypothesized to arise from a primary deficiency of caudal mesoderm. [2,65] This theory is supported by many authors. [66,67,35,23,68,9,69,15,70] Dias and Walker (1992) [71] suggested that a teratogenic event occurred during gastrulating that interfered with the formation of the notochord, resulting in abnormally developed caudal structures and concomitant neural tube defects. A noteworthy article from Padmanabhan (1998), [55] presented the entire spectrum from caudal dysgenesis to sirenomelia in retinoic acid induced malformations in the rat, the severity of caudal malformations depending on dose and timing of drug administration. However, multiple papers exist that separate the two entities, and assert that sirenomelia and caudal dysgenesis are unrelated and have their own etiopathogenetic origin. Duncan *et al.* (1991) [72] showed that patient survival, twinning and the high incidence of maternal diabetes were arguments to support its differentiation. Twickler *et al.* (1993) [73] described sonographic differences and maternal specific characteristics to separate both entities. Perez-Aytes *et al.* (1997) [75] separated both entities based on differences in angiography and Bruce *et al.* (2009) [75] described the characteristics of 9 cases with caudal dysgenesis syndrome and 6 with sirenomelia and concluded that both anomalies share similar features

but are two different entities. This conclusion was based on comparison of the available clinical information, maternal follow-up, macroscopic and microscopic findings from autopsies, surgical pathology reports, radiographs and differences in placenta morphology. At present, opinions remain divided as to whether the two conditions are etiopathogenically related and if so to what extent.

Overview of etiopathogenetic mechanisms

Due to lack of knowledge about the exact etiology and the clinical heterogeneity of sirenomelia regarding concomitant disorders, there are currently many hypotheses concerning its ethiopathogenesis. [76-78,1,70] Below, we will describe the most cited ones and try to give an overview of the many existing hypotheses.

Bolk (1899) [79] postulated that sirenomelia was the result of a defect in the formation of the caudal somites and that the extension of the defect would depend on the number of absent somites. In 1904 Ballantyne [80] proposed that the absence of a tail bud led to the merge of the limb buds in sirenomelia. Russel et al (1981) [81] suggested the term 'axial mesodermal dysplasia' to describe a disturbance during early embryogenesis that affects the mesodermal cell migration during primitive streak period. Subsequently, Opitz and Gilbert (1982) [82] noted that the midline developmental field in early embryogenesis is a weakly buffered field, vulnerable to malformation. In 1987 Chandelbois and Brunet [84] reported an anterio-posterior elongation of the brain in a sirenomelic fetus with a concomitant overdeveloped nasal fossa, and hypothesized that the neural crest migration was accelerated and induced an overdevelopment of the rostral part and hypoplasia of the caudal parts. In the same year, Stocker and Heifetz (1987) [3] postulated that an 'embryologic insult' to the caudal mesoderm, occurring between 28 and 32 days of gestation, was responsible for causing sirenomelia. In 1989, O'Rahilly and Müller, [84] stated that sirenomelia occurred through a failure in lateralization that was secondary to a mesenchymal deficiency of the caudal development. Padmanabhan (1998) [55] suggested that a mechanical defect resulted from lateral compression by amniotic folds caused sirenomelia. Ohta *et al.* (2007) [85] stated that a defect in the formation of the primitive streak during late gastrulation could lead to caudal body malformations including part of the urogenital/reproductive organs as well as the hind limbs. The primitive streak contributes to the ventral ectodermal ridge (VER): a putative signaling center responsible for tail elongation by controlling cell proliferation in the underlying mesoderm. [86] Obviously, most of the theories cited here are merely conceptual conjectures without any supporting evidence. The most

propagated theory is the 'vascular steal hypothesis' which is based on the typical presence of a single aberrant umbilical artery in most cases of sirenomelia. This vessel is interpreted as a persistent vitelline artery that originates superiorly from the abdominal aorta. [14,87] This aberrant vessel would divert the blood away from the caudal parts of the developing embryo eventually resulting in underdevelopment or absence of caudal structures, as is the case in sirenomelia. In this perspective the previously mentioned study by Wei *et al.* (1996) [54] is relevant, which described that administration of ochratoxin A in chickens caused sirenomelia: this study underlines that the vascular steal hypotheses is unlikely to be the cause of sirenomelia; chickens lack placentas and the vitelline artery normally dominates the allantoic vascular supply. Heifetz (1984) [88] supported this theory when he observed a single umbilical artery in all of the 25 included sirenomelic fetuses. However, this vascular steal theory fails to explain the frequently associated anomalies, unrelated to the caudal area, that are observed in many sirenomelic fetuses. [89,77] Moreover, it seems that this single umbilical artery is not present in all cases and is not unique for sirenomelic fetuses. Multiple authors [90-93,34] described the presence of two umbilical arteries in combination with sirenomelia albeit of abnormal origin. Additionally, aberrant umbilical arteries have also been described in individuals with caudal dysgenesis [93,66] and persistent vitelline arteries in otherwise normal fetuses. [94] Perhaps the common finding of a single umbilical artery is a consequence of sirenomelia rather than its cause. Another often cited hypothesis is the 'defective blastogenesis hypothesis' which is correlated with the overall malformed caudal structures in sirenomelia and its relationship with defective development of the caudal somites and tailbud. [3] According to this theory, sirenomelia is a primary defect of blastogenesis that occurs during the final stages of gastrulation at the tailbud stage. [92] The phenotypical variability depends on the intensity and duration of the blastogenetic event. The vascular steal hypothesis and the deficient blastogenesis hypothesis do not exclude each other, it is conceivable that a defect in early blastogenesis would concomitantly affect (targeted) organs by ischemia and deficiencies in nutrients by aberrant vessel development in later embryological development. [77,15] Moreover, these pathophysiological hypotheses could be interrelated and constitute a similar etiopathogenetic continuum.

Presence of neural tube defects and holoprosencephaly

Surprisingly, about 10% of the sirenomelic fetuses show concomitant neural tube defects [34] including anencephaly, [95,96] anencephaly with cervicothoracic meningocele, [97] (myelo)meningocele, [98,43,99] craniorachisischis totalis [100-102] and spina bifida. [103,104] Browne *et al.* (2004) [105] described the first case of sirenomelia with an angiomatous lumbosacral myelocystocele. It is known that the caudal eminence contributes to the formation of neural primordial, lower limb buds, perineum, hindgut and blood vessels; [106] an extensive disturbance of the axial mesoderm would cause a concomitant alteration of neural ectoderm and subsequently a neural tube defect.

Multiple authors reported co-occurrence of sirenomelia and holoprosencephaly (HPE) in the same individual. [6,35] As mentioned before, Garrido-Allepuz *et al.* (2012) [63] reported that removal of one or both functional alleles of the Sonic Hedgehog (*SHH*) gene leads to sirenomelia in mice; *SHH* mutations are known to cause HPE. [107] Additionally, Shirani *et al.* (2006) [109] reported sirenomelia in combination with agenesis of the corpus callosum in a human fetus, which is a key feature of HPE. This would suggest an additional developmental defect of the pre-chordal plate mesoderm in these sirenomelic fetuses resulting in HPE. [104,109] Moreover, sirenomelia and HPE are reported to occur in the same geographical region, indicating a possible common etiology in these specific cases. [110] Indeed, both sirenomelia and HPE are severe anomalies that involve a defect in the median plane that appear through a failure of lateralization. [84] Opitz and Gilbert (1982) [82] postulated that the midline is a weak buffered field during early embryogenesis and is prone to a midline field defect.

Associated 'non classical' anomalies in sirenomelia

Many clinical case studies exist on the topic of sirenomelia. In the 167 found articles in a PUBMED search *(sirenomelia[Title/Abstract] AND hasabstract[text] AND "humans"[MeSH Terms])* many case studies reported the same spectrum of anomalies that we consider to be part of the classical sirenomelia sequence including a single midline lower limb, internal and external urogenital anomalies, large bowel (blind ending colon), pelvic, sacral and spinal defects, aberrant umbilical arteries and Potter facies due to oligohydramnios. It is beyond the scope of this review to include all these case studies. However, some papers are noteworthy because they report associated anomalies normally discordant in "classic" or "isolated" sirenomelia, they included a nefroblastoma, [111] neuroblastoma and gallbladder agenesis, [112] double

inferior vena cava, [113] pentalogy of Cantrell, [114] acardia, [115] dextrocardia, [116] situs inversus totalis, [117-119] limb-body wall complex, [120,121] and amniotic band disruptions sequence. [122] A comprehensive review from Källén *et al.* (1992) [35] reviewed 92 sirenomelic infants and found the following discordant anomalies: neural tube defects, hydrocephalus, cyclopia, cleft lip/palate, otocephaly, choanal atresia, oesophageal atresia, tracheal agenesis/atresia, malrotation of gut, gall bladder agenesis, persistent vitelline duct, cardiac septal defects (VSD, ASD), common arterial trunk, single ventricle, tricuspid atresia/single atrium, limb-bodywall complex, diaphragmatic hernia, prune belly, double uterus-vagina, polydactyly, radial defects, transverse arm reduction, simian creases, thoracic vertebral anomalies and absent lung lobe.

Another noteworthy study from Orioli *et al.* (2011) [12] reported associated anomalies in 249 sirenomelic fetuses which included: pre-axial polydactyly, lobster claw hands, webbed upper limbs (pterygium), radial aplasia/hypoplasia, upper and postaxial limb reductions, joint contractures, hydrocephalus, microcephaly, spina bifida, esophageal atresia/fistula, oral clefts, microtia, eye defects (microphthalmia), ocular hypertelorism, holoprosencephaly, abdominal wall defects (gastroschisis/omphalocele), diaphragm defects, anal and intestinal duplication, duodenal atresia, ectopia cordis, dextrocardia, tetralogy of Fallot, single ventricle, heart hypoplasia, persistent superior vena cava, bladder exstrophia, horseshoe kidney, hydronephrosis, spleen agenesis and lung lobulation defects. López-Valdez *et al.* (2013) [123] found hemifacial macrosomia and Bermejo *et al.* (2014) [124] found a sirenomelia with associated truncus arteriosus. The presence of many associated anomalies advocates that the etiopathogenesis of sirenomelia is complex and perhaps originates from multiple developmental field defects due to different genetic and/or environmental disturbances.

Sirenomelia and overlap with VATER/VACTERL(H) associations

The many concomitant anomalies in sirenomelia mentioned previously include some that are part of the so called VA(C)TER/VACTERL association. This association is a statistically non-random constellation and acronym that includes at least three of the following core abnormalities: costo-vertebral segmentation defects (V), anal atresia/stenosis (A), cardiovascular defects (C), tracheo-esophageal fistula and/or esophageal atresia (TE), renal anomalies (R) and, in the VACTERL association, (radial) limb anomalies (L). [125-128] Interestingly, the acronym is sometimes expanded to VACTERLS to include single umbilical artery. Moreover, the VACTERL association was extended by including hydrocephaly and designated as VACTERL-H association or

Briard Evans syndrome. [129,130] In contrast to the often sporadically occurring cases of VACTERL this VACTERL-H appears to be a hereditary entity with both autosomal recessive and X-linked forms. [130] In addition to its sporadic presentation, VACTERL association has been reported as part of syndromic conditions, including trisomy 18, Townes-Brocks syndrome and, particularly, Fanconi anemia. The prevalence of VACTERL(H) phenotypes in Fanconi anemia patients ranges from 5% to as much as 44%, depending on the mutated gene. [131-134] Multiple reports exist that describe VACTERL(-H) associations in combination with sirenomelia [10,67,29,9,15] and several more in which the concomitant conditions were not recognized as being part of the VACTERL(H) association (see previously). Intriguingly, disturbances in the *Shh* signaling pathway in mice causes a VACTERL-like phenotype, [135] in addition to its role in both sirenomelia and HPE (See previously). So far, gene mutations in infants with VACTERL-H associations and concomitant sirenomelia have not been reported.

External and internal (dys)morphological description of one additional sirenomelic specimen from our museological collection

Many anatomical museums around the world contain, maintain and exhibit third trimester fetuses with severe congenital anomalies. These museological accumulations of nature's capriciousness can be seen as irreplaceable treasure chests that provide a wealth of information for the study of rare, and mostly lethal, malformations beyond the scope of individual "clinical case studies." [136, 48] Additionally, these (predominantly) historical collections are exceptionally valuable because most specimens were collected before the ubiquitous availability and utilization of prenatal screening and therefore often presented well into the third trimester. Nowadays, these near full-term fetuses are a rare phenomenon in well-developed countries where prenatal screening is an important element in monitoring the progress and development of the unborn child during pregnancy. [137] Investigation of a museological specimen, like the one described here, can yield valuable contributions.

Our sirenomelic fetus was scanned with high-resolution radiological techniques [138] and comprehensively described according to modern dysmorphological insights. [139,140] A short external and internal dysmorphological description is summarized. This specimen concerns a full-term ambiguous sirenomelic fetus with a symmetric intrauterine growth restriction, a single axial positioned lower limb with a single foot and anteriorly faced sole, extensive hydrocephaly, pre-axial polydactyly and Potter's facies with hypertelorism, down slanting palpebral fissures, recessed

nose, microretrognatism and a low-set of ears. A pre-sacral opening was seen which could be interpreted as an imperforated anus (Fig. 4A). Radiological data revealed several skeletal anomalies, besides the distinctive features of sirenomelia, and included rib and vertebrae anomalies and polydactyly (Fig. 4B). The MRI data showed, besides the distinctive urogenital anomalies in sirenomelia, an extensive hydrocephalus. Moreover, visceral anomalies included a malrotation of the small intestine, a double superior vena cava, a large ventricular septal defect and a single umbilical artery (Fig. 4C). We diagnosed this case as VACTERL-H with concomitant sirenomelia type 2.

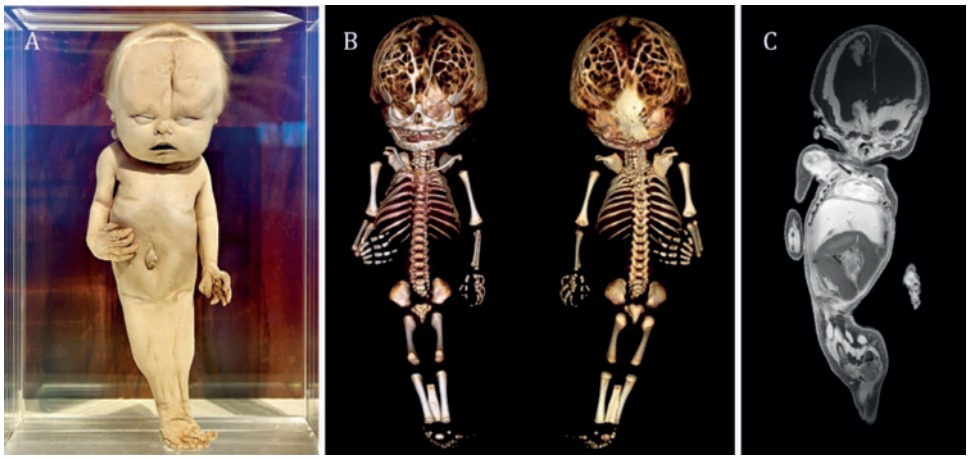


Fig. 4. [A] Photograph of the full term fetus. [B] Three-dimensional reconstructed skeletons based on the CT data showed an extensive hydrocephaly, multiple block-, hemi- and butterfly vertebrae in the cervical and thoracic region, fused ribs, cervical ribs and absence of the seventh cervical vertebra. The second and third sacral vertebrae were fused and the fourth and fifth sacral vertebrae were hypoplastic and dorsally oriented. The coccygeal vertebrae were completely absent. The left hand showed pre-axial polydactyly. The pelvic and lower limb region of the body showed the distinctive features of sirenomelia. Note: left reconstruction depicts a frontal view, the right reconstruction depicts a dorsal view. The partially translucent appearance of the skull is a digital artifact that arise at a certain threshold value that is used during three-dimensional reconstruction of CT data. [C] T1 weighted coronal MRI image showed an extensive hydrocephalus with an absent septum pellucidum and absent corpus callosum. A malrotation of the intestines with a blind ending dilated and elongated sigmoid was seen. The internal and external urogenital characteristics were completely absent. Moreover, a double superior vena cava, a large ventricular septal defect and a single right umbilical artery that inserted high in the abdominal aorta were detected.

Conclusions

Sirenomelia and the many observed caudal malformations, including VACTERL-(H) associations, in all its forms and shapes, with all its co-occurring anomalies, show a complex heterogeneous spectrum, that is not covered by any of the current pathogenetic theories. From a more meta-theoretic level, Opitz *et al.* (2002) [92] postulated that these anomalies arise as a result of a primary defect (gene mutation, environmental determinant or interaction of both) affecting multiple midline primordia in early blastogenesis onto final gastrulation. This primary defect results in so called "polytopic field defects". Opitz (1993) [141] defined developmental fields as morphogenetically reactive units present during early development that lead to a final structure that can be malformed in a similar manner albeit by different causes. Clinically therefore, a field is recognized when an identical malformation of a complex anatomical structure is proven to be caused by different factors. Thus, if one demonstrates heterogeneity in a pattern of anomaly then that pattern is a developmental field defect.

The observed phenotypic variability may depend on the intensity, time of initiation and duration of the underlying event. [142,77] Additionally, the final phenotype will be influenced on the disease modifying genes which can be divided into those uniquely affected by the primary mutation and those whose actions reflect generic responses to stress evoked by the principal mutation, then called intermediate phenotypes. [12]

It can be hypothesized that multiple "early developmental defects" such as vascular defects (vascular steal), defective blastogenesis and the many other stated hypotheses could all cause some sort of caudal malformation. Timing, intensity, duration and secondary induced effects of these pathogenetic events could cause a heterogeneous spectrum of different entities that include sirenomelia and VACTERL(H) phenotypes. It remains very difficult to point a specific cause to the origin of many caudal malformations. It is known that retinoic acid can induce an entire spectrum between sirenomelia and caudal dysgenesis, [55] however maternal diabetes seems to be a predisposition for the latter, reports exist that correlate maternal diabetes with sirenomelia (i.e. Orioli *et al.,* 2011). [12] It is imaginable that VACTERL with retinoic acid induced teratogenicity and presence of maternal diabetes can perhaps give some insight into a causal relationship. Another promising mechanism to further explore for its potential pathogenetic role is the *Shh* signaling pathway, which, at least in mouse models, seems to be involved in both sirenomelia and caudal dysgenesis as well as in VACTERL. Since each complex developing embryological structure involves concatenated networks, integrated

biological and molecular expertise is necessary to unravel these rare polytopic field defects in blastogenesis and their associated genetic mutations. It is therefore important to adequately perform clinical diagnostics and (prenatal) imaging to clarify these still complex patterns of (caudal) malformations and to get a clear overview of the complete morphological spectrum of associated conditions.

Acknowledgements

We thank Prof. Dirk Ruiter for his critical feedback on this article and thank medical student Mr. Tim Rijnhout for his pictorial support. Furthermore, we are greatly indebted to Drs. Sjaak van Asten for his technical support during radiological imaging and Drs. Dennis Bosboom and Drs. Karin Kamphuis-van Ulzen for their critical examination of the radiological images. The authors report no conflict of interest.

References

1. Gabriele T, Gianpaolo G. 2013. Sirenomelia: a review on embryogenic enviromental theories, novel three-dimensional ultrasound imaging and first trimester diagnosis in a case of mosaic 69,XXX/46,XX fetus. Arch Gynecol Obstet. 288:3-11.
2. Duhamel B. 1961. From the mermaid to anal imperforation: The syndrome of caudal regression. Arch Dis Child. 36:152–155.
3. Stocker JT, Heifetz SA. 1987. Sirenomelia. A morphological study of 33 cases and review of the literature. Perspect. Pediatr. Pathol. 10:7-50.
4. Adra A, Cordero D, Mejides A, Yasin S, Salman F, O'Sullivan MJ. 1994. Caudal regression syndrome: etiopathogenesis, prenatal diagnosis, and perinatal management. Obstet Gynecol Surv. 49:508-16.
5. Currarino G, Weinberg A. 1991. From small pelvic outlet syndrome to sirenomelia. Pediatr Pathol. 11:195-210.
6. Young ID, O'Reilly KM, Kendall CH. 1986. Etiological heterogeneity in sirenomelia. Pediatr Pathol. 5:31-43.
7. Schüler L, Salzano FM. 1994. Patterns in multimalformed babies and the question of the relationship between sirenomelia and VACTERL. Am J Med Genet. 49:29-35.
8. Jain D, Martel M, Reyes-Múgica M, Parkash V. 2002. Heterotopic nephrogenic rests in the colon and multiple congenital anomalies: possibly related association. Pediatr Dev Pathol. 5:587-91.
9. Moosa S, Lambie LA, Krause A. 2012. Sirenomelia: four further cases with discussion of associated upper limb defects. Clin Dysmorphol. 21:124-30.
10. Onyeije CI, Sherer DM, Handwerker S, Shah L. 1998. Prenatal diagnosis of sirenomelia with bilateral hydrocephalus: report of a previously undocumented form of VACTERL-H association. Am J Perinatol. 15:193-7.
11. Romano S, Esposito V, Fonda C, Russo A, Grassi R. 2005. Beyond the myth: the mermaid syndrome from Homerus to Andersen. A tribute to Hans Christian Andersen's bicentennial of birth. Eur J Radiol. 58:252-9.
12. Orioli IM. et al. 2011. Sirenomelia: An Epidemiologic Study in a Large Dataset From the International Clearinghouse of Birth Defects Surveillance and Research, and Literature Review. American journal of medical genetics. Part C, Seminars in medical genetics. 157C:358-73.
13. Pachajoa H, Rodriguez C. 2011. Possible case of sirenomelia in the Tumaco-Tolita pottery pre-Columbian culture, 2000 years before the epidemic focus of sirenomelia in Cali-Colombia. Am J Med Genet A. 155A:2327-8.
14. Kampmeier OF. 1927. On sireniform monsters, with a consideration of the causation and predominance of the male sex among them. Anat. Rec. 34:365-289.
15. Lhuaire M, Jestin A, Boulagnon C, Loock M, Doco-Fenzy M, Gaillard D, Diebold MD, Avisse C, Labrousse M. 2013. Sirenomelia: A new type, Showing VACTERL Association with thomas syndrome and a review of literature. Birth Defects Research Part A: Clinical and Molecular Teratology. 97:123–132.
16. Saint-Hilaire MI. 1836. Histoire Générale et Particulière des anomalies de l'Organisation chez l'homme et les Animaux, ou Traité de Tératologie. Bruxelles, Établissement encyclographique.

17. Forster A. 1861. Die missbildungen des Menschen systemtisch dargestellt. Jena Friedrich Mauke.
18. Kjaer KW, Keeling JW, Opitz JM, Gilbert-Barness E, Hartling U, Hansen BF, Kjaer I. 2003. Sirenomelia sequence according to the distance between the first sacral vertebra and the ilia. Am J Med Genet A. 120A:503-8.
19. A General Treatise of the Dominion of the Sea: and a Compleat Body of the Sea-laws by T. Page, W. and F. Mount, 1724.
20. Rudd NL, Klimek ML. 1990. Familial caudal dysgenesis: evidence for a major dominant gene. Clin Genet. 38:170-5.
21. Selig AM, Benacerraf B, Greene MF, Garber MF, Genest DR. 1993. Renal dysplasia, megalocystis, and sirenomelia in four siblings. Teratology. 47:65-71.
22. Akbiyik F, Balci S, Akkoyun I, Aktaş D, Cakmak O. 2000. Type 1 sirenomelia in one of male twins, with imperforate anus in the other male twin. Clin Dysmorphol. 9:227-9.
23. Gerard M, Layet V, Costa T, Roumazeilles Y, Chenal P, Cailliez D, Gerard B. 2012. Sirenomelia and caudal malformations in two families. Am J Med Genet A. 158A:1801-7.
24. Assimakopoulos E, Athanasiadis A, Zafrakas M, Dragoumis K, Bontis J. 2004. Caudal regression syndrome and sirenomelia in only one twin in two diabetic pregnancies. Clin Exp Obstet Gynecol. 31:151-3.
25. Rigon G, Vallone C, Silvestri E, Signore F. 2013. Short-term surviving sirenomelia neonate followed by a complex mesodermal malformation in a sibling. BMJ Case Rep. pii: bcr2012007941.
26. Al-Haggar M, Yahia S, Abdel-Hadi D, Grill F, Al Kaissi A. 2010. Sirenomelia (symelia apus) with Potter's syndrome in connection with gestational diabetes mellitus: a case report and literature review. Afr Health Sci. 10:395-9.
27. Gürakan B, Karaaslan E, Balci S. 1996. Sirenomelia in an infant of a diabetic mother. A case report. Turk J Pediatr. 38:393-7.
28. Lynch SA, Wright C. 1997. Sirenomelia, limb reduction defects, cardiovascular malformation, renal agenesis in an infant born to a diabetic mother. Clin Dysmorphol. 6:75-80.
29. Castori M, Silvestri E, Cappellacci S, Binni F, Sforzolini GS, Grammatico P. 2010. Sirenomelia and VACTERL association in the offspring of a woman with diabetes. Am J Med Genet A. 152A:1803-7.
30. Yeniel AÖ, Ergenoğlu AM, Sağol S. 2011. Prenatal diagnosis of caudal regression syndrome without maternal diabetes mellitus. J Turk Ger Gynecol Assoc. 12:186-8.
31. Murphy JJ, Fraser GC, Blair GK. 1992. Sirenomelia: case of the surviving mermaid. J Pediatr Surg. 27:1265-8.
32. Escobar LF, Weaver DD, Winn J. 1990. Sirenomelia sequence. In: Buyse ML (ed) Birth defects encyclopedia. Blackwell Scientific Publications, Cambridge, pp 1541–1542.
33. Schinzel AA, Smith DW, Miller JR. Monozygotic twinning and structural defects. J Pediatr. 95:921-30.
34. Thottungal AD, Charles AK, Dickinson JE, Bower C. 2010. Caudal dysgenesis and sirenomelia-single centre experience suggests common pathogenic basis. Am J Med Genet A. 152A:2578-87.
35. Källén B, Castilla EE, Lancaster PA, Mutchinick O, Knudsen LB, Martínez-Frías ML, Mastroiacovo P, Robert E. 1992. The cyclops and the mermaid: an epidemiological study of two types of rare malformation. J Med Genet. 29:30-5.

36. Sepulveda W, Romero R, Pryde PG, Wolfe HM, Addis JR, Cotton DB. 1994. Prenatal diagnosis of sirenomelus with color Doppler ultrasonography. Am J Obstet Gynecol. 170:1377-9.
37. Van Zalen-Sprock MM, van Vugt JM, van der Harten JJ, van Geijn HP. 1995. Early second-trimester diagnosis of sirenomelia. Prenat Diagn. 15:171-7.
38. Liu R, Chen XL, Yang XH, Ma HJ. 2015. Prenatal diagnosis of sirenomelia by two-dimensional and three-dimensional skeletal imaging ultrasound. J Huazhong Univ Sci Technolog Med Sci. 35:928-31.
39. Van Keirsbilck J, Cannie M, Robrechts C, de Ravel T, Dymarkowski S, Van den Bosch T, Van Schoubroeck D. 2006. First trimester diagnosis of sirenomelia. Prenat Diagn. 26:684-8.
40. Patel S, Suchet I. 2004. The role of color and power Doppler ultrasound in the prenatal diagnosis of sirenomelia. Ultrasound Obstet Gynecol. 24:684-91.
41. Lutz N, Meyrat BJ, Guignard JP, Hohlfeld J. 2004. Mermaid syndrome: virtually no hope for survival. Pediatr Surg Int. 20:559-61.
42. Guidera KJ, Raney E, Ogden JA, Highhouse M, Habal M. 1991. Caudal regression: a review of seven cases, including the mermaid syndrome. J Pediatr Orthop. 11:743-7.
43. McCoy MC, Chescheir NC, Kuller JA, Altman GC, Flannagan LM. 1994. A fetus with sirenomelia, omphalocele, and meningomyelocele, but normal kidneys. Teratology. 50:168-71.
44. Goodman SH, Ko DS, Johnson HW, Colemean GU, Murphy JJ, Fraser GC. 1996. Sirenomelia: urological abnormalities in a surviving mermaid. Can J Urol. 3:257-260.
45. Stanton MP, Penington EC, Hutson JM. 2003. A surviving infant with sirenomelia (Mermaid syndrome) associated with absent bladder. J Pediatr Surg. 38:1266-8.
46. Messineo A, Innocenti M, Gelli R, Pancani S, Lo Piccolo R, Martin A. 2006. Multidisciplinary surgical approach to a surviving infant with sirenomelia. Pediatrics. 118:e220-3.
47. Sarpong S, Headings V. 1992. Sirenomelia accompanying exposure of the embryo to cocaine. South Med J. 85:545-7.
48. Holmes MW, Hammond TT, Wogan GOU et al. 2016. Natural history collections as windows on evolutionary processes. Molecular Ecology. 25:864–881.
49. Taghavi MM, Jafari Naveh HR, Shariati M, Morteza Zade F. 2009. Sirenomelia (mermaid syndrome): an infant from parents who used a special form of snuff. Pak J Biol Sci. 12:722-5.
50. Dosedla E, Kalafusová M, Calda P. 2012. Sirenomelia apus after trimethoprim exposure: first-trimester ultrasound diagnosis-a case report. J Clin Ultrasound. 40:594-7.
51. Tica OS, Tica AA, Brailoiu CG, Cernea N, Tica VI. 2013. Sirenomelia after phenobarbital and carbamazepine therapy in pregnancy. Birth Defects Res A Clin Mol Teratol. 97:425-8.
52. Cozzolino M, Riviello C, Fichtel G, Tommaso MD. 2016. Exposure to methylergonovine maleate as a cause of sirenomelia. Birth Defects Research Part A: Clinical and Molecular Teratology. 106:643–647.
53. Hilbelink DR, Kaplan S. 1986. Sirenomelia: analysis in the cadmium- and lead-treated golden hamster. Teratog Carcinog Mutagen. 6:431-40.
54. Wei X, Sulik KK. 1996. Pathogenesis of caudal dysgenesis/sirenomelia induced by ochratoxin A in chick embryos. Teratology. 53:378-91.
55. Padmanabhan R. 1998. Retinoic acid-induced caudal regression syndrome in the mouse fetus. Reprod Toxicol. 12:139-51.
56. Alexander PG, Tuan RS. 2010. Role of environmental factors in axial skeletal dysmorphogenesis. Birth Defects Research Part C: Embryo Today: Reviews. 90:118–132.

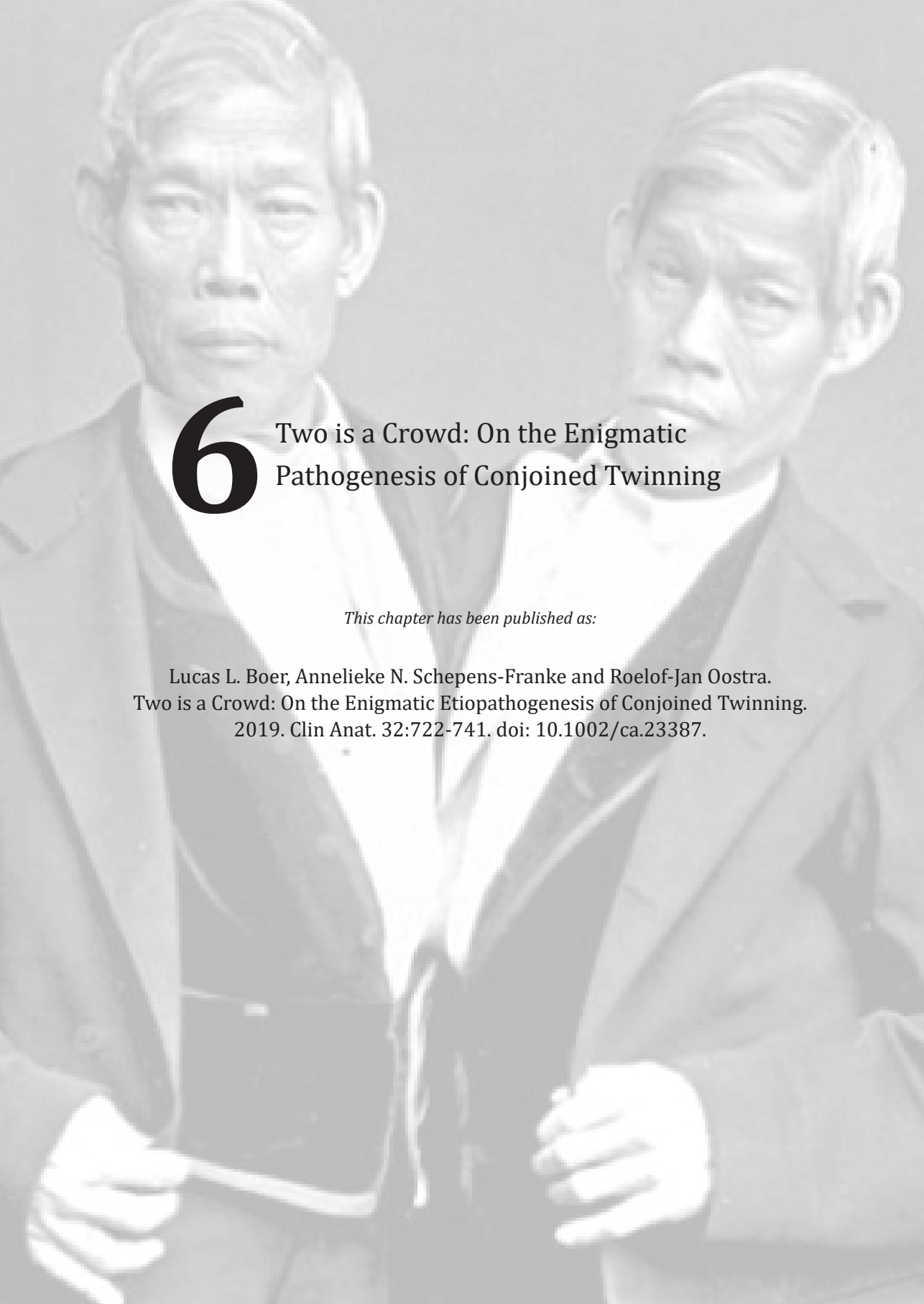
57. Alexander PG, Clark KL, Tuan, RS. 2016. Prenatal exposure to environmental factors and congenital limb defects. Birth Defect Res C. 108:243–273.
58. Orr BY, Long SY, Steffek AJ. 1982. Craniofacial, caudal, and visceral anomalies associated with mutant sirenomelic mice. Teratology. 26:311-7.
59. Abu-Abed S, Dollé P, Metzger D, Beckett B, Chambon P, Petkovich M. 2001. The retinoic acid-metabolizing enzyme, CYP26A1, is essential for normal hindbrain patterning, vertebral identity, and development of posterior structures. Genes Dev. 15:226-40.
60. Kessel M, Gruss P. 1991. Homeotic transformations of murine vertebrae and concomitant alteration of Hox codes induced by retinoic acid. Cell 4:89–104.
61. Zakin L, Reversade B, Kuroda H, Lyons KM, De Robertis EM. 2005. Sirenomelia in Bmp7 and Tsg compound mutant mice: requirement for Bmp signaling in the development of ventral posterior mesoderm. Development. 132:2489-99.
62. Suzuki K, Adachi Y, Numata T, Nakada S, Yanagita M, Nakagata N, Evans SM, Graf D, Economides A, Haraguchi R, Moon AM, Yamada G. 2012. Reduced BMP signaling results in hindlimb fusion with lethal pelvic/urogenital organ aplasia: a new mouse model of sirenomelia. PLoS One. 7:e43453.
63. Garrido-Allepuz C, González-Lamuño D, Ros MA. 2012. Sirenomelia phenotype in bmp7;shh compound mutants: a novel experimental model for studies of caudal body malformations. PLoS One. 7:e44962.
64. Kurosawa K, Tanoshima-Takei M, Yamamoto T, Ishikawa H, Masuno M, Tanaka Y, Yamanaka M. 2012. Sirenomelia with a de novo balanced translocation 46,X,t(X;16)(p11.23;p12.3). Congenit Anom (Kyoto). 52:106-10.
65. Alles AJ, Sulik KK. 1993. A review of caudal dysgenesis and its pathogenesis as illustrated in an animal model. Birth Defects Orig Artic Ser. 29:83-10.
66. Duesterhoeft SM, Ernst LM, Siebert JR, Kapur RP. 2007. Five cases of caudal regression with an aberrant abdominal umbilical artery: Further support for a caudal regression-sirenomelia spectrum. Am J Med Genet A. 143A:3175-84.
67. Charlier P, Valat AS, Boute O, Petit S, Chafiotte C, Huynh-Charlier I, Gosselin B, Devisme L. 2008. Sirenomelia as a part of VACTERL association: a study of three cases. Ann Pathol. 28:176-81.
68. Seidahmed MZ, Abdelbasit OB, Alhussein KA, Miqdad AM, Khalil MI, Salih MA. 2014. Sirenomelia and severe caudal regression syndrome. Saudi Med J. 35:36-43.
69. Das SP, Ojha N, Ganesh GS, Mohanty RN. 2013. Conjoined legs: Sirenomelia or caudal regression syndrome? Indian J Orthop. 47:413-6.
70. Kaygusuz EI, Eken MK, Sivrikoz ON, Cetiner H. 2016. Sirenomelia: a review of embryogenic theories and discussion of the differences from caudal regression syndrome. J Matern Fetal Neonatal Med. 29:949-53.
71. Dias MS, Walker ML. 1992. The embryogenesis of complex dysraphic malformations: a disorder of gastrulation? Pediatr Neurosurg. 18:229-53.
72. Duncan PA, Shapiro LR, Klein RM. 1991. Sacrococcygeal dysgenesis association. Am J Med Genet. 41:153-61.
73. Twickler D, Budorick N, Pretorius D, Grafe M, Currarino G. 1993. Caudal regression versus sirenomelia: sonographic clues. J Ultrasound Med. 12:323-30.
74. Perez-Aytes A, Montero L, Gomez J, Paya A. 1997. Single aberrant umbilical artery in a fetus with severe caudal defects: sirenomelia or caudal dysgenesis. Am J Med Genet. 14:409-12.

75. Bruce JH, Romaguera RL, Rodriguez MM, González-Quintero VH, Azouz EM. 2009. Caudal dysplasia syndrome and sirenomelia: are they part of a spectrum? Fetal Pediatr Pathol. 28:109-31.
76. Valenzano M, Paoletti R, Rossi A, Farinini D, Garlaschi E, Fulcheri N. V. 1999. Sirenomelia. Pathological features, antenatal ultrasonographic clues, and a review of current embryogenic theories. Human Reproduction Update. 5:82-6.
77. Garrido-Allepuz C, Haro E, González-Lamuño D, Martínez-Frías ML, Bertocchini F, Ros MA. 2011. A clinical and experimental overview of sirenomelia: insight into the mechanisms of congenital limb malformations. Dis Model Mech. 4:289-99.
78. Arvind Athavale S. 2012. Deciphering caudal embryonic defects: embryological analysis and reviewing literature data. Rom J Morphol Embryol. 53:1013-20.
79. Bolk L. 1899. De sympodie, een voorbeeld van pathologische segmentaal-anatomie. Geneesk Bladen. 6:301-335.
80. Ballantyne JW. 1904. Manual of antenatal pathology and Hygiene. Green, Edinburgh.
81. Russel LJ, Waever DD, Bull MJ. 1981. The axial mesodermal dysplasia. Pediatrics. 67:176–182.
82. Opitz JM, Gilbert EF. 1982. CNS anomalies and the midline as a "developmental field". Am J Med Genet. 12:443-55.
83. Chandebois R, Brunet C. 1987. Origin of abnormality in a human simelian foetus as elucidated by our knowledge of vertebrate development. Teratology. 36:11-22.
84. O'Rahilly R, Müller F. 1989. Interpretation of some median anomalies as illustrated by cyclopia and symmelia. Teratology. 40:409-21.
85. Ohta S, Suzuki K, Tachibana K, Tanaka H, Yamada G. 2007. Cessation of gastrulation is mediated by suppression of epithelial-mesenchymal transition at the ventral ectodermal ridge. Development. 134:4315-4324.
86. Gruneberg H. 1956. A ventral ectodermal ridge of the tail in mouse embryos. Nature. 177:787-8.
87. Stevenson RE, Jones KL, Phelan MC, Jones MC, Barr M Jr, Clericuzio C, Harley RA, Benirschke K. 1986. Vascular steal: the pathogenetic mechanism producing sirenomelia and associated defects of the viscera and soft tissues. Pediatrics. 78:451-7.
88. Heifetz SA. 1984. Single umbilical artery. A statistical analysis of 237 autopsy cases and review of the literature. Perspect Pediatr Pathol. 8:345-78.
89. Källén B, Winberg J. 1974. Caudal mesoderm pattern of anomalies: from renal agenesis to sirenomelia.Teratology. 9:99-111.
90. Kohler HG. 1972. An unusual case of sirenomelia. Teratology. 6:295–301.
91. Jaiyesimi F, Gomathinayagam T, Dixit A, Amer M. 1998. Sirenomelia without vitelline artery steal. Ann Saudi Med. 18:542-4.
92. Opitz JM, Zanni G, Reynolds JF Jr, Gilbert-Barness E. 2002. Defects of blastogenesis. Am J Med Genet. 115:269-86.
93. Hentschel J, Stierkorb E, Schneider G, Goedde S, Siemer S, Gortner L. 2006. Caudal regression sequence: vascular origin? J Perinatol. 26:445-7.
94. Gamzu R, Zalel Y, Jacobson JM, Screiber L, Achiron R. 2002. Type II single umbilical artery (persistent vitelline artery) in an otherwise normal fetus. Prenat Diagn. 22:1040-3.
95. Schwaibold H, Oehler U, Helpap B, Böhm N. 1986. Sirenomelia and anencephaly in one of dizygotic twins. Teratology. 34:243-7.
96. Rodríguez JI, Palacios J, Razquin S. 1991. Sirenomelia and anencephaly. Am J Med Genet. 39:25-7.

97. Pfeiffer RA, Becker V. 1988. Comments on Schwaibold's "Sirenomelia and Anencephaly in One of Dizygotic Twins".Teratology. 38:497-8.
98. Gupta R, Gupta M, Gupta S. 1992. Sirenomelia bipus (mermaid) with meningocele. Indian J Pediatr. 59:387-388.
99. Chen C, Shih SL, Jan SW, Lin YN. 1998. Sirenomelia with an uncommon osseous fusion associated with a neural tube defect. Pediatr Radiol. 28:293-6.
100. Battaglia S, Fraccaro M. 1954. Anencefalia in sirenide. Fol Hered Pathol III:197-204.
101. Rodríguez JI, Palacios J.1992. Craniorachischisis totalis and sirenomelia. Am J Med Genet. 43:732-6
102. Halder A, Pahi J, Chaddha V, Agarwal SS. 2001. Sirenomelia sequence associated with craniorachischisis totalis, limb reduction and primitive heart. Indian Pediatr. 38:1041-5.
103. Kulkarni ML, Sureshkumar C, Sindhur PS. 1994. Sirenomelia with spina bifida. Indian Pediatr. 31:51-5.
104. Chen CP, Shih SL, Liu FF, Jan SW. 1997. Cebocephaly, alobar holoprosencephaly, spina bifida, and sirenomelia in a stillbirth. J Med Genet. 34:252–255.
105. Browne M, Fitchev P, Adley B, Crawford SE. 2004. Sirenomelia with an angiomatous lumbosacral myelocystocele in a full-term infant. J Perinatol. 24:329-31.
106. Müller F, O'Rahilly R. 2004. The primitive streak, the caudal eminence and related structures in staged human embryos. Cells Tissues Organs. 177:2-20.
107. Roessler E, Belloni E, Gaudenz K, Jay P, Berta P, Scherer SW, Tsui LC, Muenke M. 1996. Mutations in the human Sonic Hedgehog gene cause holoprosencephaly. Nat Genet. 14:357-60.
108. Shirani S, Rekabi V, Kamalian N. 2006. Sirenomelia with agenesis of corpus callosum. Arch Iran Med. 9:269-70.
109. Martínez-Frías ML, García A, Bermejo E. 1998. Cyclopia and sirenomelia in a liveborn infant. J Med Genet. 35:263-4.
110. Castilla EE, Mastroiacovo P, López-Camelo JS, Saldarriaga W, Isaza C, Orioli IM. 2008. Sirenomelia and cyclopia cluster in Cali, Colombia. Am J Med Genet A. 146A:2626-36.
111. Kimura A, Kudo E, Hamamoto Y, Hirai H, Watanabe K. 1975. Sympus monopus accompanied by nephroblastoma-A case report. Acta Pathol Jpn. 25:375-84.
112. Drossou-Agakidou V, Xatzisevastou-Loukidou C, Soubasi V, Kostopoulou E, Laporda A, Pantzaki A, Agelidou S, Kremenopoulos G. 2004. Rare manifestations of sirenomelia syndrome: a report of five cases. Am J Perinatol. 21:395-401.
113. Goodlow OG, McCoy Sibley RI, Allen BG, Kamanda WS, Gullattee AC, Rayfield WC. 1988. Sirenomelia: Mermaid Syndrome. J Natl Med Assoc. 80:343-344, 345-346.
114. Egan JF, Petrikovsky BM, Vintzileos AM, Rodis JF, Campbell WM. 1993. Combined pentalogy of Cantrell and sirenomelia: a case report with speculation about a common etiology. Am J Perinatol. 10:327-9.
115. Zanforlin Filho SM, Guimarães Filho HA, Araujo Júnior E, Pires CR, Mattar R, Nardozza LM. 2007. Sirenomelia sequence: early prenatal diagnosis of one rare case associated with acardiac malformation. Arch Gynecol Obstet. 275:315-6.
116. Cavaliere A, Dinatale A, Cardinale G, Ermito S, La Galia T, Circosta B, Imbruglia L, Rapisarda G. 2009. A prenatally diagnosed case of sirenomelia with dextrocardia and omphalocele. J Prenat Med. 3:42-3.
117. Langer B , Stoll C, Nicolau R, Gasser B, Schlaeder G. 1996. Sirenomelia and Situs inversus: case report and review of the Literature. Fetal Diagn Ther. 11:79–84.

118. Sivridis E, Giatromanolaki A, Androulakis I, Hatzimichael A, Anastasiadis P. 2002. Sirenomelia in uneventful pregnancy. Clin Exp Obstet Gynecol. 29:140-2.
119. Rougemont AL, Bouron-Dal Soglio D, Désilets V, Jovanovic M, Perreault G, Laurier Oligny L,Fournet JC. 2008. Caudal dysgenesis, sirenomelia, and situs inversus totalis: a primitive defect in blastogenesis. Am J Med Genet A.146A:1470-6.
120. Tang TT, Oechler HW, Hinke DH, Segura AD, Franciosi RA. 1991. Limb body-wall complex in association with sirenomelia sequence. Am J Med Genet. 41:21-5.
121. Martínez-Frías ML, Cucalón F, Urioste M. 1992. New case of limb body-wall complex associated with sirenomelia sequence.Am J Med Genet. 44:583-5.
122. Managoli S, Chaturvedi P, Vilhekar KY, Iyenger J. 2003. Mermaid syndrome with amniotic band disruption. Indian J Pediatr. 70:105-7.
123. López-Valdez JA, Estrada-Juárez H, Moreno-Verduzco ER, Aguinaga-Ríos M. 2013. A fetus with hemifacial microsomia and sirenomelia. The same mesodermal defect spectrum? Fetal Pediatr Pathol. 32:152-7.
124. Bermejo R, Cartagena F, Valiente M, Mármol E, Acién P. 2014. Sirenomelia Associated withTruncus Arteriosus. J Clin Case Rep. S1:005.
125. Khoury MJ, Cordero JF, Greenberg F, James LM, Erickson JD. 1983. A population study of the VACTERL association: evidence for its etiologic heterogeneity. Pediatrics. 71:815-20.
126. Czeizel A. 1984. VACTERL-association. Acta Morphol Hung. 32:75-96.
127. Botto LD, Khoury MJ, Mastroiacovo P, Castilla EE, Moore CA, Skjaerven R, Mutchinick OM, Borman B, Cocchi G, Czeizel AE, Goujard J, Irgens LM, Lancaster PA, Martinez-Frias ML, Merlob P, Ruusinen A, Stoll C, Sumiyoshi Y. 1997. The spectrum of congenital anomalies of the VATER association: An international study. Am J Med Genet. 71:8–15.
128. Sözübir S, Güven F, Ozkamaci T, Yildiz F, Tugay M, Say A, Pektas OZ. 2000. Sirenomelia with esophageal atresia. Adv Clin Path. 4:165-8.
129. Briard ML, le Merrer M, Plauchu H, Dodinval P, Lambotte C, Moraine C, Serville F. Association of VACTERL and hydrocephalus: a new familial entity. 1984. Ann Genet. 27:220-3.
130. Evans JA, Stranc LC, Kaplan P, Hunter AG. 1989. VACTERL with hydrocephalus: Further delineation of the syndrome(s). Am J Med Genet. 34:177–182.
131. Alter BP, Rosenberg PS, Brody LC. 2007. Clinical and molecular features associated with biallelic mutations in FANCD1/BRCA2. J. Med. Genet. 44:1-9.
132. Savage SA, Ballew BJ, Giri N, Chandrasekharappa SC, Ameziane N, de Winter J, Alter BP. 2016. Novel FANCI mutations in Fanconi anemia with VACTERL association. Am J Med Genet A. 170A:386-91.
133. McCauley J, Masand N, McGowan R, Rajagopalan S, Hunter A, Michaud JL, Gibson K, Robertson J,Vaz F, Abbs S, Holden ST. 2011. X-linked VACTERL with hydrocephalus syndrome: further delineation of the phenotype caused by FANCB mutations. Am J Med Genet A. 155A:2370-80.
134. Mikat B, Roll C, Schindler D, Gembruch U, Klempert I, Buiting K, Bramswig NC, Wieczorek D. 2016. X-linked recessive VACTERL-H due to a mutation in FANCB in a preterm boy. Clin Dysmorphol. 25:73-6.
135. Kim JH, Kim PCW, Hui C. 2001. The VACTERL association: lessons from the Sonic hedgehog pathway. Clin. Genet. 59:306-315.
136. Suarez AV, Tsutsui ND. 2004. The value of museum collections for research and society. BioScience. 54:66–74.

137. Benson CB, Doubilet PM. 2014. The history of imaging in obstetrics. Radiology. 273(2 Suppl):S92 110.
138. Boer LL, Schepens-Franke AN, van Asten JJA, Bosboom DGH, Kamphuis-van Ulzen K, Kozicz TL, Ruiter DJ, Oostra RJ, Klein WM. 2017. Radiological imaging of teratological fetuses: what can we learn? Insights Imaging. 8:301-310.
139. Gorlin RJ, Cohen MM, Levin LS. 1990. Syndromes of the Head and Neck. New York: Oxford University Press.
140. Hall S. 2006. Human malformations and related anomalies. Second edition. New York: Oxford University Press.
141. Opitz JM. 1993. Blastogenesis and the 'primary field' in human development. BD:OAS vol. XXIX. New York: Alan R. Liss, Inc., for the National Foundation March of Dimes. p 3–37.
142. Kulkarni ML, Manaf KMA, Prasannakumar MG, Kulkarni PM. 2004. Sirenomelia with radial dysplasia. Indian J. Pediatr. 71:447–449.



6 Two is a Crowd: On the Enigmatic Pathogenesis of Conjoined Twinning

This chapter has been published as:

Lucas L. Boer, Annelieke N. Schepens-Franke and Roelof-Jan Oostra.
Two is a Crowd: On the Enigmatic Etiopathogenesis of Conjoined Twinning.
2019. Clin Anat. 32:722-741. doi: 10.1002/ca.23387.

Abstract

In this article, we provide a comprehensive overview of multiple facets in the puzzling genesis of symmetrical conjoined twins. The etiopathogenesis of conjoined twins remains matter for ongoing debate and is currently cited—in virtually every paper on conjoined twins—as partial fission or secondary fusion. Both theories could potentially be extrapolated from embryological adjustments exclusively seen in conjoined twins. Adoption of these, seemingly factual, theoretical proposals has (unconsciously) resulted in crystallized patterns of verbal and graphic representations concerning the enigmatic genesis of conjoined twins. Critical evaluation on their plausibility and solidity remains however largely absent. As it appears, both the fission and fusion theories cannot be applied to the full range of conjunction possibilities and thus remain matter for persistent inconclusiveness. We propose that initial duplication of axially located morphogenetic potent primordia could be the initiating factor in the genesis of ventrally, laterally, and caudally conjoined twins. The mutual position of two primordia results in neo-axial orientation and/or interaction aplasia. Both these embryological adjustments result in conjunction patterns that may seemingly appear as being caused by fission or fusion. However, as we will substantiate, neither fission nor fusion are the cause of most conjoined twinning types; rather what is interpreted as fission or fusion is actually the result of the twinning process itself. Furthermore, we will discuss the currently held views on the origin of conjoined twins and its commonly assumed etiological correlation with monozygotic twinning. Finally, considerations are presented which indicate that the dorsal conjunction group is etiologically and pathogenetically different from other symmetric conjoined twins. This leads us to propose that dorsally united twins could actually be caused by secondary fusion of two initially separate monozygotic twins. An additional reason for the ongoing etiopathogenetic debate on the genesis of conjoined twins is because different types of conjoined twins are classically placed in one overarching receptacle, which has hindered the quest for answers.

Introduction

The presence of ancient depicted cave drawings, carved figurines and ceramics of human conjoined twins, as well as their opulent occurrence in the animal kingdom, strongly suggest that these malformations existed long before the human race finished descending from its ancestors. [1-3] Initially, the birth of a conjoined twin was seen as an inauspicious sign of impending disaster. [4] This superstition-filled era was followed by a prolonged period through the Middle Ages and well into the nineteenth century when conjoined twins were regarded as freaks or monstrosities and were exhibited at circuses and sideshows with substantial financial reward. [5] Conjoined twins rarely survive early infancy—approximately 30% dies *in utero*, 40% to 60% are stillborn and 35% survives 1 day. [6,4] It is therefore that the birth and subsequent survival of a conjoined twin is matter for worldwide news, as they are seen as wonders and marvels of nature's creation or even perceived as incarnations of deities. [7,8] This wonderment on conjoined twins, which exists up to the present day, is of all times and all communities—eliciting strong emotions that vary between admiration to (maternal) rejection, repellence and hostility or even infanticide. [9-11]

Early academic interest in gross teratological conditions—including conjoined twins—predominantly flourished in Europe between the 18th and early 20th century: the heydays of descriptive teratology. [12] Between these pinnacles, prospectors who thought about possible etiopathogenetic causes were hampered by a lack of early (molecular) embryological knowledge, especially regarding the processes of (in)complete twinning. However, throughout multiple centuries the etiopathogenesis of conjoined twins has crystallized into two currently conjectured theories: partial fission [13] versus secondary fusion. [14] Although both theories are postulated throughout literature, controversies remain existing. [15] Both the fission and fusion theories have crystallized in patterns of paraphrased and graphic representations in virtually every paper concerning conjoined twins. [16] However, both theories show limitations, have overlapping dogmas and parlance—creating a potential susceptible situation for semantic interpretations. Most notably, the hypothetical deductions of these theories have (unconsciously) transited to allegedly verified factual embryological or (dys)morphological descriptions. An epistemic evolution changing an initial and tentative explanation or conceptualization into an accepted and undisputed theory is currently being observed. These "truths" should be critically reviewed and evaluated on their plausibility and (seeming) solidity.

In this article we provide a comprehensive overview of multiple facets in the puzzling genesis of symmetrical conjoined twins. We will discuss the currently held etiopathogenetic views on the origin of conjoined twins and its commonly assumed etiological correlation with monozygotic twinning. In addition, arguments are given which indicate that the dorsal conjunction group is etiologically and pathogenetically different in comparison to other symmetric conjoined twins and could be caused by secondary fusion.

Formation of early embryonic organizers

Due to the different approach in the possible genesis of conjoined twins presented in this paper, we feel the necessity to first highlight some basic considerations on the formation of early embryonic organizers. One highly regulative cell lineage during embryogenesis is the hypoblast (the anterior visceral endoderm (AVE) in the mouse) which controls epiblastic cell movements, ultimately leading to primitive streak formation and bilateral symmetry. [17] In normal development, an isolated central epiblast disk cannot generate axial structures in the absence of the hypoblast. [18] It has to be noted that particularly in ducks, about 2% of the fertilized eggs form conjoined twins. [19] The high prevalence of conjoined twins is assumed to be caused by orientation changes of the egg during critical periods of symmetrisation. These movements change direction of rotation subsequently resulting in the formation of two organizing centers. [20] Waddington (1933) [21] found that 90° rotation of the hypoblast prior to gastrulation in birds causes the orientation of the primitive streak to bend in the direction of the rotated hypoblast. The hypoblast transiently induces expression of pre-neural markers in the epiblast which contributes to delayed streak formation. [22] In the mouse, the AVE is essential for the correct positioning of the primitive streak: AVE imparts antero-posterior polarity and potency on the primitive streak. [23] In knockout mouse embryos where AVE cells arrest or fail to be induced, the primitive streak is ectopic or even duplicated; highlighting the pivotal role of the AVE in streak positioning and formation. [24,25]

Besides the hypoblast, another major structure in embryogenesis is the primitive streak which plays a key role in the formation of the axial and paraxial mesoderm and the definitive endoderm from the epiblast. [26] This structure will subsequently establish the whole fate map for ensuing embryological development initiating its ultimate morphology. [27] The highly regulated primitive streak formation relies on a critical and concatenated, network of mainly three signaling activities at both transcriptional and signaling levels: BMP4 signaling activates the Wnt pathway which in turn

activates the Activin–Nodal pathway. [28,29] Activation of secretion factors like Vg1, nodal, Wnt8C, FGF8 and chordin completed with transcription factors such as brachyury and goosecoid adjacent to the site of streak establishment are required for streak formation. [30] In addition, during gastrulation, the left-right asymmetry is established by complex genetic signaling pathways and cilia-mediated preferential flow at Hensen's node. This culminates in the exclusively left-sided expression of the *NODAL* gene—which is mediated by SHH—in the lateral plate mesoderm. [31]

Types of conjoined twins

The first discriminative in conjoined twins is the fact that some are symmetrical and others are not. The latter are characterized by gross underdevelopment of one of the twin members, commonly known as 'parasites' or 'heteropagi.' [32] We have chosen to exclude parasitic twins from the present discussion due to their complex and possibly heterogeneous nature.

The most commonly used classification divides symmetric conjoined twins into four general conjunction groups: ventral, lateral, caudal and dorsal conjunction. In these four groups 11 more or less well defined entities can be discriminated. [14] However many conjunction types show overlapping latero-ventral, latero-caudal and intermediate conjunction patterns, ultimately creating a divergent variability and heterogeneous phenotypical spectrum of conjunction; indicating a continuum between the different types of twins. [33]

Nondorsal conjunction

Classically, nondorsally conjoined twins can be divided into ventral, lateral and caudal conjunction types. Ventrally conjoined twins are joined at the peri-umbilical regions and, with increasing degrees of union, the thorax, neck, face and/or head can be additionally involved. A gradual spectrum exists between the different forms of ventral union. The mildest form of ventral conjunction is xipho-omphalopagus (Fig. 1A) characterized by joined livers and a common peritoneal cavity. [34] Xipho-omphalopagi are successful candidates for surgical separation. [35] When conjunction becomes more profound omphalopagus arises (Fig. 1B). The liver and diaphragm are involved in the union which can be additionally complicated by pericardiac and cardiac displacements—although no cardiac conjunction is present. [36] In approximately one-third of the omphalopagi shared intestines are seen. [37] Omphalopagi could be considered as candidates for surgical separation as well. [38] Thoracoileopagus (Fig. 1C) twins show the same union as omphalopagi,

but they share a single complex and composite heart with equal contributions from both twins. [5] Besides a compound heart, the liver, diaphragm and proximal intestines are joined. [14] Thoracoileopagi are hardly ever separable because of the cardiac involvement . [37] Prosopothoracoileopagi (Fig.1D) are united ventrally from the face and/or neck to the umbilicus. At the extreme end of the ventral union spectrum is cephalothoracoileopagus (Fig. 1E/F). These twins are united throughout the entire head, presenting with two (complete) compound faces on opposite sides of a single conjoined head; each twin contributing half of all conjoined structures. Both prosopo- and cephalothoracoileopagi are nonviable due to the often complex cardiovascular nature and the intricate degree of union, although the central nervous systems are individually owned by each twin.

Lateral conjoined twins are characterized by conjunction at the lateral aspects of the abdomen, thorax, neck, face and/or heads and classically consists of two gradually overlapping entities: parapagus dicephalus with two heads (Fig. 1G) and parapagus diprosopus with two laterally oriented faces in one compound head (Fig. 1H). In contrast to the former entities, laterally conjoined twins share vast parts of their body. In addition, dicephalic twins can be further divided into dicephalus tetrabrachius (four arms), tribrachius (three arms) and dibrachius (two arms). Finally, the affix dipus and tripus can be included in the nomenclature to describe the amount of present lower limbs. The extensive union in both dicephali and diprosopi usually precludes the possibility of separation and most die in the perinatal period. [37]

The third entity in the spectrum of nondorsally united twins are the caudally conjoined twins called ileoischiopagus (Fig.1I). These twins are joined at the peri-umbilical and pelvic region—sharing the lower abdomen, pelvis and perineum. Classically, two vertebral columns are located in a 180 degrees opposite position. Twins may be oriented face to face, creating considerable variation in the angle between the two spines ranging from 15 to 180 degrees. Ileoischiopagi usually share four upper and four lower limbs and two separate hearts. [14] Assessment of the pelvic osteology, genitourinary system, lower intestine and rectum and the degree of vascular sharing are the most important considerations for separation. [37]

Noteworthy is that all nondorsally conjoined twins share the peri-umbilical region and therefore have a single umbilical cord, which is sometimes flanked by a single overarching omphalocele. [34] It is this premise, together with the often encountered intermediate conjunction patterns (Fig. 1J) which converge the ventrally, laterally and caudally united twins into one overlapping phenotypical spectrum. In addition, this spectrum is affected by two embryological adjustments—exclusive seen in the nondorsally conjoined group (See further).

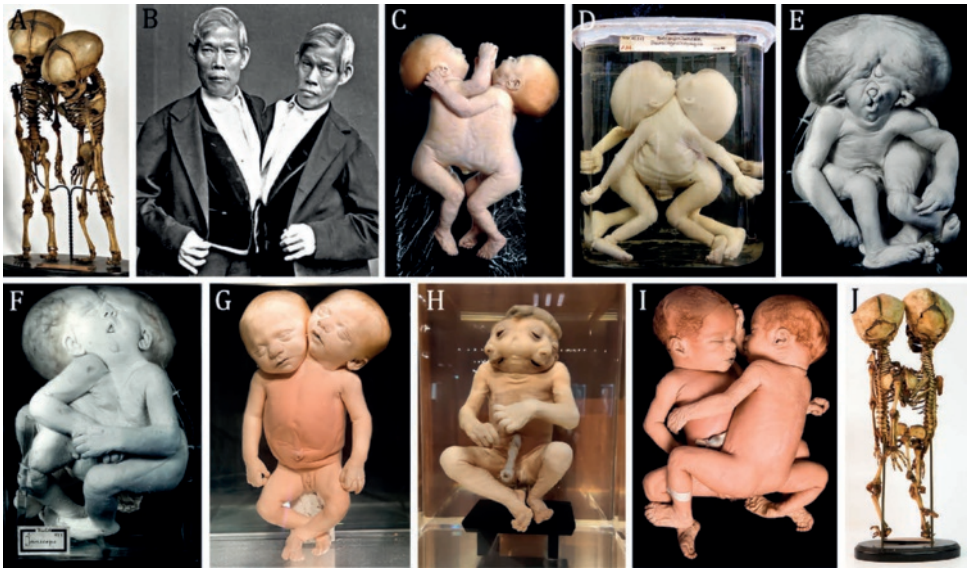


Fig. 1. Nondorsally united twins. [A] Skeleton of a xipho-omphalopagus twins united at the mid-ventral portion of the trunk. Specimen from the Vrolik Collection in Amsterdam (The Netherlands). [B] Photograph of perhaps the most famous conjoined twins: Chang end Eng Bunker (1811-1974) born in Siam (Thailand) and the reason why the expression "Siamese twins" was coined. Chang and Eng were omphalopagi twins united in the epigastric region and mid abdominal area. [C] Thoracoileopagus twins in which union starts mid-sternally and extends to the umbilicus. Specimen from the Anatomical Museum in Nijmegen (The Netherlands). [D] Prosopothoracoileopagus twins united ventrally from the face and/or neck to the umbilicus; the lower abdomen, genitalia, vertebral columns, limbs and face are individually owned by each twin. Specimen from the Narrenturm collection in Wien (Austria). [E] Cephalothoracoileopagus "ventral view, united throughout the entire head, two (complete) faces on opposite sides of a single conjoined head are noticeable. [F] Cephalothoracoileopagus "dorsal" view. Specimen from the Vrolik Collection in Amsterdam (The Netherlands). [G] Parapagus dicephalus twins with two heads on a single compound body. Specimen from the Anatomical Museum in Nijmegen (The Netherlands). [H] Parapagus diprosopus twin with two laterally oriented faces in one compound head. Specimen from the Anatomical Museum in Nijmegen (The Netherlands). [I] Ileoischiopagus tetrapus twins joined at the peri-umbilical and pelvic region—sharing the lower abdomen, pelvis and perineum. Specimen from the Anatomical Museum in Nijmegen (The Netherlands). [J] Skeleton of a thoracoileoischiopagus tribrachius tripus. Specimen from the Vrolik Collection in Amsterdam (The Netherlands). Note that all nondorsally united twins always have a single umbilicus and that vast amounts of the general body plan altered dramatically.

Dorsal conjunction

Classically, three types of dorsally united twins are discriminated. Noteworthy is that all dorsally united twins show individual internal organs and two separate umbilical cords; it is this peculiarity which discriminates the dorsally conjoined group from the nondorsally united twins.

Craniopagus twins are joined at the head, more specifically the cranial vault (Fig. 2A). The site of cranial union can be subdivided in frontal, temporal, parietal or occipital union but infinite variations exist in both axial and rotational orientation, ultimately leading to heterogenic phenotypes with marked non-homologous connections such as fronto-parietal, temporo-parietal and occipito-parietal union. [14] The juncture may include the meninges, the superior sagittal sinus and, in some cases, show cerebral deformities and conjoined brain tissue—the latter often shows separable leptomeninges overlying the interdigitated gyri. [39,40] Generally, it can be stated that the more extensive the union, the greater the decrease of relative calvarial volume and the greater the reciprocal pressure on the two developing brains. [39] Union never involves the foramen magnum, the base of the skull, face and vertebrae; the latter are however involved in cranio-rachipagus twins (see further). The most important considerations for separation of craniopagi are the intricacy of shared dural venous sinuses and the amount of conjoined brain tissue. [37]

Pygopagus twins are united at the sacrum, coccyx and perineum (Fig. 2B). Union often involves the dural sheath and the terminal portion of the spinal cord. [14] Structures originating from secondary neurulation (e.g. the conus medullares and filum terminale) which arise after the closure of the caudal neuropore are often communal in pygopagi. [41] The degree of dural and spinal cord conjunction and perineal, genitourinary and sacro-coccygeal morphology are the most important considerations for a possible separation. [39]

Rachipagus twins are conjoined at the back (Fig. 2C). Only two reports of non-parasitic rachipagi exist, described by Bétoulières *et al*. (1960) [42] and Durin *et al*. (2005). [43] Both cases concern rachipagi with cranial conjunction (cranio-rachipagus). This entity belongs to one of the rarest forms of conjoined twinning. Union may include the entire vertebral columns and occipital regions.

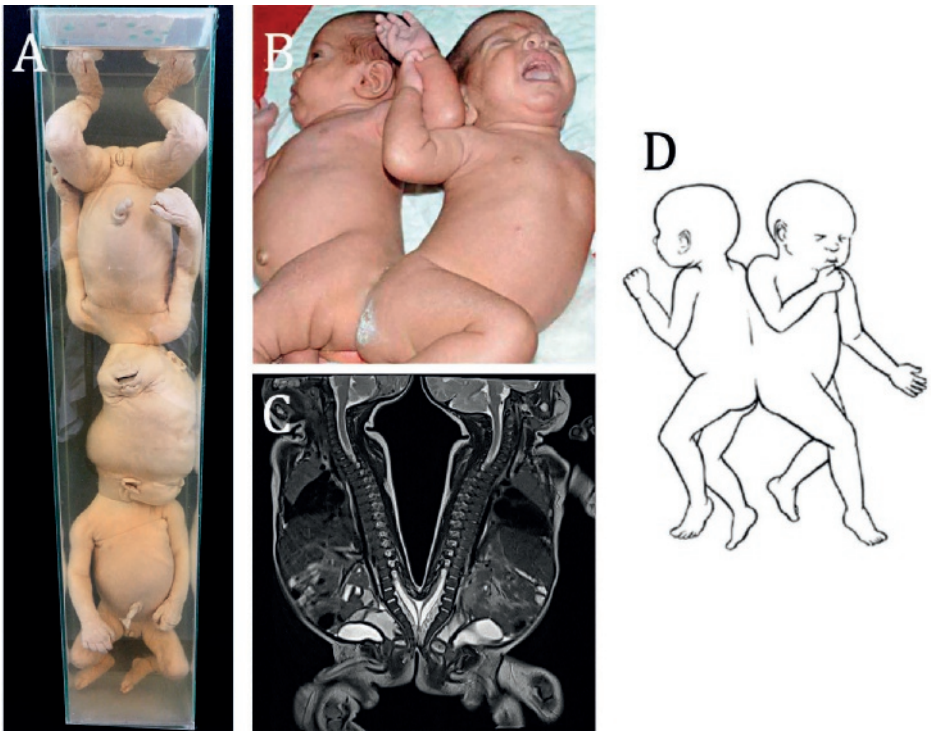


Fig. 2. Dorsally united twins. [A] Craniopagus twins with non-homologous union at the head. Specimen from the Narrenturm collection in Wien (Austria). [B] Pygopagus twins joined at the sacrum, coccyx and perineum, facing away from each other. [C] MRI of the child depicted in B which revealed a spina bifida from the fourth lumbar vertebra downwards and low-lying spinal cords tethered at the fifth lumbar vertebra to the first sacral vertebra. The filum terminale was fused at the second and third sacral vertebra within a single thecal sac. Figures B and C re-used form Awasthi *et al.* (2015) [44] with permission from Springer nature. [D] Drawing of rachipagus twins (Figure adapted from Spencer, 2003), [14] united at the spine and facing away from each other. Note that all dorsally united twins always have two separate umbilical cords, individual internal organs and lack in gross underdeveloped regions or dysmorphologies, which are almost invariably present in nondorsally united twins.

Embryological adjustments in conjoined twins

Two embryological mechanisms—seen in ventrally, laterally and caudally united twins—exists that both are responsible for adjustment and alteration of external and internal (embryological) morphology: neo-axial orientation and interaction aplasia. [45,14] Because of their exclusiveness in conjoined twins, these embryological adjustments could possibly imply certain etiopathogenetic clues about their origin. Whatever the true pathogenic mechanism of conjoined twinning may be, it is reasonable to assume that during early embryonic development a certain "conjoined twinning event" occurs which results in

aberrant hypoblast configurations that leads to duplication of the first visible initiations of gastrulation, being the primitive streak, node and/or pit on a single cell-mass or bilaminar embryonic disk. Hypothetically, duplication and subsequent outgrow of these morphogenetic primordia could occur in a direct opposite manner, resulting in ventrally and caudally conjunction types, or angulated and somewhat parallel configurations, resulting in laterally conjoined phenotypes (Fig. 3).

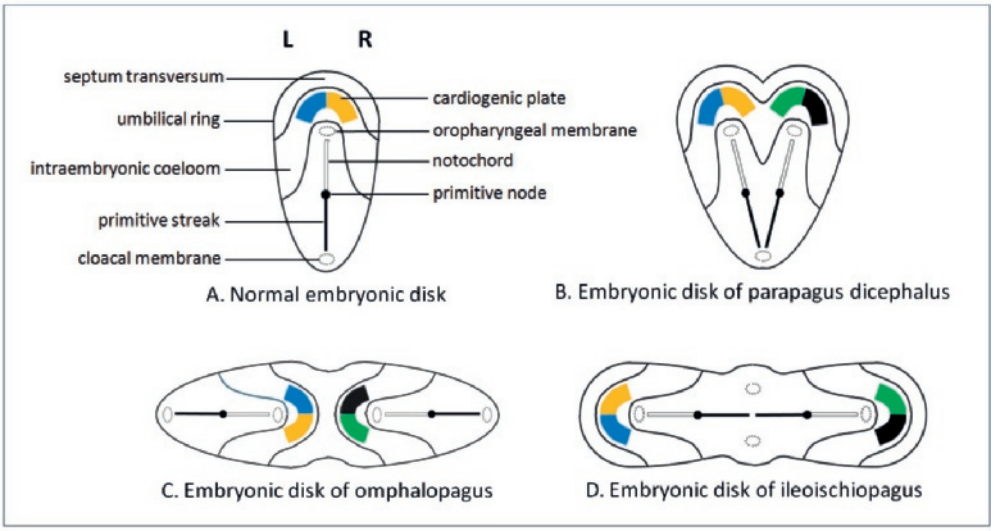


Fig. 3. [A] Schematic dorsal view of an embryonic disk at a late stage of gastrulation and normal configuration of early structures as depicted in many textbooks about embryology. [B] When axial primordia (primitive streaks, nodes and/or pits) are duplicated and located in a more or less parallel and angulated manner, with cranially located heart fields, laterally united twins will arise. The depicted embryonic disk shows the configuration of a parapagus dicephalus. Note the single cloacal membrane and the two oropharyngeal membranes. When this embryonic configuration persists the ultimate phenotype will therefore include two heads, two hearts, two vertebral columns and a single united lower body with a single umbilicus. [C] When duplication of axially located primordia arise in an opposing manner and development proceeds, ventrally conjoined twins will arise. The depicted embryonic disk shows the possible configuration of an omphalopagus; only the diaphragm and liver are conjoined, resulting from the united septum transversum and umbilical ring. Note the presence of two oropharyngeal and cloacal membranes and two heart fields. The presence of two primitive streaks initiate duplicated notogenesis, ultimately leading to two complete vertebral columns and two complete neurulation processes. The ultimate phenotype of omphalopagi will include two heads, two hearts and two lower bodies with a single umbilicus, as is clearly comparable with the depicted embryonic disk. [D] Embryonic disk configuration of an ileoischiopagus. When two primordia arise in an opposing manner, although now with laterally located heart fields and oropharyngeal membranes and medially located cloacal membranes, caudal conjoined twins will arise. These twins have two separate hearts, two heads, two vertebral columns and a conjoined and shared caudal area with a single umbilical cord. (Figures adapted from Oostra *et al.*, 2012). [46]

Neo-axial orientation

Embryonic disks with two axial primordia in an opposing configuration—as is the case in ventrally and caudally conjoined twins—are subjected to neo-axial orientations (Fig. 4). This embryonic adjustment refers to the mechanism by which opposing homologous structures are divided in the median plane after which the two halves will divert laterally. Compound organs and structures are thus formed by equal contributions of both embryos. The formed structures are located in a plane perpendicular to the original, thereby altering their original topographical location in a 90° axial rotation. [14] From a gross morphological point of view, two more or less normal structures are formed, although each half of these structures originally belongs to one of the twins. Neo-axial orientation is demonstrated in all ventrally and caudally conjoined twins and is most dramatically demonstrated in cephalothoracoileopagi (joined at the head, thorax and abdomen), in which two compound faces on opposite sides of a united head are seen (see Fig. 1E/F).

Interaction aplasia

The second mechanical adjustment—again typical for nondorsally conjoined twins—is interaction aplasia. This mechanism is best demonstrated in laterally conjoined twins (i.e. parapagi). In contrast to the mechanism of neo-axial orientation, occurring when primordia have opposite positions, interaction aplasia occurs when two primordia have any other mutual positions than exactly opposite, most typically when their positions are parallel. In interaction aplasia of contiguous primordia, organs and structures in the conjunction area fail to develop. The degree of aplasia depends on the approximation of the two primordia and their mutual angle. When approximation increases, interaction aplasia becomes more prominent (Fig. 5). Suppression of the structure and/or organ formation is assumed to result from aberrant concentrations of morphogens in and around the two longitudinal axes conflicting their concentration gradients and/or their (molecular) pathways. [47] Primordia become obliterated by these overlapping gradients and subsequently fail to form a developmental field. [48,14]

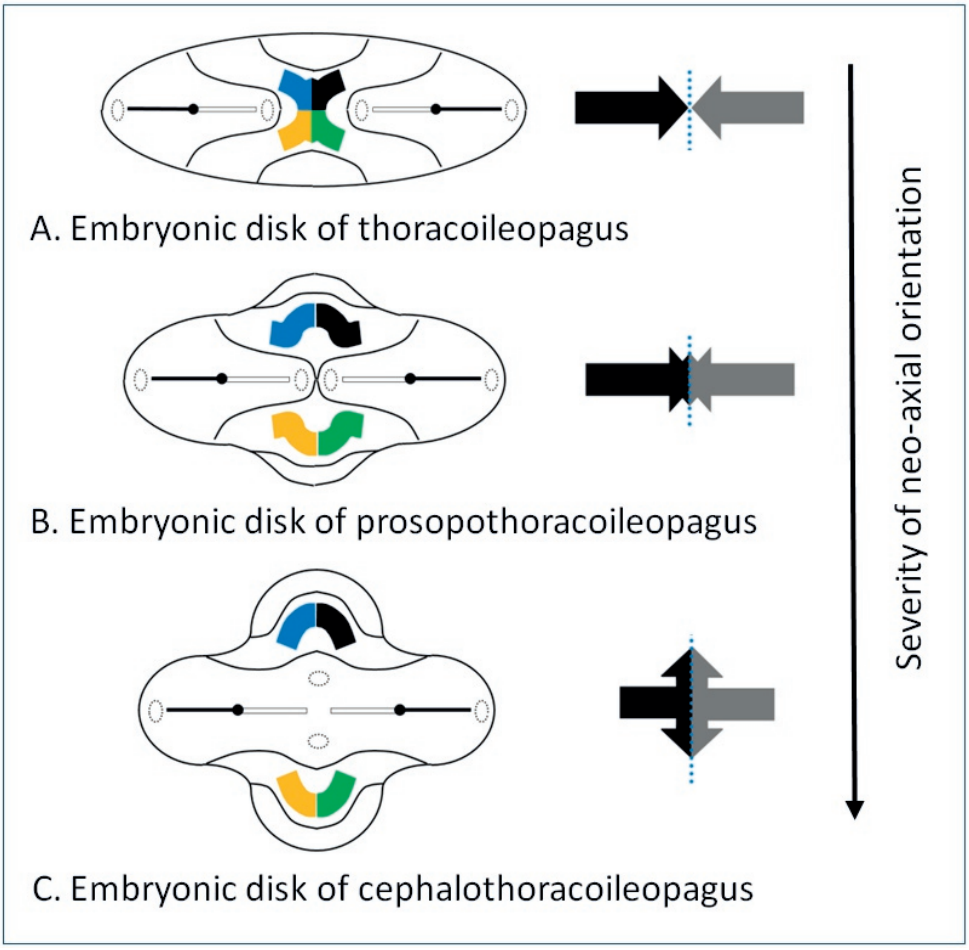


Fig. 4. Embryonic disk models with opposing duplication of axial structures. [A] Embryonic disk configuration of a thoracoileopagus with contiguous heart fields and neo-axial orientation of structures derived from the anterior most parts of the embryonic disc, such as the sternums, livers and diaphragms. This configuration will lead to a single complex and compound heart originated from cardiac primordia of both twins. Two separate heads and two lower bodies with a single umbilicus are found. [B] Embryonic disk configuration of a prosopothoracoileopagus. When the initial reciprocal distance of two opposing primordia is more approximate than in thoracoileopagus, more intricate neo-axial orientation will be initiated. This configuration will lead to neo-axially oriented heart fields and thus to two compound hearts, in addition to the compound sternums, livers and diaphragms. The presence of two separate oropharyngeal membranes, close to each other, will lead to two largely separate heads without neo-axial orientation. [C] Embryonic disk configuration of a cephalothoracoileopagus. If the initial distance between the opposing primordia is even more approximated, neo-axial orientation will also involve facial and cranial structures. Note that the arrows represent the direction of relative growth of the embryonic disk. (Figures adapted from Oostra *et al.*, 2012).[46]

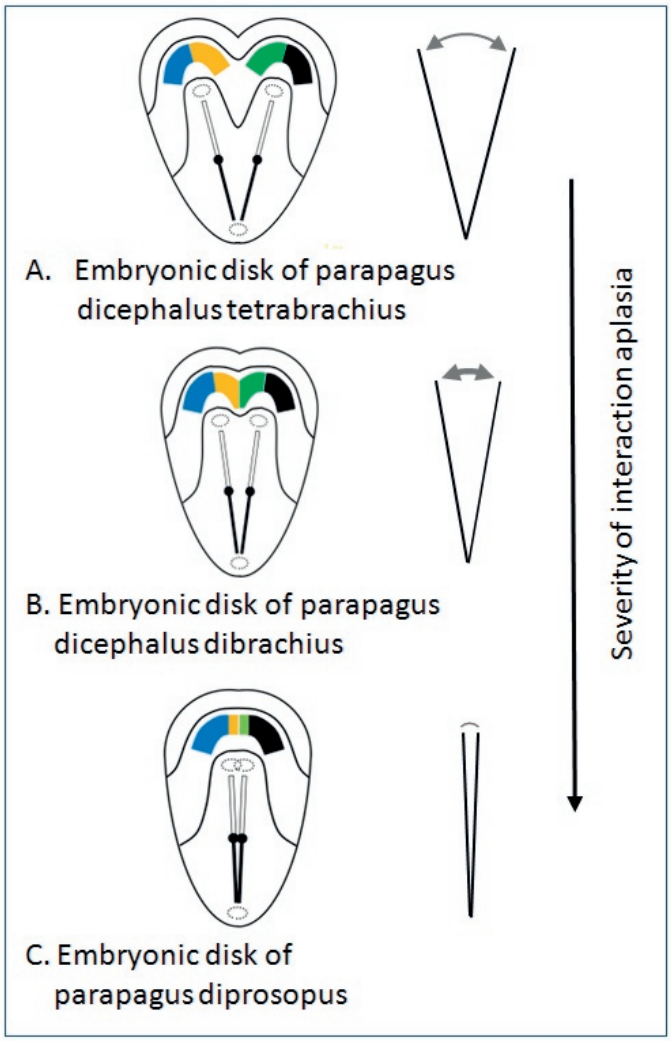


Fig. 5. Embryonic disk models with two angulated axial structures. [A] Embryonic disk configuration of a parapagus dicephalus tetrabrachius with two angulated axial primordia. Note the single cloacal membrane and the two, cranially located, heart fields and oropharyngeal membranes. Phenotypically, this configuration will lead to a parapagus twin with two heads, four arms, two separate hearts, more or less intricate junctions at the level of the lower thoraxes, diaphragms and livers and a single lower body with a single umbilicus. [B] Embryonic disk configuration of a parapagus dicephalus dibrachius. If the angulation of the two axial primordia approximates more acute than in figure A, their mutual distance is less and interaction aplasia will be more intense. Note that the heart fields of both twins become contiguous. This configuration will lead to a parapagus twin with two heads, two arms, a shared composite heart, profound junction at the level of the thorax(es), diaphragm(s) and liver(s), and a single lower body with a single umbilicus. [C] Embryonic disk configuration of a parapagus diprosopus. If the initial position of the primordia is even more approximated, interaction aplasia of almost two complete body halves will occur. This configuration will lead to twins with a head with two (partial) laterally oriented faces on the ventral side and a more or less singular heart, diaphragm and liver. (Figures adapted from Oostra *et al.*, 2012). [46]

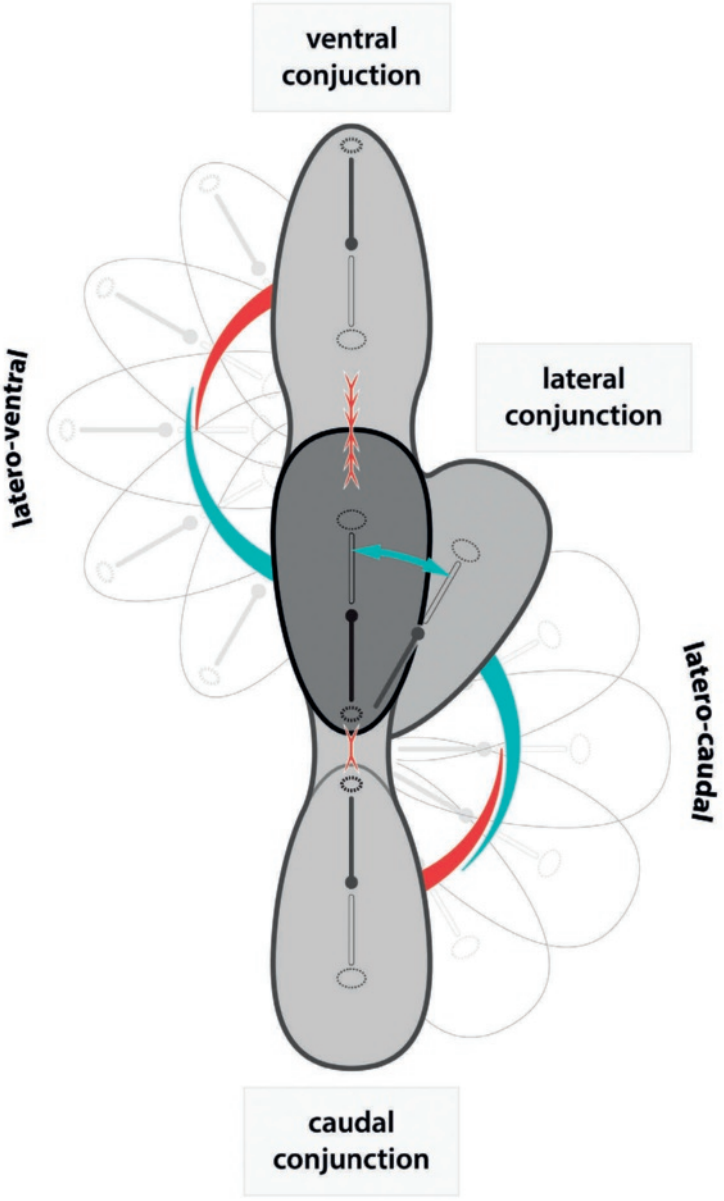


Fig. 6. Schematic representation of a continuous model between lateral, ventral and caudal united twins showing overlapping latero-caudal and latero-ventral phenotypes. Interaction aplasia (indicated by the turquoise arrow) will decrease when the positions of the duplicated primordia become more opposite to each other. Interaction aplasia is thus absent in the caudal and ventral phenotypes. On the other hand, whereas neo-axial orientation (red arrows facing each other) is absent in laterally united twins, this adjustment is profoundly present in the ventral and caudal conjunction group. However, the latter is affected much less severe. This is due to the fact that embryonic growth is much greater towards the future head primordia than it is in caudal directions—as is indicated by the red arrows in the model.

A phenotypical continuum in nondorsally united twins

As stated above the nondorsally united twins can all be included in a spectrum with infinite intermediate phenotypes and simultaneously concomitant neo-axial orientation and interaction aplasia. [33] This is depicted in figure 6. The intermediate phenotypes can all be extrapolated from the initial reciprocal distance and angle of the "duplicated axial primordia." In that respect it can be stated that no two pairs of conjoined twins are identical. For instance, many thoracoileopagi with axial primordia that are not exactly opposing each other show some degree of interaction aplasia, resulting in hypoplasia of compound organs and structures (e.g. junctions between two arms) on the concave aspect of the twins (Fig. 7A). Cephalothoracoileopagi with laterally deviating axes often show hypoplasia in one of the compound faces, mimicking the phenotype of holoprosencephaly (Fig. 7B). Finally, ileoischiopagi often show considerable caudo-lateral oriented variations and subsequent interaction aplasia, resulting in a composite lower limb and peno-scrotal aplasia on one side of the conjoined pelvises (Fig. 7C).

Etiology and pathogenesis of conjoined twins

Many embryological theories are extrapolated by reasoning backwards from late phenotypical stages to early embryological development. [49,50] Although this method could be beneficial to exploit certain embryological explanations, it makes the enigmatic genesis of conjoined twins prone to conflux in regard to the actual cause and result. "You are not a twin because the inner cell mass splits, the inner cell mass splits because you are a twin." [51] This quote reflects the exact problem in the everlasting enigma of imperfect twinning. What is the actual embryological cause? And what will be the final phenotypical result of this early defect? Therefore, the two paramount questions in respect to conjoined twins are: Why (etiologically) and how (pathogenetically) do these entities arise? Regarding the mechanism of conjoined twinning, there are currently two postulates: partial fission and secondary fusion. The fission theory, which assumes that conjoined twins originate around the primitive streak stage between days 15 to 17 of embryonic development, [52] is more profoundly postulated in textbooks, whereas the model of secondary fusion is a widely accepted premise in the genesis of conjoined twins when exploring current research papers. [14]

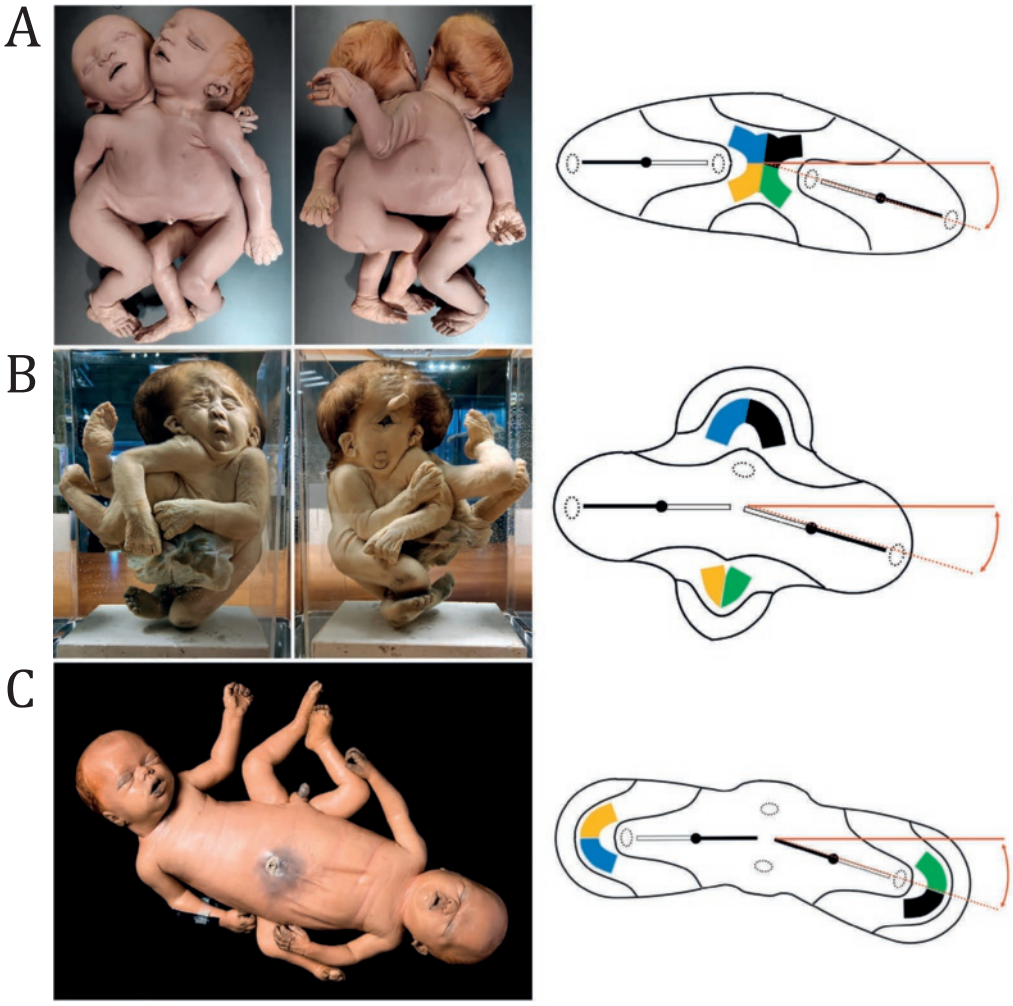


Fig. 7. [A] Thoracoileopagus tribrachius tetrapus twins with lateral deviations resulting in the formation of hypoplastic compound and composite structures. In this case a composite arm forms at the concave side of the twins due to interaction aplasia of the compound shoulder girdle. [B] Cephalothoracoileopagus twins with lateral deviations resulting in profound hypoplasia of craniofacial structures at the concave side of the twins, which is phenotypically reminiscent of holoprosencephaly. [C] Ileoischiopagus tripus twins with lateral deviations resulting in a composite leg and hypoplastic penile structures at the concave side of the twins due to interaction aplasia of the compound pelvic girdle. All depicted specimens are from the Anatomical Museum in Nijmegen (The Netherlands). Figures of the embryonic disks are adapted from Oostra *et al*. (2012). [46]

Fission theory: monozygotic twinning and incomplete fission of a single embryo

The fission theory suggests that all types of monozygotic twins and conjoined twins are entities in a single etiopathogenetic continuum. [13] Classically, depending on the time of fission, separate monozygotic twins can be divided into three entities. [53,54] The developmental stage at which splitting occurs would determine chorionicity and amnionicity. [55] In the morula stage, splitting is thought to result in two genetically identical blastocysts. Each blastocyst will implant separately, ultimately leading to a dichorionic-diamniotic (DC-DA) placentation. This type of monozygotic twinning accounts for approximately 18 to 36% of all monozygotic twins and is thought to occur within 3 days of embryonic development. [56] If fission of the embryoblast (without interfering the trophoblast) would occur in the peri-implantation period—after the third but before the seventh day of development—two embryoblasts in a single blastocyst would form and hence a monochorionic-diamniotic (MC-DA) placentation. This type accounts for about 60 to 80% of monozygotic twins. [56] The third developmental stage at which fission could occur is the most uncommonly encountered group of monozygotic twins: this type is seen in about 1 to 4% of human monozygotic twins. [13] Splitting is thought to occur shortly prior or during the formation of the primitive streak (around day 15 of development). The bilaminar embryonic disk is believed to "split in two," giving rise to two embryos which develop within a single amniotic cavity and inherently show a monochorionic-monoamniotic (MC-MA) placentation. When fission occurs after day 15 of development—when there is presence of a bilaminar embryonic disk as well as three extra embryonic spaces (amniotic cavity, primitive yolk sac and chorionic cavity) and a single (caudally located) connecting stalk—it is assumed that if fission will be incomplete and will subsequently give rise to the various forms of conjoined twins. [13] Noteworthy is that the postzygotic fission model places all conjoined twins in a single receptacle (together with all three types of separate monozygotic twinning) and extensive embryonic development is only minimally taken into consideration.

Several mechanisms have been proposed that could explain the occurrence of splitting as the cause of separate monozygotic twinning. Blickstein and Keith (2007) [57] proposed that a small proportion of oocytes might have an inborn tendency to undergo splitting upon fertilization, leading to the constant prevalence of spontaneous monozygotic conceptions among different populations. The revolutionary idea of an imprinted twinning gene needs further investigations. [58] But indeed, monozygotic twinning occurs

at a relatively constant rate of 3 to 5 in 1,000 births worldwide, supporting the view that it represents a random and/or genetic event. [59] A paper by Liu *et al*. (2018) [60] described a four-generation pedigree of monozygotic female twins revealing novel genetic variants specific to monozygotic twins in the X chromosome.

Apparently, there are certain factors (e.g. environmental, mechanical and genetic) which potentially play a key role in the occurrence of different types of monozygotic twins. Proposed triggers for splitting include gene mutations, abnormalities in cell surface proteins, and abnormalities in the formation of the zona pellucida. [61-63] Increased frequency of monozygotic twins is observed after infertility therapy such as intracytoplasmic sperm injection, [64-66] indicating a possible association of zona pellucida damage and monozygotic twinning. Enders (2002) [67] described that one possible cause for the increase in monozygotic twinning following in vitro fertilization such as assisted hatching, is the constriction of the inner cell mass during mechanical hatching of the blastocyst from the zona pellucida as opposed to the natural digesting that occurs *in vivo*. Indeed, *in vivo* developed mouse cleavage stages following focal damage to the zona pellucida frequently yielded two blastocyst-like structures on subsequent recovery from the uterus. [68] This phenomenon can be explained by partial herniation of the blastocyst followed by its shearing from the zona pellucida. These herniated trophectodermal vesicles may include inner cell mass tissue and subsequently can form an additional blastocyst. [68]

Fusion theory: secondary fusion of two, initially separate, embryonic disks

In contrast to the fission theory, the fusion theory—predominantly embraced in current research papers—suggests that conjoined twins result from two, initially separate monozygotic embryos, which coalesce and become secondarily and homologously fused. [69] This fusion theory was espoused by Spencer (2003) [14] and is now a widely accepted theory, cited in virtually every paper on this topic. Spencer (2003) [14] proposed that conjoined twins originate when the inner cell mass divides (implying an early fission) during the first week after fertilization into two separate monozygotic embryonic primordia staying close enough together to share either the amniotic cavity or the yolk sac. When these embryos continue their rapid growth, they might come in contact with one another and become reunited to result either in ventrally, laterally, caudally or dorsally conjoined twins. Spencer (2000) [70] substantiated the concept of secondary fusion with a theoretical model called the "spherical theory." This model delineates that two monozygotic embryonic disks lie

adjacent to each other and "float" on the outer surface of a spherical yolk sac resulting in a—always homologous—ventral, lateral or caudal configuration. When two monozygotic embryonic disks "float" on a shared amniotic cavity the possible secondary fusion of two, initially separate, primitive neural folds can occur, resulting in dorsally united twins (Fig. 8). In addition, Spencer (1992) [71] described that secondary fusion will not occur randomly and that intact skin will not fuse with intact skin. Union only occurs where surface ectoderm is either absent (primordia of the heart and septum transversum) or is destined (preprogrammed) to undergo apoptosis (neural tube, the oropharyngeal and cloacal membranes) or is influenced by differential growth (the periphery of the embryonic disk). Furthermore, union always occurs in the midline, "the two lateral halves of specific structures of one embryo united to the opposing halves of the same structures of the other embryo." [14] According to Spencer (2003), [14] the presence of supernumerary umbilical vessels and the presence of two umbilical cords in dorsally conjoined twins is a strong argument for the fusion theory of two initially separate embryonic disks which coalesce and will fuse secondarily.

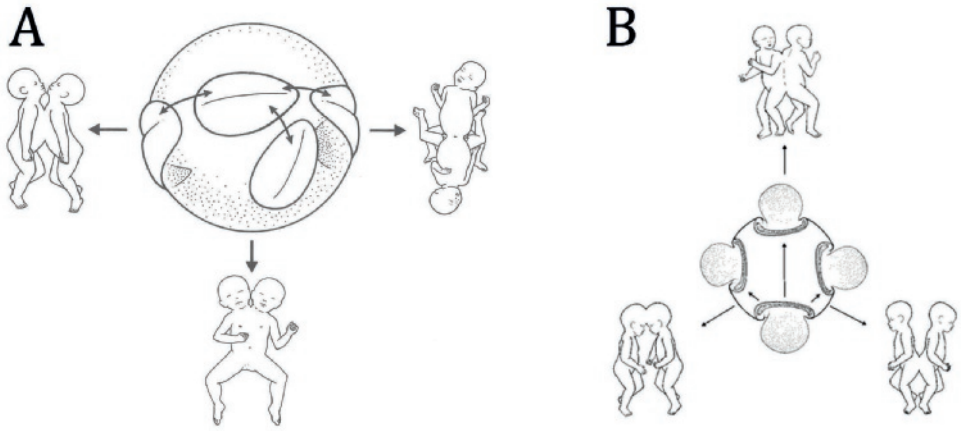


Fig. 8. The spherical theory as the etiological basis for conjoined twins devised by Spencer. [A] When two embryonic disks lie adjacent to each other and "float" on the outer surface of a spherical yolk sac, ventral, lateral or caudal conjunction types could occur. [B] When two embryonic disks "float" on a shared amniotic cavity the possible secondary fusion of two, initially separate, primitive neural folds can occur, resulting in dorsally united twins. Note the presence of two yolk sacs. Figures re-used from Spencer (2000) [70] with permission by John Wiley and Sons.

"Crowding" theory: induction of two axial primordia

In addition to the fission and fusion theories, a third conjecture to explain conjoined twins may be the initial "crowding and thereby duplication of morphogenetic potent primordia." [72,73,29] Interestingly, already in 1866, Fisher described duplication of the primitive streak in one embryonic cell mass as the cause of conjoined twinning. [74] Wilder (1908) [75] concluded that there is "neither a fusion of parts already formed nor a gradual development from the normal towards the abnormal during embryonic life, but the parts appear double or reduced from their first appearance, and their development is controlled in the same way as are the bilateral structures and other architectural characteristics of normal beings." Moreover, Ysander and Wikstrom (1925) [76] concluded that "the manner in which twins will be definitely joined depends on the distance between the two developing centers, their independence of each other, and the angle between their polar axes." The "crowding of organizers" presumes the early induction of two instead of one multifaceted organizer on the surface of one embryonic cell mass. When applied to nondorsally united twins the initial reciprocal distance and mutual position determines the site and degree of conjunction. Depending on the configuration of the initial "duplications" (opposite or angulated), neo-axial orientation and/or interaction-aplasia occurs. [48, 14, 46] The premise of initial duplication of certain "axial primordia" is strengthened by many experimental studies. Since the initial transplantation experiments by Spemann and Mangold (1924), [26] amphibians, and especially *Xenopus,* have served as model systems for the analysis and manipulation of axis formation in the vertebrate embryo. [77] In subsequent decades, experimentally-induced duplication of organizing centers in mammals and non-mammals have been described abundantly (Table I).

In addition, Ziv *et al*. (1992) [79] showed that mesoderm induction is mediated through morphogens distributed in a gradient manner and suggested that during normal development only one axis is obtained because of carefully controlled inhibitory processes. Seleiro *et al*. (1996) [27] described genes of the transforming growth factor β (TGFβ) super-family as the earliest steps of developmental patterning in vertebrates and Beddington (1994) [80] suggested that the node can serve as a "stem cell" source of axial mesoderm. Besides describing axes duplication, Pöpperl *et al*. (1997) [84] stated that a number of secreted protein factors—such as certain Wnt family members, noggin and Vg1—can induce the formation of a second axis in *Xenopus*. The embryos exhibited two different forms of axis duplication: either the axes were in opposing orientation, giving a head-to-head duplication or they were angulated and fused caudally.

Table I. Overview of recent studies (since 1991) in which duplicated axial structures are found after molecular and genetic alterations.

Author and year of publication	*Species*	*Short description of their findings*
Sokol *et al.* (1991) [78]	*Xenopus*	Injection of Wnt mRNA induced complete axis duplication
Ziv *et al.* (1992) [79]	Chick	Produced ectopic axes after injecting Activin containing medium
Beddington (1994) [80]	Mouse	Induced ectopic notochords by implanting grafts of transgenically marked mid-gastrulation nodes
Karnovsky and Klymkowsky (1995) [81]	*Xenopus*	Injection of RNA encoding an epitope-tagged form of plakoglobin induced axis duplication
Toyama *et al.* (1995) [82]	Zebrafish	Injection of *Nodal* mRNA produced duplication of the notochord and somites
Seleiro *et al.* (1996) [27]	Chick	Induced a complete second axis after implanting grafts with Vg1 protein
Molenaar *et al.* (1996) [83]	*Xenopus*	Injection of β-catenin RNA consistently induced axis duplication
Pöpperl *et al.* (1997) [84]	Mouse	Ectopic expression of Cwnt8C caused axis duplication in *b-actin-Cwnt8C* transgenic mice
Zeng *et al.* (1997) [85]	*Xenopus*	Suppression of wildtype *Axin* resulted in duplication of the body axis
Nascone and Mercola (1997) [86]	*Xenopus*	Microinjection of mRNA's encoding Wnt signaling pathway components *wnt8* or β-catenin duplicated the inductive properties of the Niewkoop and Spemann regions and created conjoined twins
Fang *et al.* (2000) [87]	Frog	Ectopic injection of noggin RNA in blastomeres induced complete duplications of axes including heads and eyes
Perea-Gomez *et al.* (2002) [88]	Mouse	Demonstrated that *Cerl*$^{-/-}$;*Lefty1*$^{-/-}$ compound mutants developed ectopically primitive streaks
Merill *et al.* (2004) [89]	Mouse	Demonstrated that in *Tcf3*$^{-/-}$mutants duplication of nodes and notochords occurred
Tisler *et al.* (2017) [77]	*Xenopus*	Induced conjoined tadpoles after injection of Wnt-pathway components into the ventral marginal zone of cleavage stage embryos

These configurations are exactly the same as observed in human conjoined twins. Moreover, it has been shown that *axin*—encoding for an inhibitor of the WNT-signaling pathway—regulates embryonic axis formation in mouse and *Xenopus.* Besides induction of axial duplications, Perea-Gomez *et al*. (2002) [88] demonstrated that *Nodal* antagonists in the anterior visceral endoderm prevents formation of multiple primitive streaks. Nodal is known to play an important signaling role from the node, in the anterior primitive streak to the lateral plate mesoderm. [90] In addition, Gardner (2001) [91] demonstrated that the axis of polarity of the developing mouse embryo may be established as early as in the zygote or during the first cleavage stage. McCrea at al. (1993) [92] described that embryonic axis formation initiates before major activation of the zygotic genome. Noteworthy is that prior to zygotic genome activation, early mammalian development relies on maternal-effect genes to orchestrate the oocyte-to-embryo transition. [93] It can be even hypothesized that these critical mRNA's could be dislocated, inducing polarity changes and subsequent duplications. [94,95] Vandenberg *et al.* (2012) [96] found that apical-basal and planar polarity proteins are required for left-right axis orientation in *Xenopus*. Studies in table I clearly indicate that it is possible to experimentally initiate duplicated axial structures such as primitive streaks, nodes or notochords on one embryonic disk and obtain phenotypes that are indistinguishable from conjoined twinning.

Discussion

Normal human pregnancy concerns single offspring, it is therefore that (imperfect) twinning is in itself a congenital anomaly. [97] Although—relatively many—gross congenital anomalies have a known cause, the etiology and pathogenesis of (conjoined) twinning remains enigmatic. Many case reports exist on the topic of conjoined twinning, especially regarding separation and pre- and post-operative management. Interesting is the underrepresentation of papers which (extensively) discuss their potential etiology and pathogenesis or correlate human embryology in its delineated contemplations. Apart from the "crowding" concept presented within this paper, there are currently two conjectures for their possible genesis: partial fission versus secondary fusion. These mutually exclusive hypotheses are—curiously enough—widely spread in medical textbooks on embryology and cited abundantly in current literature. Despite their purported plausibility, they show clear omissions, lack a substantiated (theoretical) correlation with (human) embryological development and are not unequivocally demonstrated experimentally. Furthermore, traditional assumptions such as all conjoined twins having a

common etiology and pathogenesis are often adopted without any critical appraisal. This could cause erroneous theories to evolve into accepted and apparent factual etiological and pathogenetic models. These models should be critically reviewed an re-evaluated to break the current paradigmatic stalemate.

Comments on the fission theory

The fission mechanism behind monozygotic twinning is still poorly understood. Corner (1955) [98] stated that selective cellular death can act as a dissecting knife dividing the embryo into two. A paper by Herranz (2015) [55] argued that the commonly accepted Corner model of post zygotic fission lacked scientific proof. He stated that factors initiating cleavage are unspecified, coexistence of separate embryos within a single zona pellucida seems unlikely, post zygotic splitting becomes more unlikely with the passage of time, and splitting has never been observed *in vitro*. However, a paper by Kyono (2013) [99] found evidence from *in vitro* fertilization studies that monozygotic DC-DA twins would occur at the blastocyst stages and not during early morula stages, doubting the long held credo that DC-DA twins would develop after embryo splitting in the early stages of embryonic development. Furthermore, Herranz (2015) [55] proposed a new theory to explain the timing of monozygotic twinning. Monozygotic twinning would be a fertilization event; "due to an alteration of the zygote–blastomere transition, the first zygotic division, instead of producing two blastomeres, generates twin zygotes. Second, monochorionicity and monoamnionicity would not depend on embryo splitting, but on fusion of membranes." Critical notes on the paper of Herranz were espoused by Denker (2015) [100] and Gardner (2014) [101] which both concluded that the traditional Corner model and Herranz's model were unsubstantiated. However, none of the above authors postulate an alternative explanation.

With respect to the different types of monozygotic twins the veracity of the currently used fission model remains rather debatable. However, this ubiquitous model could indeed be plausible in DC-DA and MC-DA twins. This argument can be strengthened by the fact that after e.g. assisted fertility treatment—which often yield multiple gestations—both monozygotic DC-DA and MC-DA twins occur. [102,103] However—and to the best of our knowledge—we did not came across any reports of MC-MA twins after infertility therapy, indicating that the model of postzygotic fission is perhaps not applicable for MC-MA twins. The only MC-MA like cases reported are those resulting from a dividing membrane in a MC-DA gestation which ruptures, creating

a functional MC-MA configuration and a "pseudomonoamniotic" gestation. [104] A paper by Galjaard *et al*. (2014) [105] reported on two intermediate forms of chorionicity and amnionicity that may arose due to zygotic cleavage within the time interval just between di- and monochorionic and di- and monoamniotic twinning. Although the presence of pseudo and intermediate types of MC-MA twins (which are truly exceptional cases), we feel inclined to suggest that zygotic fission could be applicable in DC-DA and MA-DA twins but is not necessarily etiologically responsible for the formation of MC-MA twins and conjoined twins, and that the spectrum between these entities is not immediately obvious. It is appoint worthy that the acclaimed homology in the etiopathogenesis of monozygotic and conjoined twins is currently only based on the congruent configuration of its embryonic membranes. [54] Although this premise is true, it is not because of this peculiarity that they necessarily have a common etiological background (see further). The model of (in)complete fission is rather hard to imagine embryologically and some questions remain inconclusive. For instance, it remains a mystery why fission occurs at these specific days of development? Is fission a time specific event or could this process cease after a certain threshold of development? What causes partial zygote splitting after day 15 of development and is it even possible at this stage? And how can the amniotic epithelium insinuate between (partially) fissioned hypo- and epiblasts? In addition to the fission model being plausible in DC-DA and MC-DA twins, the assumption that incomplete fission could explain conjoined twinning is merely based on the Y-shaped contour of laterally conjoined twins, with their "split" and duplicated upper body halves and singular lower body half, creating the illusion of incomplete fission confined to the anterior part of the embryonic disc as its pathogenesis. That it truly concerns an illusion is underpinned by the fact that the—on external examination—seemingly singular and hence "unsplit" lower body half of even the most intricately conjoined parapagus twins, being diprosopus, in fact shows longitudinal duplications down to the caudal most end of the vertebral column (Fig. 9). Moreover, the explanatory potential of this illusion fails when applied to ventrally and caudally conjoined twins. Since, as we have shown throughout this paper, all nondorsally conjoined twins are part of a phenotypical continuum and are subsequently affected by the same embryological adjustments (neo-axial orientation and/or interaction aplasia), this means that incomplete fission cannot be the cause of laterally, nor of any other type of nondorsally conjoined twins.

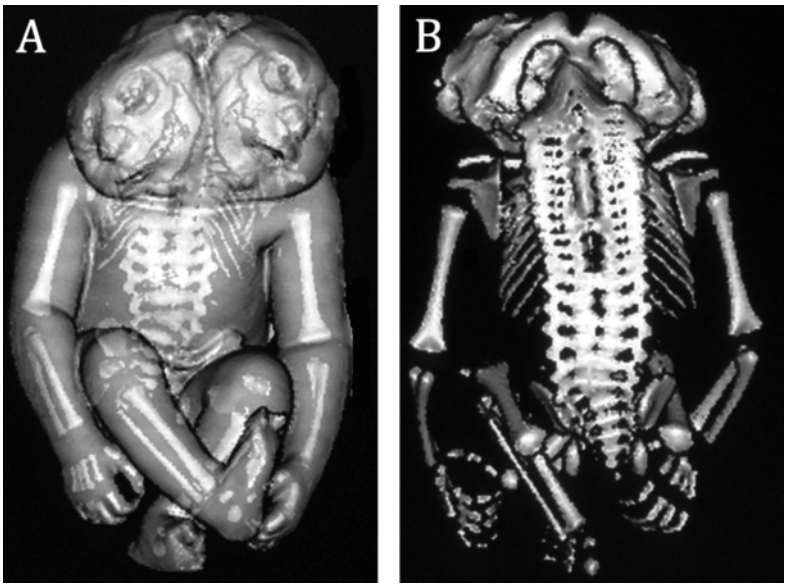


Fig. 9. Digitally reconstructed images of a spiral CT from a parapagus diprosopus from the Vrolik collection in Amsterdam (The Netherlands). [A] Ventral view of the specimen in which the outer contour is combined with the reconstructed skeleton. [B] Dorsal view showing complete duplication of the vertebral column. Note the concomitant craniorachischisis which is often present in parapagi disprosopi.

Comments on the fusion theory

Although we did not came across any literature concerning experimental *in vitro* fusion of developing placental mammals, studies generating parabiotic zebrafish embryos indicate that it is physically possible to fuse blastula stage zebrafish embryos ultimately creating parabiosis which develop as partially fused embryos sharing a common blood circulation. [106] Noteworthy is that fusion always required (micro)surgical procedures inducing an artificial component. [107] A study by Gianasi *et al*. (2018) [108] detected for the first time that a sea cucumber (*Cucumaria frondosa*) could undergo natural zygote fusion in hatched blastulae.

In addition, according to Spencer (2003), [14] the presence of supernumerary umbilical vessels and the presence of two umbilical cords in dorsally united twins is an important argument for the fusion theory of two initial separate embryonic disks. However, only a small percentage of nondorsally conjoined twins show supernumerary vessels. Many twins show normal configurations of the umbilical vessels or even less than three vessels. [109] The connecting stalk will be formed on day 13-14 by condensed

extra-embryonic mesoderm in which subsequently umbilical vessels will develop and into which the allantois grows. [110] Strangely, Spencer (2003) [14] stated that conjoined twinning originates in the first week after fertilization. According to the fission theory of monozygotic twinning—described above—if complete splitting occurs in the first week of development, a monochorial-diamniotic placentation will occur. With this configuration it is impossible to get secondarily fused twins as the amniotic membranes would interfere within this process. Furthermore, in this period gastrulating processes have not yet occurred so there is neither a bi- or trilaminar embryonic disk nor a connecting stalk with umbilical vessels. The initial presence of two caudally located body stalks is inherent if the secondary fusion takes place after or around 14 days of embryological development. Many reports describe that conjoined twins arise after day 14 of development, independent if fusion or fission might have occurred. It is however difficult to envision how the process of two separate embryonic disks, with two connecting stalks and all their embryological primordia, unite to form one seemingly "fused" individual with one umbilicus and often profound embryological adjustments. If this process would actually happen around the primitive streak stage of development, one would expect duplication of umbilical vessels and para-umbilical structures in all conjoined twins.

Although we excluded non-symmetrical twins, a paper by Logroño *et al*. (1997) [111] has to be noted. They found three different alleles in four loci at the site of junction of a parasitic conjoined twin with fluorescent in situ hybridization techniques. It is noteworthy because it was this finding which was the most decisive argument for secondary fusion. [14] Although these findings could be correct, dizygosity is not immediately implicated when finding genetic differences between members of (parasitic) conjoined twins, nor does it imply that fusion is the cause of their conjunction. Traditionally, it is presumed that monozygotic twins are genetically identical and subsequent phenotypical discordances are ascribed to environmental influences alone (shared or non-shared), thereby altering and modifying the expression of the otherwise identical genetic endowment. [112] Recent insights indicate that this explanation is far too simple. [53] Genetic divergence due to post-zygotic point mutations does occur. [113] Gringras and Chen (2001) [112] reviewed genetic alterations in monozygotic twins and found heterokaryotypical divergence, chromosomal mosaicisms, epigenetic modifications such as DNA methylation, histone acetylation and skewed or non-random X-inactivation causing discordance in monozygotic twins. Moreover, divergent epigenetic modifications can lead to differential expression of inherited disease genes. [114,115] Furthermore, phenotypic discordance in monozygotic twins may, in part,

be caused by *de novo* mutations of copy number variants and copy number variants mosaicisms. [116] Copy number variants account for a major portion of the genome, are strongly polymorphic and relatively unstable, with mutation rates 100 to 10,000 times higher than those for single base substitutions. [117] Additionally, unequal exchange of cells during gestation might potentially lead to discordant fetomaternal microchimerism and thus possibly induce discordances in monozygotic twins. [112] Evidence has been accumulating showing that spontaneous chimerism is far more common than previously realized. [118] It is therefore not surprising that monozygotic twins can show a high degree of discordance for complex genetic traits and disorders. Taking the above mentioned in mind, it is imaginable that genetic differences occur in members of conjoined twins, irrespective of their pathogenesis, because of its monozygotic nature subtle gene differences do not directly imply dizygosity. [58]

The theory of secondary fusion and its accompanying spherical etiology, as postulated by Spencer (2000) [70] is rather difficult to (embryologically) envision in the nondorsally united twins. Giving this model less credibility. Moreover, it is intriguing to question—in accordance with the spherical theory—how conjoined twins can be affected so dramatically by neo-axial orientation and/or interaction aplasia when two (complete?) embryological entities coalesce. Secondly, it remains a mystery why some zygotes float on a shared yolk sac and have two amniotic cavities and others float on a shared amniotic sac with two yolk sacs. [14] No rational (embryological) explanation is present to validate this assumption. Finally, it is unclear how and why initially separated embryonic disks would coalesce homologously.

Interestingly Spencer (2003) [14] stated, with respect to laterally conjoined twins, that: "the two lateral halves of specific structures of one embryo united to the opposing halves of the same structures of the other embryo" but strangely enough she subsequently admits that the secondary fusion theory is rather hard to imagine within the lateral group and cannot be immediately explained within this model. Since all non-dorsal types of conjoined twinning form a phenotypic continuum, as we have demonstrated above, precluding one type from this explanatory postulate makes it highly unlikely that it remains applicable to the other types. As with the fission theory, the concept of secondary fusion as the causative explanation for nondorsally conjoined twins is based on an illusion, in this case created by superficially connected ventrally and caudally conjoined twins. However, the illusion fails as soon as one is confronted with more intricate neo-axial orientation and especially with interaction aplasia.

However, the secondary fusion theory could be the underlying mechanism in dorsally conjoined twins which show several characteristics,

unique to this group, that may suggest an etiopathogenesis that fundamentally differs from that of other types of conjoined twinning. First, dorsally conjoined twins always have two separate umbilical cords and show no phenotypic overlap with other conjoined twinning groups. Secondly, there is no or very restricted neo-axial orientation and/or interaction aplasia, in that a low percentage show superficially shared brains and dural venous sinuses. [40] In addition, dorsally united twins frequently show a non-homologous union such as a temporo-parietal or occipito-frontal union in craniopagus. [119] This is in contrast to the ventrally, laterally and caudally conjoined twins, which are always united in a homologous fashion. As proposed by Spencer (2003), [14] dorsally conjoined twins could theoretically arise when two—initially separated—rapidly growing monozygotic MC-MA embryos get in mutual contact with the still open parts of their neural grooves and become secondarily fused. Assuming that secondary fusion is the causal mechanism for dorsally conjoined twinning, three different entities can be discerned which depends on the time and specific site of fusion. Fusion at the cranial neuropore forms craniopagi, fusion at the caudal neuropore results in pygopagi and fusion in the midportion of the neural tube will create rachipagi.

The presence of two separate umbilical cords implies the presence of two primordial connecting stalks on day 14 of embryological development and thus two initially separate embryonic disks. Furthermore, the clear absence of pronounced morphological adjustments in the plain of conjunction and the occasional non-homologous conjunction all plead for secondary fusion at the sites of neural tube closure of two initially separate embryos. Spencer (2003), [14] assuming a single pathogenetic mechanism for all types of conjoined twinning, concluded that: "The dorsally united twins present the most compelling argument for the fusion theory—and against fission—as the origin of conjoined twins," a view point that rapidly gained ground. [14]

Noteworthy is one truly exceptional case of a craniopagus, described by Bolk (1926) [120] (reviewed by Oostra *et al*. (1998)), [33] historically diagnosed as cranioamniopagus. This twin was united in two separate sites: a non-homologous union at the head was accompanied by an overarching omphalocele with concordant cloacal exstrophy (Fig. 10). In this case a secondary fusion of the cranial neuropore could have occurred in an initially very superficially conjoined xipho-omphalopagus; this site of union may have acted as a "hinge point" creating exactly the right distance and mutual opposability between the two twins to facilitate the occurrence of secondary fusion at the site of the cranial neuropores. These hinge points are inherently absent in more profoundly united ventrally as well as in laterally or caudally conjoined twins.

Fig. 10. Cranioamniopagus twin from the Vrolik collection in Amsterdam (The Netherlands).

Initial axial duplications may be responsible in the genesis of ventrally, laterally and caudally conjoined twins

With the rejection of both the fusion and the fission theories as causative explanations, we propose that initial duplication of axially located morphogenetic potent primordia in one inner cell mass is the initiating factor in the genesis of nondorsally conjoined twins. Moreover, we also propose this mechanism to be responsible for (at least some cases of) separate MC-MA twinning, in which we assume the initial reciprocal distance between the axial primordia to be large enough to prevent mutual developmental interference from occurring. Conjoined and separate MC-MA twinning are equally rare with prevalences of 1-4% of all monozygotic twins. [13] But more compelling is the rare but repeatedly reported occurrence of MC-MA twins with a single placentally inserted but bifurcated umbilical cord, connected with two separate MC-MA twins (reviewed by Fraser *et al*. 1997), [121] which could be interpreted as a transitional twinning type between separate and conjoined MC-MA twins. Since we demonstrated in the previous paragraphs the plausibility of DC-DA and MC-DA monozygotic twinning to result from

fission of the early embryoblast but excluded this mechanism as causative for any form of conjoined twinning, the pathogenic connection made here between MC-MA monozygous twins and nondorsally conjoined twins implies that monozygous twinning is a heterogeneous phenomenon.

Interestingly, this "molecular and morphological crowding" of axial primordia has been abundantly described in animal experimental studies as ectopic or duplicated axial structures in a single entity, including the primitive streak, node or notochord (see references table I). These experiments induced duplications by altering various secretion and transcription factors all involved in embryonic axis formation. Curiously, these findings have rarely been correlated with the genesis of human conjoined twins. It can be assumed that duplicated primordia are localized in a certain (pre)destined pattern, both following their own fate while inducing their own signaling pathways. [122] Subsequently, these signaling pathways could potentially interfere with each other and create dysmorphological phenotypes. [47,123] It is known that loss of function mutations of genes expressed by the anterior visceral endoderm results in the formation of extra primitive streaks. [124]

Conclusion

A pitfall in the ongoing etiopathogenetic debate on the genesis of conjoined twins is the fact that different types of conjoined twins are classically placed in one overarching receptacle. This approach has seriously hindered the quest for explanatory models.

We have shown that all nondorsally conjoined twins are part of a single phenotypical spectrum and probably have a single etiology and pathogenesis and that both the fission as well as the secondary fusion hypotheses to explain the pathogenesis of nondorsally conjoined twins is based on illusions.

Although the following needs further empirical evidence, we consider that the etiopathogenesis of dorsally united twins could be attributed to secondary fusion of two initially separate monozygotic twins. Based on what is presented in this paper we propose that initial duplication of axially located morphogenetic potent primordia could be the initiating factor in the genesis of ventrally, laterally and caudally conjoined twins as well as monozygotic MC-MA twins. The model of postzygotic fission could be possible in the genesis in DC-DA and MC-DA twins. However, one must be aware, although we state that MC-MA twins and conjoined are part of a continuum, that these entities could still be etiologically heterogeneous. In addition, it is conceivable that very little additional diagnostics are performed after the birth of conjoined twins—perhaps because it is obvious that it concerns a united twin with a

supposedly perspicuous etiology when one looks perfunctory to literature. However, new cases should be critically evaluated with addition radiological imaging and genetic diagnostics. [125] Finally, determination of the chorionicity and amnionicity in new cases is crucial to proof our propositions.

Limitations of this study

The first footnote in this study is the lack of prior comprehensive appraisals on the correlation of human conjoined twins and an elaborate view on early embryogenesis. In addition, although the embryonic disk models presented within this paper are applicable and imaginable in all nondorsally conjoined twins, they remain abstract renderings which are created by rationally reasoning backwards to early embryogenesis. This starting point could be too simplistic; the multifold of complicated molecular mechanisms during human embryogenesis could impossibly be overseen within this simplified model. Furthermore, the propositions that nondorsally united twins originate from the duplication of axially located structures and the assumption that dorsally united twins originate through a process of secondary fusion are still a mere conceptual conjecture. However, this paper tries to break the paradigm in the current pathogenetic models in the genesis of united twins, and criticizes the general view that all types of (conjoined) twinning, irrespective of the applied explanatory model, are placed in one overarching receptacle. Progress in embryological understanding will never occur if oversimplified theories are reinforced by standard concepts being repeated over and over. [118,126]

Acknowledgements

The authors thank Drs. Elke de Boer, Dr. Willemijn Klein and Prof. Dirk Ruiter for their critical comments on the manuscript. Furthermore, we thank Dr. Laurens de Rooy for his help in providing photographs from specimens located in the Vrolik museum in Amsterdam (The Netherlands). We thank Eduard Winter for providing the photographs from the specimens located in the Narrenturm collection in Wien (Austria). We thank Frank Muller (ZorginBeeld) for providing pictures 1C and 1D.

References

1. Berrin K, Larco M. 1997. The spirit of ancient Peru: treasures from the Museo Arqueoló-gico Rafael Larco Herrera. New York: Thames and Hudson.
2. Canfield D, Brignolo L, Peterson PE, Hendrickx AG. 2000. Conjoined twins in a rhesus monkey (Macaca mulatta). J Med Primatol. 29:427–430.
3. Pachajoa H, Hernandez-Amaris MF, Porras-Hurtado GL, Rodriguez CA. 2014. Siamese twins with craniofacial duplication and bilateral cleft lip/palate in a ceramic representation of the Chimú culture (Peru): a comparative analysis with a current case. Twin Res Hum Genet. 17:211-4.
4. Millar AJW, Rode H, Thomas J, Hewitson J. 2009. Thoracopagus Conjoined Twins. In: Parikh DH, Crabbe DCG, Auldist AW, Rothenberg SS. (eds) Pediatric Thoracic Surgery. Springer, London.
5. Spitz L. 2005. Conjoined twins. Prenat Diagn. 25:814-9.
6. Casale P, Grady RW, Waldhausen JH, Joyner BD, Wright J, Mitchell ME. 2004. Cloacal exstrophy variants. Can blighted conjoined twinning play a role? J Urol. 172:1103-6, discussion 1106-7.
7. Kokcu A, Cetinkaya MB, Aydin O, Tosun M. 2007.Conjoined twins: historical perspective and report of a case. J Matern Fetal Neonatal Med. 20:349-56.
8. Tubbs RS, Ditty B, Bosmia AN, Bosmia AN. 2015. Ischiopagus and diprosopus in India: two pairs of conjoined twins perceived as incarnations of Hindu deities. J Relig Health. 54:87-92.
9. Bondeson J. 1992. The Biddenden Maids: a curious chapter in the history of conjoined twins. J R Soc Med. 85:217-221.
10. Mayer A. 2001. The Monstrous Birth: The Theory of Maternal Impression and Congenital Malformations. Proceedings of the 10th Annual History of Medicine Day: pp. 48–52.
11. DeSesso JM. 2019. The arrogance of teratology: A brief chronology of attitudes throughout history. Birth Defects Res. 111:123-141.
12. Beckwith JB. 2002. Congenital malformations: from superstition to understanding. Virchows Arch. 461:609-19.
13. Kaufman MH. 2004. The embryology of conjoined twins. Childs Nerv Syst. 20:508-25.
14. Spencer R. 2003. Conjoined Twins: Developmental Malformations and Clinical Implications. JHU Press, Baltimore and London.
15. Mutchinick OM, Luna-Muñoz L, Amar E, Bakker MK, Clementi M, Cocchi G, da Graça Dutra M, Feldkamp ML, Landau D, Leoncini E, Li Z, Lowry B, Marengo LK, Martínez-Frías ML, Mastroiacovo P, Métneki J, Morgan M, Pierini A, Rissman A, Ritvanen A, Scarano G, Siffel C, Szabova E, Arteaga-Vázquez J. 2011. Conjoined twins: a worldwide collaborative epidemiological study of the International Clearinghouse for Birth Defects Surveillance and Research. Am J Med Genet C Semin Med Genet. 157C:274-87.
16. Mian A, Gabra NI, Sharma T, Topale N, Gielecki J, Tubbs RS, Loukas M. 2017. Clin Anat. 2017. Conjoined twins: From conception to separation, a review. 30:385-396.
17. Bellairs R. 1953. Studies on the development of the foregut in the chick blastoderm. 2. The morphogenetic movements. J. Embryol. Exp. Morph. 1:369-385.
18. Azar Y and Eyal-Giladi A. 1981. Interaction of epiblast and hypoblast in the formation of the primitive streak and the embryonic axis in chick, as revealed by hypoblast rotation experiments. J. Embryol. Exp. Morphol. 61:133-144.

19. Ulshafer RJ, Clavert A. 1997. The use of avian double monsters in studies on induction of the nervous system. J Embryol Exp Morphol. 53:237-43.
20. Clavert J. 1962. Symmetrization of the Egg of Vertebrates. Adv Morphogen 2:27-60.
21. Waddington CH. 1933. Induction by the endoderm in birds. Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismen. 128:502–521.
22. Stern CD, Downs KM. 2012.The hypoblast (visceral endoderm): an evo-devo perspective. Development. 139:1059-69.
23. Rodriguez AM, Downs KM. 2017. Visceral endoderm and the primitive streak interact to build the fetal-placental interface of the mouse gastrula. Dev Biol. 432:98-124.
24. Bertocchini F, Stern CD. 2002. The hypoblast of the chick embryo positions the primitive streak by antagonizing nodal signaling. Dev Cell. 3:735-44.
25. Stower MJ, Srinivas S. 2014. Heading forwards: anterior visceral endoderm migration in patterning the mouse embryo. Philos Trans R Soc Lond B Biol Sci. 369.
26. Spemann H and Mangold H. 1924. Über Induktion vonEmbryonalanlagen durch Implantation artfremder Organisatoren. Roux's Arch. Dev. Biol. 100:599-638.
27. Seleiro EA, Connolly DJ, Cooke J. 1996. Early developmental expression and experimental axis determination by the chicken Vg1 gene. Curr Biol. 6:1476-86.
28. Ben-Haim N, Lu C, Guzman-Ayala M, Pescatore L, Mesnard D, Bischofberger M, Naef F, Robertson EJ, Constam DB. 2006. The nodal precursor acting via activin receptors induces mesoderm by maintaining a source of its convertases and BMP4. Dev Cell. 11:313-23.
29. Martyn I, Kanno TY, Ruzo A, Siggia ED, Brivanlou AH. 2018. Self-organization of a human organizer by combined Wnt and Nodal signalling. Nature. 558:132-135.
30. Skromne I, Stern CD. 2002. A hierarchy of gene expression accompanying induction of the primitive streak by Vg1 in the chick embryo. Mech Dev. 114:115-8.
31. Sutherland MJ, Ware SM. 2009. Disorders of left-right asymmetry: heterotaxy and situs inversus. Am J Med Genet C Semin Med Genet. 151C:307-17.
32. Sharma G, Mobin SS, Lypka M, Urata M. 2010. Heteropagus (parasitic) twins: a review. J Pediatr Surg. 45:2454-63.
33. Oostra RJ, Baljet B, Verbeeten BW, Hennekam RC. 1998. Congenital anomalies in the teratological collection of Museum Vrolik in Amsterdam, The Netherlands. V: conjoined and acardiac twins. Am J Med Genet. 80:74-89.
34. Lai HS, Lee PH, Chu SH, Chen MT, Lin TW, Duh YC, Chen WJ. 1997. Successful early separation of a premature xipho-omphalopagus conjoined twins: a case report. Can J Surg. 40:139-141.
35. Shukla RM, Mukhopadhyay B, Mandal KC, Saha K, Roy D, Mukherjee PP. 2010. Emergency separation of a xipho-omphalopagus twin in a developing country. Afr J Paediatr Surg. 7:197-9.
36. McHugh K, Kiely EM, Spitz L. 2006. Imaging of conjoined twins. Pediatr Radiol. 36:899-910.
37. Winkler N, Kennedy A, Byrne J, Woodward P. 2008. The imaging spectrum of conjoined twins. Ultrasound Q. 24:249-55.
38. Patil PS, Kothari P, Gupta A, Kekre G, Dikshit KV, Kamble R, Deshmukh S. 2016. Successful Separation of Omphalopagus Twins: A Case Report. J Neonatal Surg. 5:5.
39. O'Connell JE. 1976. Craniopagus twins: surgical anatomy and embryology and their implications. J Neurol Neurosurg Psychiatry. 39:1-22.
40. Stone JL, Goodrich JT. 2006. The craniopagus malformation: classification and implications for surgical separation. Brain. 129:1084-95.

41. Fieggen G, Millar A, Rode H, Ngiloi P, Andronikou S, Peter J. 2003. Spinal cord involvement in pygopagus conjoined twins: case report and review of the literature. Childs Nerv Syst. 19:183-7.
42. Bétoulières P, Caderas de Kerleau J, Gevaudand L. 1960. Etude radiologique de squelette d'un monstre double janicéphale-rachipage. Montpellier Med J. 58:30-39.
43. Durin L, Hors Y, Jeanne-Pasquier C, Barjot P, Herlicoviez M, Dreyfus M. 2005. Prenatal diagnosis of an extremely rare type of conjoined twins: cranio-rachi-pygopagus twins. Fetal Diagn Ther. 20:158-60.
44. Awasthi R, Iyengar R, Rege S, Jain N. 2015. Surgical management of pygopagus conjoined twins with spinal bifida. Eur Spine J. 24 Suppl 4:S560-3.
45. Machin GA, Sperber GH. 1987. Invited editorial comment: lessons from conjoined twins. Am J Med Genet. 28:89-97.
46. Oostra RJ, Keulen N, Jansen T, van Rijn RR. 2012. Absence of the spleen(s) in conjoined twins: a diagnostic clue of laterality defects? Radiological study of historical specimens. Pediatr Radiol. 42:653-9.
47. Levin M, Roberts DJ, Holmes LB, Tabin C. 1996. Laterality defects in conjoined twins. Nature. 28;384(6607):321.
48. Machin GA. 1993. Conjoined twins: implications for blastogenesis. Birth Defects Orig Artic Ser. 29:141-79.
49. Opitz JM, Herrmann J, Pettersen JC, Bersu ET, Colacino SC. 1979. Terminological, diagnostic, nosological, and anatomical-developmental aspects of developmental defects in man. Adv Hum Genet. 9:71-164.
50. Spranger J, Benirschke K, Hall JG, Lenz W, Lowry RB, Opitz JM, Pinsky L, Schwarzacher HG, Smith DW. 1982. Errors of morphogenesis: concepts and terms. Recommendations of an international working group. J Pediatr. 100:160-5.
51. Dudek W Ronald. BRS Embryology 6th Edition. 2014. Wolters Kluwer. Lippincott Williams & Wilkins, Philadelphia.
52. Sadler TW. 2010. Third month to birth: The fetus and placenta. In: Sadler, TW., editor. Langman's Medical Embryology. 11. Philadelphia: Lippincott Williams & Wilkins.
53. Czyz W, Morahan JM, Ebers GC, Ramagopalan SV. 2012. Genetic, environmental and stochastic factors in monozygotic twin discordance with a focus on epigenetic differences. BMC Med. 17:10-93.
54. McNamara HC, Kane SC, Craig JM, Short RV, Umstad MP. 2016. A review of the mechanisms and evidence for typical and atypical twinning. Am J Obstet Gynecol. 214:172-191.
55. Herranz G. 2015. The timing of monozygotic twinning: a criticism of the common model. Zygote. 23:27-40.
56. Singh S, Murphy B, O'Reilly R. 2002. Epigenetic contributors to the discordance of monozygotic twins. Clinical Genetics. 62:97-103.
57. Blickstein I, Keith LG. 2007. On the possible cause of monozygotic twinning: lessons from the 9-banded armadillo and from assisted reproduction. Twin Res Hum Genet. 10:394-9. Review.
58. Shur N. 2009. The genetics of twinning: from splitting eggs to breaking paradigms. Am J Med Genet C Semin Med Genet. 151C:105-9.
59. Tarlatzis BC, Qublan HS, Sanopoulou T, Zepiridis L, Grimbizis G, Bontis J. 2002. Increase in the monozygotic twinning rate after intracytoplasmic sperm injection and blasto-cyst stage embryo transfer. Fertil Steril, 77 p. 196.

60. Liu S, Hong Y, Cui K, Guan J, Han L, Chen W, Xu Z, Gong K, Ou Y, Zeng C, Li S, Zhang D, Hu D. 2018. Four-Generation Pedigree of Monozygotic Female Twins Reveals Genetic Factors in Twinning Process by Whole-Genome Sequencing. Twin Res Hum Genet. 21:361-368.
61. Kilby M, Baker P, Critchley H and Field D. 2006. Consensus views arising from the 50th study group: multiple pregnancy. RCOG Press, London; 283–286.
62. Mercan R, Oktem O, Salar Z, Nuhoglu A, Balaban B, Urman B. 2011. Conjoined twins after intracytoplasmic sperm injection and transfer of day-3 embryos. Fertil Steril. 96:111-4.
63. Jim C, Berkovich S. 2015. Labeling of Chromosomes in Cell Development and the Appearance of Monozygotic Twins. Biomed Res Int. 628092.
64. Sills ES, Tucker MJ, Palermo GD. 2000. Assisted reproductive technologies and monozygous twins: implications for future study and clinical practice. Twin Res. 3:217-23.
65. Song B, Wei ZL, Xu XF, Wang X, He XJ, Wu H, Zhou P, Cao YX. 2017. Prevalence and risk factors of monochorionic diamniotic twinning after assisted reproduction: A six-year experience base on a large cohort of pregnancies. PLoS One. 12:e0186813.
66. Hviid KVR, Malchau SS, Pinborg A, Nielsen HS. 2018. Determinants of monozygotic twinning in ART: a systematic review and a meta-analysis. Hum Reprod Update. Hum Reprod Update. 24:468-483.
67. Enders AC. 2002. Implantation in the nine-banded armadillo: how does a single blastocyst form four embryos? Placenta. 23:71-85.
68. Malter HE, Cohen J. 1989. Blastocyst formation and hatching in vitro following zona drilling of mouse and human embryos. Gamete Res. 24:67–80.
69. Guttmacher AF, Nichols BL. 1967. Teratology of conjoined twins. Birth Defects Orig Art Ser. 3:3-9.
70. Spencer R. 2000. Theoretical and analytical embryology of conjoined twins: part I: embryogenesis. Clin Anat. 13:36-53.
71. Spencer R. 1992. Conjoined twins: theoretical embryologic basis. Teratology. 45:591-602.
72. Kapur RP, Jack RM, Siebert JR. 1994. Diamniotic placentation associated with omphalopagus conjoined twins: implications for a contemporary model of conjoined twinning. Am J Med Genet. 52:188-95.
73. Bidondo MP, Groisman B, Tardivo A, Tomasoni F, Tejeiro V, Camacho I, Vilas M, Liascovich R, Barbero P. 2016. Diprosopus: Systematic review and report of two cases. Birth Defects Res A Clin Mol Teratol. 106:993-1007.
74. Fisher GJ. 1866. Diploteratology. Trans New York State Med Soc 133:207-298.
75. Wilder HH. 1908. The morphology of cosmobia: speculations concerning the significance of certain types of monsters. Am J Anat 8:353–440.
76. Ysander F, Wikstrom R. 1925. The deformity of the visceral skeleton in a prosopo-thoracopagous monster. Uppsala Lakare Forh. 30:211-226.
77. Tisler M, Schweickert A, Blum M. 2017. Xenopus, an ideal model organism to study laterality in conjoined twins. Genesis. 55(1-2).
78. Sokol S, Christian JL, Moon RT, Melton DA. 1991. Injected Wnt RNA induces a complete body axis in Xenopus embryos. Cell. 67:741-52.
79. Ziv T, Shimoni Y and Mitrani E. 1992. Activin can generate ectopic axial structures in chick blastoderm explants. Development. 115:689-694.
80. Beddington RS. 1994. Induction of a second neural axis by the mouse node. Development. 120:613-620.

81. Karnovsky A, Klymkowsky MW. 1995. Anterior axis duplication in Xenopus induced by the over-expression of the cadherin-binding protein plakoglobin. Proc Natl Acad Sci USA. 92:4522–4526.
82. Toyama R, O'Connell ML, Wright CV, Kuehn MR, Dawid IB. 1995. Nodal induces ectopic goosecoid and lim1 expression and axis duplication in zebrafish. Development. 121:383-91.
83. Molenaar M, van de Wetering M, Oosterwegel M, Peterson-Maduro J, Godsave S, Korinek V, Roose J, Destrée O, Clevers H. 1996. XTcf-3 transcription factor mediates beta-catenin-induced axis formation in Xenopus embryos. Cell. 86:391-9.
84. Pöpperl H, Schmidt C, Wilson V, Hume CR, Dodd J, Krumlauf R, Beddington RS. 1997. Misexpression of Cwnt8C in the mouse induces an ectopic embryonic axis and causes a truncation of the anterior neuroectoderm. Development. 124:2997-3005.
85. Zeng L, Fagotto F, Zhang T, Hsu W, Vasicek TJ, Perry WL 3rd, Lee JJ, Tilghman SM, Gumbiner BM, Costantini F. 1997. The mouse Fused locus encodes Axin, an inhibitor of the Wnt signaling pathway that regulates embryonic axis formation. Cell. 90:181-92.
86. Nascone N, Mercola M. 1997. Organizer induction determines left-right asymmetry in Xenopus. Dev Biol. 189:68-78.
87. Fang H, Marikawa Y, Elinson RP. 2000. Ectopic expression of Xenopus noggin RNA induces complete secondary body axes in embryos of the direct developing frog Eleutherodactylus coqui. Dev Genes Evol. 210:21-7.
88. Perea-Gomez A, Vella FD, Shawlot W, Oulad-Abdelghani M, Chazaud C, Meno C, Pfister V, Chen L, Robertson E, Hamada H, Behringer RR, Ang SL. 2002. Nodal antagonists in the anterior visceral endoderm prevent the formation of multiple primitive streaks. Dev Cell. 3:745-56.
89. Merrill BJ, Pasolli HA, Polak L, Rendl M, García-García MJ, Anderson KV, Fuchs E. 2004. Tcf3: a transcriptional regulator of axis induction in the early embryo. Development. 131:263-74.
90. Kawasumi A, Nakamura T, Iwai N, Yashiro K, Saijoh Y, Belo JA, Shiratori H, Hamada H. 2011. Left-right asymmetry in the level of active Nodal protein produced in the node is translated into left-right asymmetry in the lateral plate of mouse embryos. Dev Biol. 353:321-30.
91. Gardner RL. 2001. Specification of embryonic axes begins before cleavage in normal mouse development. Development. 128:839–847.
92. McCrea PD, Brieher WM, Gumbiner BM. 1993. Induction of a secondary body axis in Xenopus by antibodies to beta-catenin. J Cell Biol. 123:477-84.
93. Lu X, Gao Z, Qin D, Li L. 2017. A Maternal Functional Module in the Mammalian Oocyte-To-Embryo Transition. Trends Mol Med. 23:1014-1023.
94. Driever W, Nüsslein-Volhard C. 1988. The bicoid protein determines position in the Drosophila embryo in a concentration-dependent manner. Cell. 54:95-104.
95. Goldman CH, Gonsalvez GB. 2017. The Role of Microtubule Motors in mRNA Localization and Patterning Within the Drosophila Oocyte. Results Probl Cell Differ. 63:149-168.
96. Vandenberg LN, Levin M. 2012. Polarity proteins are required for left-right axis orientation and twin-twin instruction. Genesis. 50:219-34.
97. Weber MA, Sebire NJ. 2010. Genetics and developmental pathology of twinning. Semin Fetal Neonatal Med. 15:313-8.
98. Corner GW. 1955. The observed embryology of the human single-ovum twins and other multiple births. Am.J.Obstet.Gynecol. 70:933–51.

99. Kyono K. 2013. The precise timing of embryo splitting for monozygotic dichorionic diamniotic twins: when does embryo splitting for monozygotic dichorionic diamniotic twins occur? Evidence for splitting at the morula/blastocyst stage from studies of in vitro fertilization. Twin Res Hum Genet. 16:827-32.
100. Denker HW. 2015. Comment on G. Herranz: The timing of monozygotic twinning: a criticism of the common model. Zygote (2013). Zygote.;23:312-4.
101. Gardner RL. 2014. The timing of monozygotic twinning: a pro-life challenge to conventional scientific wisdom. Reprod Biomed Online. 28:276-8.
102. Wehbe SA, Tucker MJ, Palermo GD, Sills ES. 2003. Monozygotic twin delivery following reduction from quadramniotic-dichorionic gestation established after ICSI and embryo transfer: Case report. Hum Reprod. 18:444-6.
103. Yanaihara A, Ohgi S, Motomura K, Taniguchi R, Hatakeyama S, Yanaihara T. 2017. Dichorionic triplets following frozen-thawed poor-stage embryo transfer: a report of two cases and a review. Reprod Biol Endocrinol. 3;15:80.
104. Patil AS, Martin J, Tsukahara K, Skljarevski A, Miller K, Towns R, Schubert FP. 2015. Pseudomonoamniotic Pregnancy: Case Report and Review of Etiologic Considerations. Fetal Pediatr Pathol. 34:413-21.
105. Galjaard S, Moerman P, Corveleyn A, Devlieger R, Lewi L. 2014. Partial monochorionic and monoamniotic twin pregnancies: a report of two cases. Ultrasound Obstet Gynecol. 44:722-4.
106. Demy DL, Ranta Z, Giorgi JM, Gonzalez M, Herbomel P, Kissa K. 2013. Generating parabiotic zebrafish embryos for cell migration and homing studies. Nat Methods. 10:256-8.
107. Kamran P, Sereti KI, Zhao P, Ali SR, Weissman IL, Ardehali R. 2013. Parabiosis in mice: a detailed protocol. J Vis Exp. 6;(80).
108. Gianasi BL, Hamel JF, Mercier A. 2018. Full allogeneic fusion of embryos in a holothuroid echinoderm. Proc Biol Sci. 30;285 (1879).
109. Konstantinova B. 1976. Morphological and cytogenetic studies on conjoined twins. Acta Genet Med Gemellol (Roma). 25:55-8.
110. Müller J. 1848. Embryology, with the Physiology of Generation. Taylor, Walton and Maberly, London.
111. Logroño R, Garcia-Lithgow C, Harris C, Kent M, Meisner L. 1997. Heteropagus conjoined twins due to fusion of two embryos: report and review. Am J Med Genet. 73:239-43.
112. Gringras P, Chen W. 2001. Review Mechanisms for differences in monozygous twins. Early Hum Dev. 64:105-17.
113. Acuna-Hidalgo R, Bo T, Kwint MP, van de Vorst M, Pinelli M, Veltman JA, Hoischen A, Vissers LE, Gilissen C. 2015. Post-zygotic Point Mutations Are an Underrecognized Source of De Novo Genomic Variation. Am J Hum Genet. 97:67-74.
114. Petronis A, Gottesman II, Kan P, Kennedy JL, Basile VS, Paterson AD, Popendikyte V. 2003. Monozygotic twins exhibit numerous epigenetic differences: clues to twin discordance? Schizophr Bull. 29:169-78.
115. Castillo-Fernandez JE, Spector TD, Bell JT. 2014. Epigenetics of discordant monozygotic twins: implications for disease. Genome Med. 6:60.
116. Bruder CE, Piotrowski A, Gijsbers AA, Andersson R, Erickson S, Diaz de Ståhl T, Menzel U, Sandgren J, von Tell D, Poplawski A, Crowley M, Crasto C, Partridge EC, Tiwari H, Allison DB, Komorowski J, van Ommen GJ, Boomsma DI, Pedersen NL, den Dunnen JT, Wirdefeldt K, Dumanski JP. 2008. Phenotypically concordant and discordant monozygotic twins display different DNA copy-number-variation profiles. Am J Hum Genet. 82:763-71.

117. Itsara A, Wu H, Smith JD, Nickerson DA, Romieu I, London SJ, Eichler EE. 2010. De novo rates and selection of large copy number variation. Genome Res. 20:1469-81.
118. Boklage CE. 2009. Traces of embryogenesis are the same in monozygotic and dizygotic twins: not compatible with double ovulation. Hum Reprod. 24:1255-66.
119. Walker M, Browd SR. 2004. Craniopagus twins: embryology, classification, surgical anatomy, and separation. Childs Nerv Syst. 20:554–566.
120. Bolk L. 1926. Een zeer merkwaardig menschelijk dubbelmonstrum: cranioamniopaag. Ned Tijdschr Geneeskunde. 70:1878-1889.
121. Fraser RB, Liston RM, Thompson DL, Wright JR Jr. 1997. Monoamniotic twins delivered liveborn with a forked umbilical cord. Pediatr Pathol Lab Med. 17:639-44.
122. Tabata T, Takei Y. 2004. Morphogens, their identification and regulation. Development. 131:703-12.
123. Gilbert-Barness E, Debich-Spicer D, Opitz JM. 2003. Conjoined twins: morphogenesis of the heart and a review. Am J Med Genet A. 120A:568-82.
124. Schoenwolf GC, Bleyl SB, Brauer PR, Francis-West PH. 2009. Larsen's Human Embryology (8th ed.), Churchill Livingstone, New York.
125. Kompanje EJ. 2006. Very rare congenital malformations: responsibilities of scientists. The case of rachipagus conjoined twins. Fetal Diagn Ther. 21:319-20.
126. Hoekstra C, Zhao ZZ, Lambalk CB, Willemsen G, Martin NG, Boomsma DI, Montgomery GW. 2008. Dizygotic twinning. Hum Reprod Update 14:37-47.

The Future of Dutch Teratological Collections

7 Radiological Imaging of Teratological Fetuses: What can we Learn?

This chapter has been published as:

Lucas L. Boer, Annelieke N. Schepens-Franke, Jack. J.A. van Asten,
Dennis G.H. Bosboom, Karin Kamphuis-van Ulzen,
Tamas L. Kozicz, Dirk J. Ruiter,
Roelof-Jan Oostra and Willemijn M. Klein.
Radiological imaging of teratological fetuses: what can we learn? 2017.
Insights Imaging. 8:301-10. doi: 10.1007/s13244-017-0551-8.

Abstract

The objective of this study was to determine the advantages of radiological imaging a collection of full-term teratological fetuses in order to increase its scientific and educational value. Anatomical museums around the world exhibit full-term teratological fetuses. Unfortunately, these museums are regularly considered as "morbid cabinets." Detailed dysmorphological information concerning the exhibited specimens is often lacking. Moreover, fetuses with severe and complex congenital anomalies are frequently diagnosed incompletely, incorrectly or not at all. To verify diagnoses and to enrich their educational and scientific value we imaged 41 out of the 72 teratological specimens present in the collection of our Anatomical Museum in Nijmegen (The Netherlands) by means of magnetic resonance imaging (MRI) and computed tomography (CT). Additionally, contemporary dysmorphological insights and 3D models are implemented in the teratology education of medical students and residents. We conclude that full-term teratological fetuses become increasingly rare and deserve a prominent place in every anatomical museum; they are suitable for contemporary teratological research and education. Modern radiological techniques markedly enhance their scientific and didactic value.

Introduction

Many anatomical museums around the world exhibit teratological specimens of 3rd trimester fetuses. Among the institutionalized collections, especially noteworthy are the 18th century collection of the Federal Pathologic-Anatomical Museum in Vienna (Austria), [1,2] the 18th century collection of the Hunterian Museum of the Royal College of Surgeons in London [3] and the 19th century *Vrolik* collection residing in the Museum *Vrolik* of the University Medical Centre of Amsterdam. [4] They all contain a rich trove of teratological specimens. Although some academic institutions have abandoned their anatomical collections because of apparent legal issues, safety reasons, financial cuts or newly defined priorities, these museums are much more than time capsules with accumulations of curiosities. [5] They can be regarded as vibrant, inspirational, instructive and interdisciplinary academic working environments with scientific and educational potentials that can be exploited in (bio)medical curricula or in resident training programs. [6] However, one might wonder if anatomical museums should still exhibit full-grown dysmorphic fetuses as these types of anomalies are rarely occurring events in modern times. Moreover, one could question whether historical teratological specimens still have a contemporary value in a period of daily evolving medical innovations and molecular technology. These are issues anatomical museums have to deal with on a daily basis. [7,8]

Congenital anomalies have intrigued mankind since the earliest times. Already in ancient cultures terracotta ornaments were fabricated depicting congenital anomalies. These were clearly based upon existing cases, indicating that they were perceived as divinities, omens or even punishments of supernatural origin. [9,10] Nowadays, people with very diverse backgrounds visit teratological collections residing in medical museums. Knowledge of both the normal and abnormal embryological development is important for both teachers and physicians while medical students and patients have a constantly growing medical knowledge and ask more sophisticated questions. [6] This implies that a teratological collection can be of great value to educate people about human development. Although several anatomical museums expose teratological specimens, most institutions lack detailed external and internal (dys)morphological descriptions or imaging. Because of this, collections are often stigmatized as “morbid cabinets”.

The teratological collection of the Museum for Anatomy and Pathology in Nijmegen (The Netherlands) currently possesses 72 specimens. Between the 1950’s and 1980’s it was collected by Dr. Albert Verhofstad († 2008) who was affiliated at the Radboud university medical center in Nijmegen. The

collection originates from before the ubiquitous availability and utilization of (high-resolution) ultrasound for prenatal screening and therefore most specimens are full-grown fetuses or newborns. Nowadays full-grown fetuses with severe congenital anomalies are rarely born in well-developed countries. This implies that teratological collections become more valuable with time.

In the past, our collection of teratological fetuses too was often seen as a "morbid cabinet" by both students and the general public. In the exhibition, there was neither a clear choice of the exhibited fetuses nor was there any systematic approach recognizable; questions about the nature and pathogenesis of several congenital anomalies could not be answered. Therefore, in order to systematically expose the teratological collection of our museum, we defined 9 anomaly groups in which all 72 specimens could be categorized. This categorization led to a new exhibition in which 35 of the most educational specimens found a permanent expository position (Fig.1) (see Addendum).

Fig. 1. Photograph of the teratological collection in the Museum for Anatomy and Pathology of the Museum for Anatomy and Pathology in Nijmegen (The Netherlands). The exhibit displays 35 teratological fetuses, 10 specimens of animal teratology, historical books on teratology, 3D models and plaster casts.

In order to increase the scientific and educational value of the specimens of this unique teratological collection, we wanted to elucidate the internal (dys)morphological characteristics. Instead of invasive exploration, CT and MR techniques were used to generate detailed images of the internal (dys)morphology of the teratological fetuses. Radiological imaging proved to be an excellent method to investigate these delicate specimens in a non-invasive manner.[11] Recently, the museum opened an innovative exhibition of specimens documented with these images. This exhibition is accessible for both medical students and the general public. Radiological imaging and information about normal development and pathogenesis can be obtained by consulting a touchscreen in which all specimens are described in full detail (Fig. 2). Common information is given on physical billboards throughout the exposition.

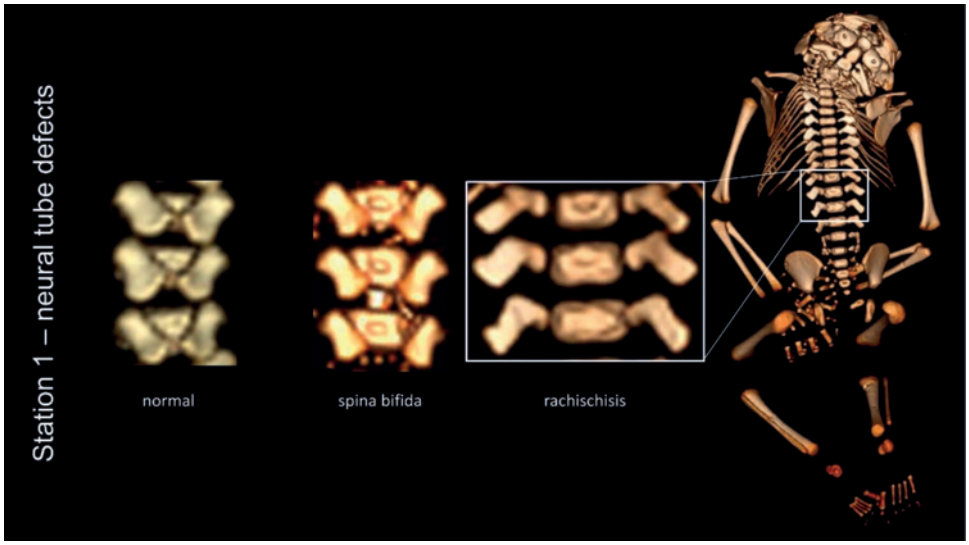


Fig. 2. Screenshot of the interactive information of neural tube defects using radiological imaging: vertebrae of a fetus with rachischisis can be compared to vertebrae of a normal fetus and a fetus with spina bifida.

Here we report on the radiological imaging results and we describe four fetuses (case 1-4) in which new diagnoses or interesting morphological characteristics were established. Furthermore, we discuss the scientific and educational benefits that can be gained from radiological imaging of dysmorphic fetuses. The purpose of this paper is to present an approach to create an innovative teratological exposition in order to de-stigmatize and to more profoundly educate the (bio)medical student and professional.

Materials and methods

The entire collection of 72 teratological fetuses was visually inspected and redescribed according to contemporary syndromological views by a panel of experts in 2012 (RJO, ANSF and LLB). Verification of the identified syndromes and sequences was obtained by consulting peer groups and contemporary handbooks on clinical syndromology. [12,13] Several anomaly groups were defined according to the classification of congenital anomalies described by the European Surveillance of Congenital Anomalies. [14] Radiological imaging was used to generate detailed images of the internal (dys)morphology. An inclusion criterion for radiological imaging was that the specimen had not been previously subjected to autopsy in the area of interest. Furthermore, the group with neural tube defects consisted of 20 fetuses. From these fetuses, six fetuses were selected: three that displayed iniencephaly and three with a variety of other neural tube defects. This resulted in the radiological imaging of 41 specimens. Radiological imaging consisted of a total body MRI and total body CT; scanning protocols are described below. Prior to scanning, the specimens were taken out of their jars, thoroughly rinsed with demineralized water and placed in disposable plastic bags to prevent dehydration during imaging. After imaging, the specimens were replaced in a 4% formaldehyde solution. Radiological data were reviewed by three radiologists with expertise in pediatric neurological (KKvU), cardiothoracic (DGHB) and abdominal/musculoskeletal (WMK) radiology, all with previous expertise in fetal postmortem imaging.

1) MRI protocol

Specimens were scanned on a TIM TRIO 3 Tesla MRI scanner (SIEMENS, Erlangen, Germany). Specimens smaller than 30 cm in length were placed in a standard circular head and neck coil, specimens over 30 cm in length were measured with an additional body coil. MRI scan parameters of the clinical fetal postmortem MRI were transformed to optimize the spatial resolution of the specimens as they had stayed in a 4% formaldehyde solution for 50-60 years. [15,16] An overview of the MRI parameters is given in table I.

2) CT protocol

The specimens underwent a total body CT scan in a CT scanner (Aquilion One Vision Edition, Toshiba, Japan). Two protocols were used to cover the different sizes of the specimens; one volume scan mode for fetuses smaller than 16 cm and one helical scan mode for fetuses larger than 16 cm. 3D reconstructions were made using a filter convolution (FC) of 30 for bone and a FC of 07 for soft tissue, both with adaptive iterative dose reduction (AIDR) in 3D. An overview of the CT protocol parameters is listed in table II.

Table I: Summary of the MRI sequence parameters

Sequence	Voxel (mm^3)	Fov (mm)	TE (ms)	TR (ms)	NA	Flipangle (o)	TA (min)
T1w flash 3D	~0.5x0.5x0.5	300-400	5	11-13	6-8	25	90
T2w TSE 3D (SPACE)	~1.2x0.7x0.5	300-400	184-479	3280	4-9	Var Exc.	30-45

Fov: field of view, TE: echo time, TR: repetition time, NA: number of averages, flipangle: RF power, TA: total acquisition time.

Table II: CT parameters

Protocol	Potential (kV)	Current (mA)	Rotation (s)	ΔSlice (mm)	Increment (mm)	Pitch	Collim. (mm)
Scan < 16cm	80	300	1	0.5	0.25	0 (vol.)	scanlen·0.5
Scan > 16cm	80	400	1	0.5	0.25	0.813	80 · 0.5

Results

We performed CT and MRI scanning on 41 of the 72 teratological specimens. The radiological imaging had no effects on the condition of the specimens and no complications in the specimen conservation were discovered. CT and MR images were found to be of very high quality. Although, we encountered some problems with postmortem artifacts, e.g. shrinkage of the brain, decalcified skeletons and unusable radiographic skeleton surveys, most radiological data was of sufficient quality to rediagnose and describe the internal characteristics of each fetus. An overview of the scanned fetuses is given in table III. We give an extensive description of a selection of four cases below.

Skeletal dysplasias

Case 1 concerns a full-term large male stillborn which showed on external examination a disproportionate micromelic shortening of all extremities, a narrow "bell-shaped" thorax, protuberant abdomen and relatively large scrotum. All extremities showed redundant skin folds with severe brachydactyly and mildly affected trident hands. Craniofacial abnormalities included macrocephaly, severe frontal bossing, prominent cheeks and chin, ocular proptosis, a depressed nasal bridge and a severe hypoplastic midface with hypertelorism, a prominent tongue and slightly recessed ears (Fig. 3A). The former diagnosis of this specimen, before re-examination in 2012, was achondroplasia. The CT images revealed that the calcification of the bones was severely diminished (Fig. 3B). MRI images did reveal the contours of the bones. Based on the combination of the micromelic shortening of all extremities, bowing of both femora, short ribs, bell-shaped thorax with small chest cavity and presumably hypoplastic lungs, platyspondyly of the vertebra and the polymicrogyria we diagnosed the condition as Thanatophoric Dysplasia (TD) type I (Fig. 3B-F). Neonatal death was most likely due to respiratory insufficiency and/or compression of the spinal cord or brainstem by spinal stenosis.

Table III: Diagnostic revision in 41 scanned teratological fetuses

Anomaly group ↓	Previous diagnosis ↓	Diagnosis after radiology ↓
Ventral body wall defects	- ventral body wall defect with cleft lip and encephalocele - ventral body wall defect with neural tube defect - ventral body wall defect (3x)	- amniotic band sequence with concomitant ectopia cordis, unilateral CLP[1] and unilateral temporal encephalocele - vascular disruption sequence with concomitant occipital encephalocele and gastroschisis - OEIS[2] complex with concomitant omphalocele - OEIS complex with concomitant gastroschisis and ambiguous genitalia - OEIS complex with concomitant gastroschisis and spina bifida
Skeletal dysplasias (osteochondrodysplasias)	- achondroplasia (3x)	- thanatophoric dysplasia Type I *(Case 1)* - osteogenesis imperfecta Type II *(Case 2)* - short-rib polydactyly syndrome Not Otherwise Specified
Congenital teratomas	- teratoma	- oropharyngeal teratoma / epignathus
Conjoined twins	- conjoined twin (9x)	- cephalothoracoileopagus - prosopo-ileopagus - thoracoileopagus tribrachius - thoracoilieopagus tetrabrachius - ischiopagus tripus - ischiopagus tetrapus - diprosopus tetrophthalmus diotis with concomitant craniorachischisis totalis - parapagus dicephalus dibrachius dipus *(Case 3)* - craniopagus
Syndromes with multiple congenital anomalies	- syndrome (2x) - phocomelia	- Meckel-Gruber Syndrome - bilateral schisis (most likely trisomy 13) - tetra-amelia syndrome *(Case 4)*
Sirenomelia	- sirenomelia (7x)	- isolated sirenomelia type I (3x) - isolated sirenomelia type II - VACTERL[3] association with concomitant sirenomelia type II - VACTERL-H[4] association with concomitant sirenomelia type I - VACTERL-H association with concomitant sirenomelia type II
Holoprosencephaly	- cyclopia (6x)	- alobar HPE[5] (4x) - alobar HPE with concomitant otocephaly (2x)
Neural tube defects	- iniencephaly (3x) - occipital encephalocele / exencephaly - craniorachischis - craniorachischis totalis	- iniencephaly - iniencephaly with concomitant semi-lobar HPE and omphalocele - iniencephaly with concomitant myelomeningocele - occipital encephalocele / exencephaly - craniorachischis - craniorachischis totalis
Unknown specimen	- unknown diagnose	- unknown diagnose
	[1] CLP = cleft lip and palate *[2] OEIS = omphalocele-exstrophy-imperforate anus-spinal defects* *[3] VACTERL = vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies and limb abnormalities* *[4] VACTERL-H = vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies and limb abnormalities with hydrocephaly* *[5] HPE = holoprosencephaly*	

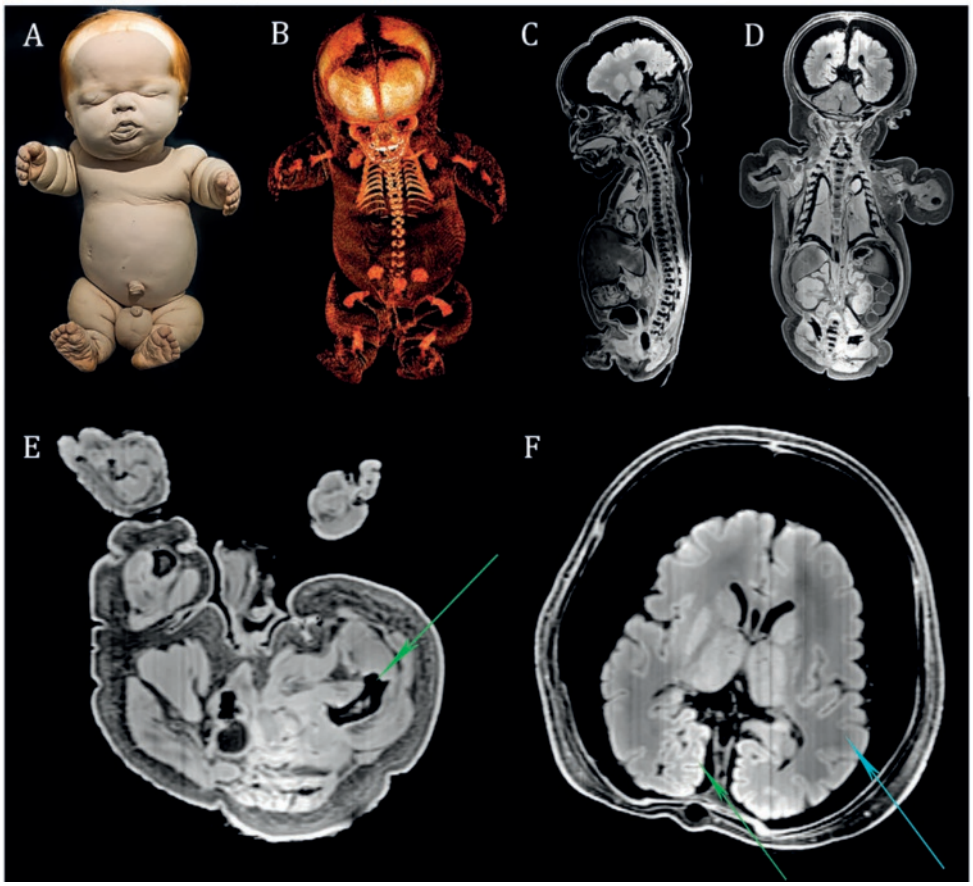


Fig. 3. [A] Photograph of the full-term fetus of case 1. [B] 3D reconstructed skeleton based on the CT data. Although there was severely diminished bone calcification, CT images visualized extremely shortened ribs, short and small scapulae and iliac bones, and femoral and humeral bowing. [C] Sagittal T1-weighted MR image which showed a small foramen magnum with slight cranio-cervical caudal transition, platyspondyly of the vertebra with short vertebral arches resulting in spinal canal stenosis. [D] Coronal T1-weighted MR images showed a severely hypoplastic thorax with presumably severe lung-hypoplasia. [E] Transverse T1-weighted MR image on the level of the femoral heads showed broad and irregular metaphyseal plates and extreme femoral bowing (green arrow) sometimes referred to as 'telephone receiver' femora. [F] Transverse T1-weighted MR image of the brain which showed courser gyri of the temporal lobes (turquoise arrow) and excessive gyration of the occipital lobes (green arrow) and can be interpreted as polymicrogyria. Note the shrunken brain and small lungs which were both interpreted as normal postmortem artifacts and probably strengthened by the formalin fixation. However, the presence of lung hypoplasia cannot be ruled out.

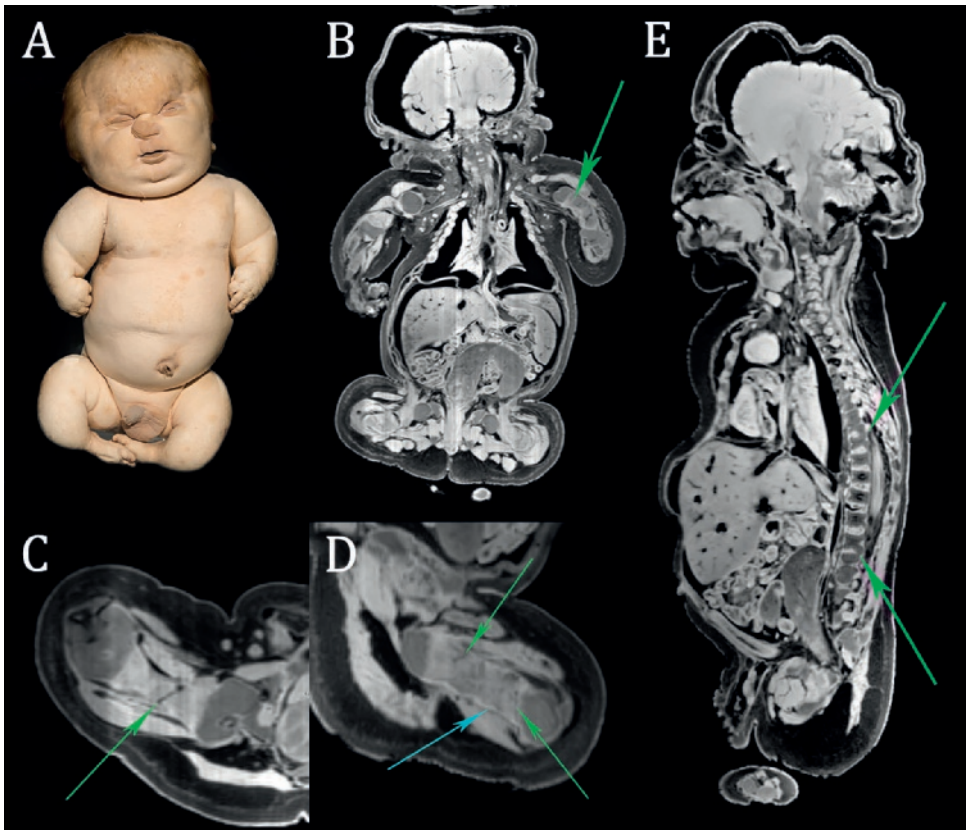


Fig. 4. [A] Photograph of the full-term fetus of case 2 demonstrating a characteristic facial appearance, protruding abdomen and severely bowed extremities. [B] Coronal T1-weighted MR image showed an aberrant and irregular humerus (green arrow). Note the severely shrunken lungs insight the hypoplastic thorax; intepreted as shrinkage of the lungs due to postmortem artifects and formalin fixation. However, the presence of lung hypoplasia cannot be ruled out. [C] Transverse T1-weighted MR image of the broadened and shortened right femoral bone which showed a fracture (green arrow). [D] Transverse T1-weighted MR image of the severely shortened and aberrant left femoral bone which showed multiple fractures (green arrows) and irregular cortical bone (turquoise arrow). [E] Sagittal T1-weighted MR image showed multiple fractures in the vertebral column (green arrows). Note the distorted calvarium due to limited mineralization and the shrunken brain due to the formalin fixation.

Case 2 concerns a full-term male neonate which showed on external examination a protruding abdomen, excessive bowing of all extremities and mesomelic shortened arms. Craniofacial abnormalities included a hypoplastic midface, microstomia, recessed ears and a somewhat flattened face. The head appeared to be positioned directly on the thorax with absence of the neck. The lower extremities were positioned in a frog-like position (Fig.4A). The diagnose of this specimen, before re-examination in 2012, was achondroplasia. The calcification of the bones was severely diminished, resulting in non-diagnosable CT images (not shown). MR images however did reveal the contours of the bones. Based

on the distinct presence of multiple prenatal fractures, poorly mineralized and deformed cranial vault, secondarily healed osseous structures and small chest with presumably lung hypoplasia (Fig. 4B-E) the diagnose Osteogenesis Imperfecta (OI) type II was made with reasonable certainty.

Conjoined twin

Case 3 concerns a small full-term female conjoined twin with two heads, two arms and two legs (Fig. 5A). The diagnose of this specimen, before re-examination in 2012, was conjoined twin. After redescribing and imaging this fetus we diagnosed this specimen as parapagus dicephalus dibrachius dipus (See discussion). Both CT (Fig. 5B) and MR (Fig. 5C-G) data were of excellent quality to describe the intricate internal dysmorphological characteristics. Additionally, based on the MR data a schematic drawing was made of the morphology of the "fused" heart in order to get insight into the complex hemodynamic situation (Fig. 6).

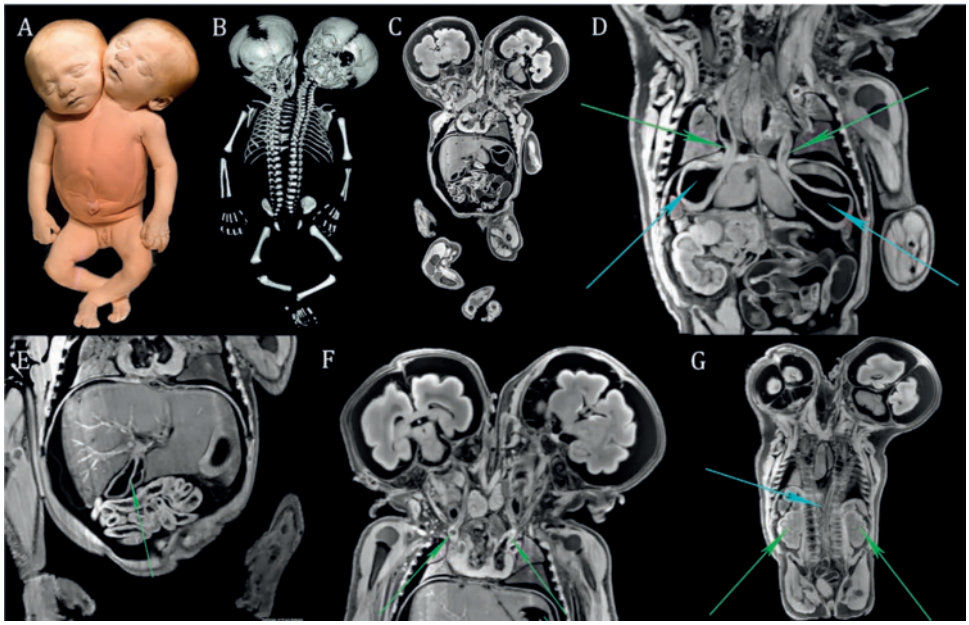


Fig. 5. [A] Photograph of the full-term conjoined twin of case 3. [B] 3D reconstructed skeleton based on the CT data showed butterfly- and block vertebra with fused ribs between the two separate vertebral columns, two heads, two arms and two legs with one broad pelvis. [C] Coronal T1-weighted MR image showed two normal brains, one shared "fused" heart and liver with one overarching diaphragm. [D] Coronal T1-weighted MR image showed two separate esophagi (green arrows) and two separate stomachs (turquoise arrow). [E] Coronal T1-weighted MR image showed a "fused" liver with two gallbladders (green arrow). [F] Coronal T1-weighted MR image showed two ascending aortas (green arrows). [G] Coronal T1-weighted MR image showed two kidneys and adrenal glands (green arrows) and one anus. The two descending aortas fused at the level of the 11th thoracic vertebra (turquoise arrow). Note that the right descending aorta is smaller than the left descending aorta.

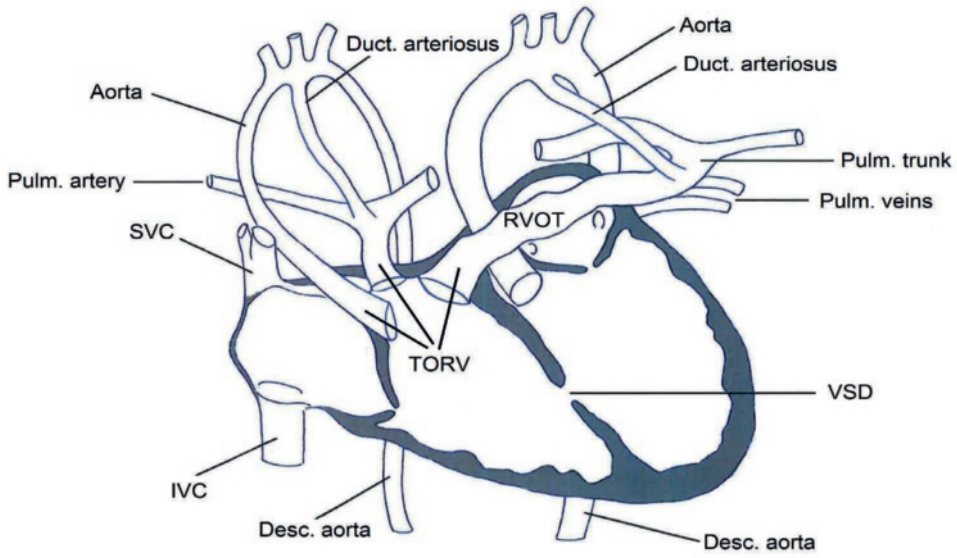


Fig 6. Schematic drawing of the fused heart of case 3. There were two ventricles and two atria. A normally located left aorta arose from the left ventricle. At the right atrium small pulmonary veins were seen. From the right ventricle the second, relative smaller, right aorta, right pulmonary trunk and a Right Ventricle Outflow Tract (RVOT) of the left pulmonary trunk were seen; this could be interpreted as a Triple Outflow Right Ventricle (TORV). There were two ducti arteriosi. On the right side no clear pulmonary veins were seen. There was a Ventricular Septal Defect (VSD) resulting in a complex hemodynamic situation.

Tetra-amelia syndrome

Case 4 concerns a full-term female neonate which showed on external examination total absence of all four limbs, micrognatia, microstomia, mild Potter's facies and hypertelorism. A left oriented deviation of the relative small body is noticeable (Fig. 7A). The diagnose of this specimen, before re-examination in 2012, was phocomelia. The CT images reveal that the calcification of the bones is severely diminished (Fig. 7B). MR images reveal the contours of the bones (Fig. 7C and D). Additionally, we found a concomitant diaphragmatic hernia, skeletal anomalies and a Arnold-Chiari malformation. We diagnosed the condition as tetra-amelia syndrome with a concomitant diaphragmatic hernia; a rarely described association. [17]

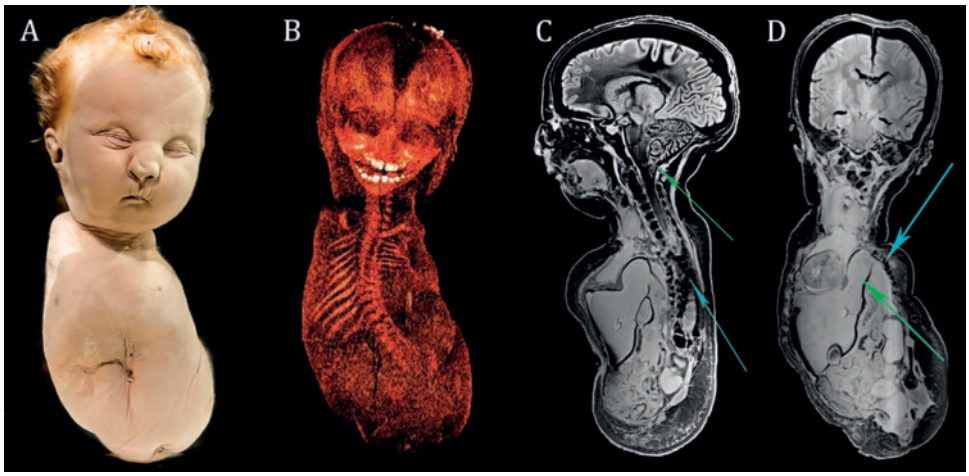


Fig. 7. [A] Photograph of the full-term fetus of case 4. [B] 3D reconstructed skeleton based on the CT data. Although there was severely diminished bone calcification, CT images visualized small pelvic bones, partial absence of the sacral bone and a thoracolumbar convex shaped curvature. The scapulae and clavicles were normally developed with absence of the humeral, ulnar and radial bones including the hands. In addition, the femora, tibial and fibular bones including the feet were absent. [C] Sagittal T1-weighted MR image showed an Arnold-Chiari malformation (green arrow) and distorted vertebral column (turquoise arrow). [D] Coronal T1-weighted MR image showed a severe diaphragmatic hernia (green arrow) which deviated the heart completely to the right lateral thoracic wall. The left pleural cavity was almost entirely occupied by the liver, stomach, spleen and left adrenal gland, the left lung was merely noticeable. Moreover, a distortion of the left ribs was seen (turquoise arrow).

Discussion

Although many anatomical museums display teratological fetuses on a smaller or larger scale, these displays usually lack comprehensive pathogenetic storylines, additional radiological imaging of the exposed specimens, and most importantly, they often neglect their potential value in biomedical curricula. Furthermore, the diagnoses that fetuses bear are often incomplete, incorrect or outdated. As we demonstrated here, radiological imaging combined with contemporary dysmorphological knowledge was in most cases valuable or even essential to arrive at the correct diagnosis and to unveil the internal and sometimes unexpected peculiarities. Nowadays, many congenital and inherited anomalies can be diagnosed genetically. However, embalmed museological specimens frequently have fragmented and contaminated DNA which is unsuitable for genetic exploration of candidate genes. We tried Molecular Inversion Probe techniques (MIP) for targeted sequencing of genomic regions with potential candidate genes of multiple fetuses, unfortunately, without satisfying result.

Cases 1 and 2 concern two distinct skeletal dysplasias, or osteochondrodysplasias, most of which originate from genetic defects that cause aberrant histological formation, growth and maturation of osseous and/or cartilaginous tissues. They usually affect all skeletal elements equally, leading to a decreased postural length (dwarfism). Therefore, skeletal dysplasias can be seen as generalized qualitative disorders of the skeleton, without primarily affecting the body plan. [18] Although achondroplasia is a specific diagnosis among the more than 300 skeletal dysplasias presently known, it has long been used as a generic term for any type of skeletal dysplasia, as it was in the cases described here. Despite the decalcification of the skeleton, which was probably largely caused by decalcification of the bone tissue due to the acidification of formalin through time, [19] radiological imaging made it possible to diagnose TD type I in case 1 and OI type 2 in case 2. TD is genetically related to (true) achondroplasia but it is much more severe, whereas OI is caused by a genetic defect in collagen formation which leads to (extremely) brittle bones. The imaging results demonstrate the pathogenesis, severity and potential lethality of the conditions in these cases, which markedly adds to their didactic value.

Case 3 concerns a pair of conjoined twins. Despite being a rare congenital malformation with an incidence of 1:200,000 live births and 1:200 monozygotic twins, it is a widely known phenomenon among scientists and laymen alike. [20] Throughout many centuries multiple rather enigmatic pathogenic hypotheses have been postulated, none of which satisfactorily explains their pathogenesis and conjunctional morphology. An intriguing though not undisputed theory was postulated by Spencer in 2003. [21] Her model hypothesizes the presence of two (instead of one) embryonic primordial disks "floating" on the surface of a shared yolk sac (resulting in ventral and lateral conjunction types) or on a shared amniotic cavity (resulting in dorsal/neural conjunction). This "spherical coalescence" theory therefore postulates a secondary, symmetrical or asymmetrical, homologous conjunction of initially separate embryonic disks and subsequent embryonic fusion. The nature and extent of the conjunction result from the initial reciprocal distance and position of the two primordial disks on the yolk sac or amniotic cavity. Case 3 concerned a parapagus dicephalus dibrachius dipus conjoined twin, which can be concluded from external dysmorphological findings. However, radiological imaging revealed the intricate internal (dys)morphology and conjunction of organs, such as the heart and liver in this specific type, which is essential to understand the pathogenesis of conjoined twinning.

Finally, case 4 presented with tetra-amelia syndrome: an extremely rare disorder characterized by the absence of all four limbs. Infants are often

stillborn or die perinatally due to lung hypoplasia and concomitant anomalies such as microstomia and micrognathia. No estimates on prevalence are described due to its rarity. After radiological imaging we found a diaphragmatic hernia in concomitance with tetra-amelia: this is only rarely found and scarcely described in modern literature. [17,22] Although, diaphragmatic hernia is atypical in tetra-amelia syndrome, the acquired images can be used to educate the medical students the subjects of congenital diaphragmatic hernias and the secondary effect on thoracic organ development.

The most convincing argument for radiological imaging a collection of teratological fetuses is the dramatic increase of internal dysmorphological insight in a non-invasive manner. Although many teratological fetuses can be diagnosed and used in an educational setting based on their external dysmorphological appearance, radiological imaging increases the diagnostic value immensely in rediagnosing anomalies (e.g. skeletal dysplasias), specifying anomaly subtypes (e.g. in osteogenesis imperfecta) or in teaching certain embryological oriented pathogeneses (e.g. conjoined twins). Moreover, radiological findings can strengthen arguments regarding pathogenetic hypotheses and thus lead to new or improved insights.

Because of currently available prenatal screening options, pregnancies complicated by congenital anomalies are often terminated well before full-term development. Nowadays, stillborn fetuses in general, let alone fetuses with rare congenital anomalies, are almost never assigned to scientific body donation programs. This results in an absence of supplementing teratological collections which makes historical specimens of teratological full-term fetuses increasingly valuable and irreplaceable.

We posit that when well defined teratological specimens are displayed respectfully with additional pathognomonic storylines and radiological data, these exhibitions are educationally legitimate and instructional for any museum visitor. The acquired radiological data are essential to educate the student and the resident on the subject of teratology. Additionally, these high resolution radiological images can be used to help the obstetrician to recognize congenital anomalies during prenatal screening. Radiological techniques transform the “old and dusty” anatomical museums into modern academic and dynamic working environments suitable to educate the student as well as the (pediatric) radiologists in training. Moreover, radiological imaging teratological collections makes students wonder and enthusiastic about the use of radiology in their curriculum and learn to compare images with the observed (museological) specimen. Finally, radiological findings can strengthen arguments regarding embryologic oriented pathogenetic hypothesizes. By imaging and rediagnosing teratological specimens that

display a similar condition congenital anomalies can be studied beyond the limitations of single case studies and the spectrum or heterogeneity of a congenital anomaly becomes more clear. Therefore, we conclude that teratological collections are a treasure chest for radiologists, pediatricians, geneticists, pathologists and embryologists and are of interest for additional (re)describing and imaging following new imaging techniques.

Conclusion

Teratological specimens are becoming increasingly rare and deserve a prominent place in anatomical museums. These collections are very suitable for contemporary teratological research and can be used for public and medical education. As shown in this paper, radiological imaging is essential to reveal all the diagnostic ins and outs of old teratological specimens.

Acknowledgments

We thank the *Reinier Post Foundation* of the Radboud University medical center and Radboud University in Nijmegen (The Netherlands) for providing a grant entitled "*The Anatomical Museum of the Future.*" Furthermore, the authors are greatly indebted to Mr. Tim Rijnhout for his pictorial support.

References

1. Beighton P, Sujansky E, Patzak B, Portele KA. 1993. Genetic skeletal dysplasias in the Museum of Pathological Anatomy, Vienna. Am J Med Genet. 47:843-7.
2. Beighton P, Sujansky E, Patzak B, Portele KA. 1994. Bone dysplasias of infancy in the Vienna collection. Pediatr Radiol. 24:384-6.
3. Williams E, Farrell M. 2013. Hunterian Museum bicentenary: 1813-2013. Br Dent J. 215:41-3.
4. De Rooy L, Moorman A. 2011. De facelift van Museum Vrolik. Ned Tijdschr Geneeskd. 155:A4241.
5. Mareez Y, Willems L, Wells M. 2010. The role of medical museums in contemporary medical education. Anat Sci Educ. 3:249-253.
6. Carlson B. 2002. Embryology in the Medical Curriculum. Anat Rec (New Anat). 269:89–98.
7. Beckwith B. 1998. Museums, Antiquarian Books, and modern teratology. American Journal of Medical Genetics. 77:89–90.
8. Charlier P, Hervé C. 2016. What new life for the Parisian (and European) anatomical and pathological collections? Struggle for medical education. Clin Anat. 30:432-433.
9. Beckwith B. 2012. Congenital malformations; from superstition to understanding. Virchows Arch. 461: 609-619.
10. Goodrich JT. 2008. A historical review of the surgical treatment of spina bifida. Özek MM, Cinall G, Maixner WJ: *The Spina Bifida. Management and Outcome* Milan, Springer. 3–17.
11. Oostra RJ, Keulen N, Jansen T, van Rijn RR (2011) Absence of the spleen(s) in conjoined twins: a diagnostic clue of laterality defects? Radiological study of historical specimens. Pediatr Radiol. 42:653-659.
12. Hall S. 2006. Human malformations and related anomalies. Second edition. New York: Oxford University Press.
13. Gorlin RJ, Cohen MM, Levin LS. 1990. Syndromes of the Head and Neck. New York: Oxford University Press.
14. European surveillance of congenital anomalies, Italy. Online available via http://www.eurocat-network.eu/
15. Thayyil S, Sebire NJ, Chitty LS, Wade A, Olsen O, Gunny RS, Offiah A, Saunders DE, Owens CM, Chong WK, Robertson NJ, Taylor AM. 2011. Post mortem magnetic resonance imaging in the fetus, infant and child: A comparative study with conventional autopsy (MaRIAS Protocol). BMC Pediatrics. **11**:120.
16. Sieswerda-Hoogendoorn T, van Rijn RR. 2010. Current techniques in postmortem imaging with specific attention to pediatric applications. Pediatr Radiol. 40:141-152.
17. Sousa SB. 2008. Tetra-amelia and lung hypo/aplasia syndrome: New case report and review. Am. J. Med. Genet. 146A:2799–2803.
18. Oostra RJ, Boer L, van der Merwe AE. 2016. Paleodysmorphology and paleoteratology: Diagnosing and interpreting congenital conditions of the skeleton in anthropological contexts. Clin Anat. 29:878-91.
19. Moritz JD, Luftner-Nagel S, Westerhof JP, Oestmann JW and Grabbe E. 1996. Microcalcifications in breast core biopsy specimens: disappearance at radiography after storage in formaldehyde. Radiology. 200:361-3.

20. Owolobi AT, Oseni SB, Sowande OA, Adejuyigbe O, Komolafe EO, Adetiloye VA. 2005. Dicephalus dibrachius dipus conjoined twins in a triplet pregnancy. Trop J Obstet Gynaecol. 22:87–88.
21. Spencer R. 2003. Conjoined twins: Developmental Malformations and Clinical Implications. Baltimore: The Johns Hopkins University Press.
22. Niemann S, Zhao C, Pascu F, Stahl U, Aulepp U, Niswander L, Weber JL, Muller U. 2004. Homozygous WNT3 mutation causes tetra-amelia in a large consanguineous family. Am J Hum Genet. 74:558–563.

8 Detection of G1138A Mutation of the *FGFR3* Gene in Tooth Material from a 180-Year-Old Museological Achondroplastic Skeleton

This chapter has been published as:

Lucas L. Boer*, Jana Naue*, Laurens de Rooy and Roelof-Jan Oostra
Detection of G1138A Mutation of the FGFR3 Gene in Tooth Material from a 180-Year-Old Museological Achondroplastic Skeleton. 2017.
Genes (Basel). 8.pii: E214. doi: 10.3390/genes8090214.
*First shared author.

Abstract

Throughout the last four centuries, many anatomical museums across the world have collected teratological specimens that became precious objects. These can be regarded as spirits of the past which have captured the morphology of diseases through time. These valuable and irreplaceable specimens can be perfectly used in contemporary dysmorphological or genetic research. Unfortunately, due to the historical nature of these specimens and the regularly used aggressive preservation fluids, DNA degradation is often present. Furthermore, the use of material for DNA extraction is restricted to preserve the appearance of these valuable museological specimens. Thus, the most challenging part in this perspective is to harvest sufficient DNA of good quality for further testing without damaging the specimens. Besides fixated specimens, most teratological collections contain dried skeletal and teeth materials which are an excellent source to extract DNA. We here present a DNA-based method that enables genetic identification of the G1138A mutation of the *FGFR3* gene in a 180-year-old achondroplastic skeleton, confirming the previously morphologically determined disease. Nuclear DNA was extracted from a premolar tooth and the mutation was found using Sanger sequencing of a small region of the *FGFR3* gene.

Introduction

Many anatomical museums throughout the world contain, maintain, and exhibit teratological collections. These predominantly historical accumulations of nature's unpredictability can be seen as irreplaceable treasure chests waiting to be explored with contemporary (dys)-morphological knowledge and supplementary genetic research. Although some museums perform additional redescribing and rediagnosing of their teratological specimens, this is still quite exceptional, taking into account the many existing teratological collections. [1–4] Therefore, many exhibited specimens have never been viewed from a modern dysmorphological stance, let alone investigated with additional diagnostic techniques. Nevertheless, most of the historically made diagnoses, which were based on contemporary classifications of diseases and ideas of embryological development, do not fit modern medical and biological standards.

Only a limited number of inherited disorders may be morphologically revealed in the skeleton, of which achondroplasia is an example. Achondroplasia (OMIM100800) is a fully penetrant autosomal dominant Mendelian disorder and is considered one of the most common forms of short-limb dwarfism in humans. [5] It occurs with a frequency of one in 10–30,000 births, [6] and in 90% of the cases it concerns a de novo mutation which is strongly correlated with high paternal age. [7] Homozygous inherited forms of achondroplasia are lethal in early infancy due to small thoracic cage size and thereby respiratory distress. [8] Achondroplasia was initially described by Parrot in 1876. [9] In addition to being a misnomer in itself (see further), it inappropriately became a receptacle for almost any short-limbed skeletal dysplasia, leading to many misdiagnoses. With increasing genetic diagnostic sophistication, many of these disorders were recognized as entities of their own. In 1994, the gene responsible for achondroplasia was obtained by linkage analysis and mapped to a 2.5 Mb of DNA located at the telomeric region of the short arm of human chromosome 4 (4p16.3) containing the fibroblast growth factor receptor-3 gene (*FGFR3*). [10,11] A paper by Etlik *et al.* [12] reports that in 97% of the affected individuals a G-to-A transition, and in 1% a G-to-C transition at nucleotide 1138 (G1138A, G1138C) occurs in the *FGFR3* gene (dbSNP 150: rs28931614). This mutation results in the substitution of a glycine residue for an arginine residue (Gly380Arg) in the transmembrane domain of the FGFR3 protein [7] (Fig. 1); in around 2% of cases, other positions within the gene are affected. [13] As a result of these mutations, FGFR3 can be activated without binding to fibroblast growth factors [14], and since *FGFR3* normally regulates chondrocyte differentiation, proliferation, and apoptosis, diminished diaphyseal growth

ensues. [15] The growth plate cartilage is therefore dysplastic, although the name of the condition erroneously implies that the cartilage is absent. Achondroplasia is characterized by a markedly shortened stature. In males, it ranges between 118 cm and 145 cm, and in females between 112 cm and 136 cm, which is 6–7 standard deviations (SD) below the normal mean. [16] Other prominent features are rhizomelic shortened limbs, bulging forehead, and midfacial retrusion (Fig. 1, bottom part). Like many other skeletal dysplasias, it mainly affects enchondrally rather than intramembranously ossifying skeletal elements. Here, we report on the identification of the Gly380Arg mutation of the *FGFR3* gene from tooth material in a 180-year-old museological achondroplastic skeleton from Museum *Vrolik*, the anatomical museum of the University of Amsterdam (The Netherlands). This skeleton was originally acquired before 1830 by Gerard Vrolik (1775–1859), founder of *Museum Vrolikianum*, the predecessor of the current Museum *Vrolik*. Pusch *et al.* (2004) attempted a screening of ancient bone samples for achondroplasia mutations, but failed because of significant false positivity in their normal controls. [23] No other attempts have been reported that we know of. To the best of our knowledge, we are the first to unequivocally establish a genetic condition using DNA obtained from dried ancient tooth tissue.

Materials and Methods

Achondroplastic Skeleton

Specimen M717 (Figure 1) concerns a macerated and dried skeleton in which much of the cartilaginous parts and some ligaments have been preserved. According to the catalogue of the original *Vrolik* collection, [24] the skeleton (at the time numbered D239) was that of a male "Prussian dwarf" with a postural length of 116 cm, a slight thoracic scoliosis, and short bowed limbs. This supposedly resulted from rickets, [24] a default diagnosis in the 19th century for virtually any condition that included bowing of tubular bones that did not obviously result from fractures. Upon external examination, a clear midfacial hypoplasia, a bell-shaped thorax, slight thoracic scoliosis, profound lumbosacral lordosis, cuboid shaped vertebrae, and a narrow pelvis with small iliac wings and flat acetabula can be seen. The tubular bones are small and robust with genu varum and relatively long fibulae. This particular specimen was, based on external dysmorphological characteristics, diagnosed with achondroplasia. [3] To verify this diagnosis, the left upper first premolar was extracted under the most careful conditions to prevent contamination with recent DNA. DNA from this tooth was obtained using the under-mentioned

protocol. For control purposes, a tooth (collected between 1930–1950) of a control individual was used. The Museum *Vrolik* collection comprises one other specimen of an adult achondroplastic skeleton (specimen M718), [3] which we also intended to sample. However, all teeth appeared to be glued in their sockets and we were unable to extract any of them without damaging the specimen.

Sample Preparation

The whole teeth preparation and drilling was performed within a cleaned (bleach, UV-light) and closed environment under pre-polymerase chain reaction (PCR) conditions, using decontaminated equipment, human DNA-free material, and protective clothing (gloves, mouth, head, and arm protection), to allow human DNA-free working conditions. The following procedure was conducted in both the control tooth as well as in the tooth of the achondroplastic skeleton. To prevent loss of museological value of the specimens, the procedure aimed to obtain DNA-containing material in the least destructive way. Testing was performed independently to avoid cross-contamination between the two samples. The tooth was first decontaminated using a swab with bleach, followed by DNA-free water to mechanically clean it. Afterwards, the tooth was cleaned twice for 30 min in ethanol and once in DNA-free water for 30 min. The surface was further decontaminated by UV-light for 30 min. A hole was drilled from the tip in the root of the tooth (Fig. 2) using a drilling machine (Sybron Endo), and a carbon drill (diameter: 1.6 mm, E0205, Dentsply LN Bur) (both from Henry Schein Dental, New York, NY, USA) with an operating speed of 300 rpm. Due to the low speed, fixation was done by hand, allowing precise handling. Drilling was performed until the pulp chamber was reached. All the obtained tooth powder was collected. In the case of the tooth acquired from the achondroplastic skeleton, drilling within the same hole was performed twice to obtain additional material for a second extraction. The powder amount was weighted with a micro scale. The powder originated mostly from dentine material, but also cementum (from the beginning of the drilling) and material from the pulp chamber surface was retained (from drilling within the chamber). The small hole in the root of the tooth was invisible after placing the element back into the skeleton.

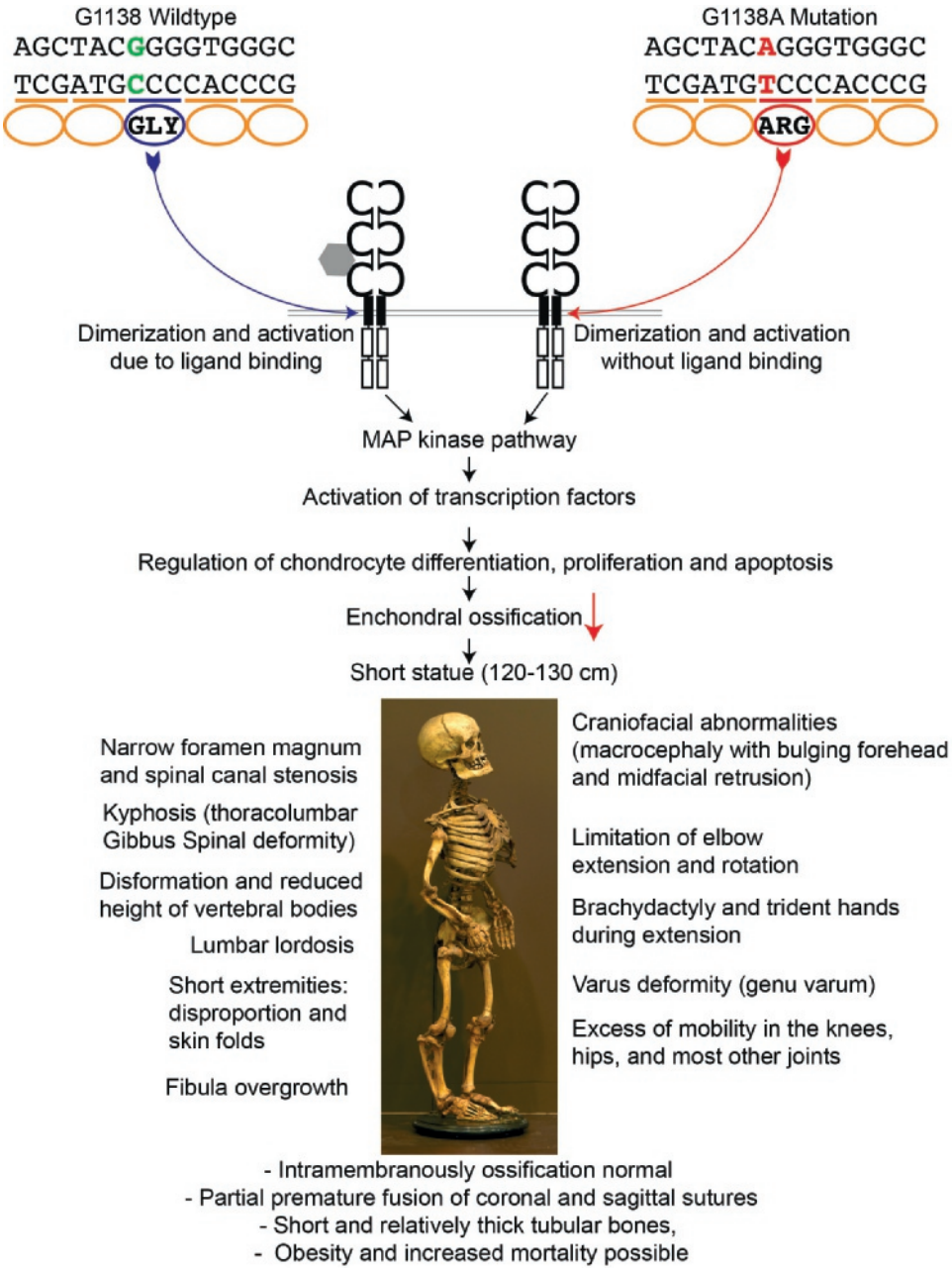


Fig. 1. Genetic background and consequences of the fibroblast growth factor receptor-3 gene (*FGFR3*) mutation. The G1138A mutation leads to an amino acid exchange within the transmembrane region of *FGFR3*, resulting in a ligand-independent activation of the downstream pathways. *FGFR3* is a negative regulator of enchondral ossification. In the case of a mutation, increased protein activity and thereby dramatically decreased enchondral ossification with normal intramembranous ossification leads to the observed anomalies in achondroplasia. [9,16–22]
SD: standard deviation.

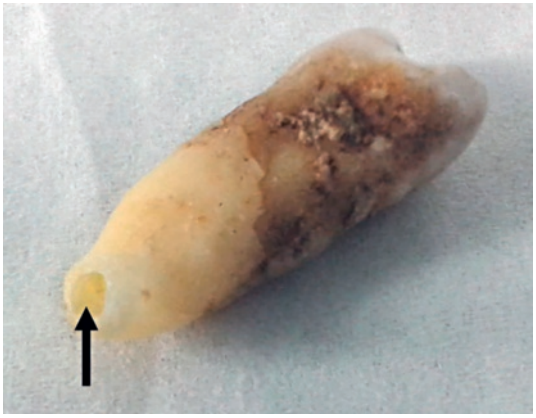


Fig. 2. Photo of the tooth extracted from the achondroplastic skeleton. Powder from the premolar tooth was obtained by drilling into the tooth from the tip of the root (black arrow).

DNA Extraction

DNA extraction was performed in another pre-PCR room. For initial decalcification, 1 mL of 0.5 M EDTA (pH 8.0) was added to the tooth powder and incubated at 30 °C under rotation for 1 h. The decalcified material was incubated overnight at 56 °C using the lysis buffer of the Gene Matrix Bone DNA Purification Kit (EURx, Gdansk, Poland). DNA extraction was performed following the manufacturer's recommendations. Final DNA elution was executed in 50 µL and 30 µL elution buffer, for the first and second DNA extraction, respectively. DNA concentration was measured using the Qubit dsDNA HS Assay Kit with the Qubit 3.0 fluoremeter (Thermo Fisher Scientific,Waltham, MA, USA).

Initial PCR Testing for the Presence of DNA

PCR setups were performed within a specific pre-PCR hood, and PCR products handled in a post-PCR area. As degradation was expected, a sensitive Alu-PCR (71 bp fragment) was used to test whether an amplifiable amount of DNA was obtained. PCR was performed using the Yb8-F and Yb8-R primer according to Walker *et al.* [25] The 10-µL reaction mix consisted of 5 µL of HotStarTaq Master Mix Kit (Qiagen, Hilden, Germany), 5 pmol of each primer, and 750 pg of DNA. PCR conditions were: 95 °C for 10 min, followed by 36 cycles of 30 s at 94 °C, 30 s at 60 °C, 30 s at 72 °C, and a final elongation for 10 min at 72 °C. The PCR product was verified on a gel.

Mutation Detection Using PCR and Sanger Sequencing

PCR of the area of interest around the positions with the expected mutation G1138A/C (rs28931614, chr4:1804392) and the less common mutation G1123T (rs75790268, chr4:1804377) was performed using a primer pair leading to a fragment of 164 bp according to Shiang *et al.* [26] (Forward (F): AGGAGCTGGTGGAGGCTGA, Reverse (R): GGAGATCTTGTGCACGGTGG). Another PCR using a different reverse primer (R1: CCAGGCCTTTCTTGGGGG) was performed to obtain a shorter fragment of 138 bp. The 10-µL reaction mix consisted of 5 µL of HotStarTaq Master Mix Kit (Qiagen), 5 pmol of each primer, 1 µL BSA (1 mg/mL) (Thermo Fisher Scientific), and between 1 ng and 15 ng of DNA. PCR was run under the following conditions: 95 °C for 10 min, followed by 36 cycles of 30 s at 94 °C, 30 s at 65 °C, 30 s at 72 °C, and a final elongation for 10 min at 72 °C. In addition, two more cycles were executed for the analysis of the second DNA extract to obtain a higher amount of PCR product for sequencing. Successful amplification was verified on gel, and the PCR product was cleaned with 10 U Exonuclease I and 1 U thermo-sensitive Alkaline Phosphatase (FastAP) (both from Thermo Fisher Scientific) for 30 min at 37 °C, which was followed by enzyme inactivation for 15 min at 85 °C. Cleaned PCR products were send to sequencing (Eurofins Genomics, Luxembourg, Luxembourg) using the reverse primers. A negative DNA extraction/PCR control and a positive PCR control (1 ng control DNA 9947A, Promega, Madison, WI, USA) were taken along, showing the expected results.

Results

Obtained Material for Analysis and Pre-Testing

Forty milligrams of dental powder were used for DNA extraction in the case of the control tooth, resulting in 270 ng DNA in total. In the case of the achondroplastic skeleton, 45 mg and 53 mg of dental powder were retrieved from the first and second drilling of the premolar, resulting in a total of 7.4 ng and 10.26 ng of DNA, respectively. Analysis of the first DNA extract using the Alu-PCR was successful, showing that amplifiable DNA was available from the tooth and that the DNA extraction was able to remove possible PCR inhibitors successfully.

FGFR3 Analysis for Mutation Detection

FGFR3 PCR and sequencing analysis of both DNA extracts of the premolar was performed multiple times for both PCR systems (164 bp and 138 bp) in order to obtain reproducible and reliable results (success rate receiving good sequences: first DNA extraction: 50% (3/6 analysis), second DNA extraction: 75% (3/4 analysis)). The 138 bp system was only tested in total three times, as a lower amplification efficiency was known from pre-experiments. Additionally, an analysis was performed for the control tooth to exclude PCR artifacts. All successful sequence results covering the position of interest are presented in figure 3 (excluding the PCR and F1 sequence reaction not covering the position in good quality). A heterozygote G-to-A mutation at position chr4:1804392 was observed, which corresponds to the expected G1138A (rs28931614) exchange within the *FGFR3* gene. Two sequencing reactions resulted in only the wild-type (G) or the mutant (A) nucleotide (Fig. 3, rows 2 and 5), which we assume was caused by allelic-dropout during the PCR reaction. Multiple analysis using two different PCR systems, two different sequencing primers, as well as independent analysis with the second DNA extract confirmed the presence of the heterozygous G1138A mutation. The analysis of the control tooth resulted in the detection of the expected wildtype sequence and gave no indication for PCR artifacts.

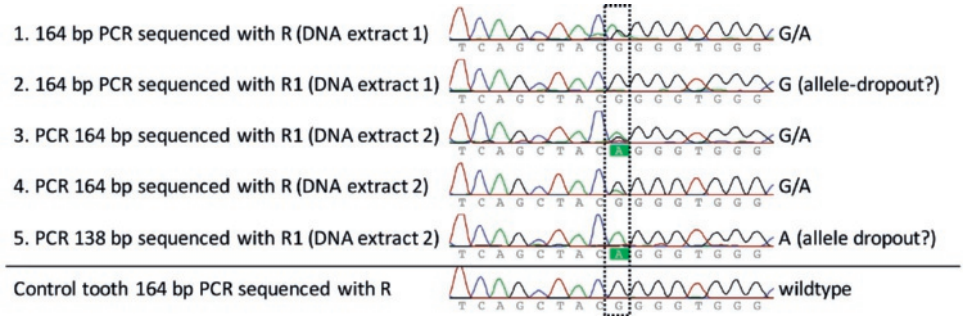


Fig. 3. Sequencing results of multiple PCRs from the premolar of the achondroplastic skeleton, as well as the analysis of a control tooth. Rows 1–5: Two independent DNA extracts of the premolar were analyzed for the presence of a mutation. Two different long PCR-systems as well as two sequencing primers were used for analysis. In two PCRs, only the wildtype G or the mutant allele A was observed, indicating allelic-dropout as the G/A was detected in the other three PCRs. The analyzed control tooth resulted in the expected wildtype.

Discussion

Old museological collections can be used eminently for further explorations regarding their excellent scientific potential. Research of old teratological specimens can yield valuable contributions in e.g., etiopathogenetic issues and potentially expand the literature beyond the restrictions of single case studies due to the plurality of specimens with similar anomalies in these collections. [27] One should, however, avoid focusing the policies regarding the management and study of these collections entirely on medical and biological aspects, thereby neglecting the irreplaceable value that these collections have as cultural-historical objects. Without being aware of the latter, researching and handling these collections may cause irreversible damage to the historical integrity of these specimens and preparations. Many historical anatomical and pathological collections in the world have already lost much information due to the unawareness of this historical and cultural aspect. In this study, the actual extraction of the tooth from the historical skeleton was therefore performed and supervised by museum specialists, making sure that the damage done to the specimen was limited to the minimum. In spite of the often beautiful and elegant appearance of these old teratological specimens, their age and frequently used formaldehyde-based preservation fluids dramatically affect the integrity of the DNA quality to the point that it is unsuitable for additional genetic testing. Nevertheless, although DNA extraction from formalin-fixated and paraffin-embedded specimens is often accompanied with poor results, analysis of gene products and/or their metabolic effects can yield valuable contributions in the verification of assumed genetic diagnoses. [28]

Another possible source where DNA can be retrieved from is the many macerated skeletons which are present in most historical collections. These skeletons were presumably only mildly macerated until all fleshy parts could be scraped off; hence the ligaments and cartilage would be retained by this delicate procedure. After the skeletons were dried, they became solid, natural positioned, infrangible time witnesses of nature's capriciousness. Due to the nature of this relatively mild cleaning process and the solidness of these skeletons through time, DNA can still reside in both bone and teeth. [29]

We managed to successfully extract nuclear DNA from dental material originating from a 180-year-old achondroplastic skeleton from Museum *Vrolik* in Amsterdam, The Netherlands. The original museum collection was privately owned and was further expanded by Gerard Vrolik's eldest son Willem Vrolik (1801–1863). When Willem Vrolik died, the collection comprised of 5123 anatomical preparations. [30] The analysis of ancient human DNA material is challenging due to multiple factors. Firstly, exogenous DNA

contamination introduced during the process needs to be avoided. Therefore, surface cleaning as well as handling under DNA-free conditions are essential. Secondly, the museological value of old specimens—which should stay intact—restricts possible sites of material extraction. Therefore, drilling through the tip of a tooth root is a good compromise. Using a molar instead of a premolar would have been favorable to obtain more DNA material, but was not possible due to the fixation of these teeth to the jaws. Non-destructive methods have been described previously, but they may change the tooth surface and cause increased porosity or lighter appearance. [31–33] Hence, we did not use this way of DNA extraction, as these consequences were not foreseeable. The third challenge is the analyzed material itself. Preservation conditions as well as the age lead to DNA degradation and cross-linking, in the case of formalin-fixated material. The use of bone material, and especially teeth, are known to be a good (and often the only available) source for DNA extraction. [34] However, the DNA quantity we obtained was small, which was also caused by the fact that we reduced the destruction of the tooth to a minimum, using only a small amount of dental material. Nevertheless, around 40 to 50 mg was enough for a successful analysis. To overcome the DNA degradation problem, only a small 138 bp and a 164 bp DNA region were amplified. Furthermore, the drilling speed was reduced to 300 rpm in order to avoid additional degradation of the DNA due to heat production. That effect was also seen by Adler *et al.* (2011), comparing mtDNA analysis after 1000 rpm and 100 rpm drilling. [35] PCR analysis confirmed the presence of the heterozygote G1138A mutation, which is pathognomonic for achondroplasia. The detection of the heterozygote version stands in accordance with the fact that the homozygous version would be lethal within early infancy. Furthermore, precautions to avoid any contamination were taken; therefore, we have no indication that the wild-type G was caused by any external DNA. We also do not see any indication that our results are caused by PCR artifacts, as analysis of the control tooth resulted in the expected wild-type. As mentioned before, we know of only one study by Pusch *et al.* (2004) in which ancient DNA material obtained from a 7000-year-old Egyptian mummy was investigated for the presence of a mutation causing achondroplasia, but they experienced false-positive PCR results in their control material. [23] Extract-induced PCR sequence changes were proposed as the most reasonable explanation, and other reasons, such as deamination processes over time, were excluded. [23] We did not see such a result in our control material. Also, no other sequence alterations in the achondroplastic skeleton were detected. However, the storage time of our control material (67–87 years) and that of the achondroplastic skeleton (180 years) is far shorter compared to the examined mummies in Pusch's

report, and the materials we used were preserved under good conditions. Furthermore, a column-based extraction method was used, which removes additional compounds very well, decreasing the risk of extract-induced sequence changes. The use of two DNA extracts, two PCR products, and two different sequencing primers demonstrated the reliability of the analysis. Unfortunately, the use of the forward primer was disadvantageous due to its close location to the position of the expected mutation. The Sequencing result indicated the presence of the mutation; however, the quality of the sequence was not sufficient for a reliable base call.

DNA analysis from ancient material is often performed in forensic cases for identification purposes or in archeological investigations. The analysis of diseases is a rarer event; however, the molecular method presented here opens new doors to verify diagnoses of museum specimens or other skeletal materials which are often based on external dysmorphological descriptions only.

Acknowledgments

We thank Hagay Shemesh from the Department of Endodontology (Academic Centre of Dentistry Amsterdam (ACTA) for providing the dental drill machine. Furthermore, we would like to thank Inge Dijkman and Lotje Broekens of Museum *Vrolik* for performing and supervising the actual extraction according to museological standards. We thank Sanne Mos for taking photographs of the skeleton.

References

1. Beighton P, Sujansky E, Patzak B, Portele KA. 1993. Genetic skeletal dysplasias in the museum of pathological anatomy, Vienna. Am. J. Med. Genet. 47:843–847.
2. Beighton P, Sujansky E, Patzak B, Portele KA. 1994. Bone dysplasias of infancy in the Vienna collection. Pediatr. Radiol. 24:384–386.
3. Oostra RJ, Baljet B, Dijkstra PF, Hennekam RC. 1998. Congenital anomalies in the teratological collection of Museum Vrolik in Amsterdam, The Netherlands. II: Skeletal dysplasias. Am. J. Med. Genet. 77:116–134.
4. Boer LL, Schepens-Franke AN, van Asten JJA, Bosboom DGH, Kamphuis-van Ulzen K, Kozicz TL, Ruiter DJ, Oostra RJ, Klein WM. 2017. Radiological imaging of teratological fetuses: What can we learn? Insights Imaging. 3:301–310.
5. Ornitz DM, Legeai-Mallet L. 2017. Achondroplasia: Development, pathogenesis, and therapy. Dev. Dyn. 246:291–309.
6. Horton WA, Hall JG, Hecht JT. 2007. Achondroplasia. 370:162–172.
7. Vajo Z, Francomano CA, Wilkin DJ. 2000. The molecular and genetic basis of fibroblast growth factor receptor 3 disorders: The achondroplasia family of skeletal dysplasias, Muenke craniosynostosis, and Crouzon syndrome with acanthosis nigricans. Endocr. Rev. 21:23–39.
8. Stanescu R, Stanescu V, Maroteaux P. 1990. Homozygous achondroplasia: Morphologic and biochemical study of cartilage. Am. J. Med. Genet. 37:412–421.
9. Parrot JM. 1876. L'achondroplasie et les lésions osseusses de la syphilis hereditaire et du rachitisme. Arch. Physiol. 2:133–139.
10. Le Merrer M, Rousseau F, Legeai-Mallet L, Landais JC, Pelet A, Bonaventure J, Sanak M, Weissenbach J, Stoll C, Munnich A. 1994. A gene for achondroplasia-hypochondroplasia maps to chromosome 4p. Nat. Genet. 6:318–321.
11. Velinov M, Slaugenhaupt SA, Stoilov I, Scott CI Jr, Gusella JF, Tsipouras P. 1994. The gene for achondroplasia maps to the telomeric region of chromosome 4p. Nat. Genet. 6:314–317.
12. Etlik O, Koksal V, Tugba Arican-Baris S, Baris I. 2008. An improved tetra-primer PCR approach for the detection of the FGFR3 G380R mutation responsible for achondroplasia. Mol. Cell. Probes. 22:71–75.
13. Richette P, Bardin T, Stheneur C. 2008. Achondroplasia: From genotype to phenotype. Joint Bone Spine. 75:125–130.
14. Webster MK, Donoghue DJ. 1996. Constitutive activation of fibroblast growth factor receptor 3 by the transmembrane domain point mutation found in achondroplasia. EMBO J. 15:520–527.
15. Deng C, Wynshaw-Boris A, Zhou F, Kuo A, Leder P. 1996. Fibroblast growth factor receptor 3 is a negative regulator of bone growth. Cell. 84:911–921.
16. Horton WA, Rotter JI, Rimoin DL, Scott CI, Hall JG. 1978. Standard growth curves for achondroplasia.J. Pediatr. 93:435–438.
17. Baujat G, Legeai-Mallet L, Finidori G, Cormier-Daire V, Le Merrer M. 2008. Achondroplasia. Best Pract. Res. Clin. Rheumatol. 22:3–18.
18. Pauli RM, Legare JM. Achondroplasia. 1998. In: Adam MP, Ardinger HH, Pagon RA, *et al.*, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2019. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1152/

19. Di Rocco F, Biosse Duplan M, Heuze Y, Kaci N, Komla-Ebri D, Munnich A, Mugniery E, Benoist-Lasselin C, Legeai-Mallet L. 2014. FGFR3 mutation causes abnormal membranous ossification in achondroplasia. Hum. Mol. Genet. 23:2914–2925.
20. Wynn J, King TM, Gambello MJ, Waller DK, Hecht JK. 2007. Mortality in achondroplasia study: A 42-year follow-up. Am. J. Med. Genet. A. 143A:2502–2511.
21. Cheema JI, Grissom LE, Harcke HT. 2003. Radiographic characteristics of lower-extremity bowing in children. Radiographics. 23:871–880.
22. Lee ST, Song HR, Mahajan R, Makwana V, Suh SW, Lee SH. 2007. Development of genu varum I achondroplasia: Relation to fibular overgrowth. J. Bone Joint Surg. Br. 89:57–61.
23. Pusch CM, Broghammer M, Nicholson GJ, Nerlich AG, Zink A, Kennerknecht I, Bachmann L, Blin N. 2004. PCR-induced sequence alterations hamper the typing of prehistoric bone samples for diagnostic achondroplasia mutations. Mol. Biol. Evol. 21:2005–2011.
24. Dusseau JL. 1865. Museé Vrolik. Catalogue de la Collection d'Anatomie Humaine, Compareé et Pathologique. De M.M. Ger. et W. Vrolik, professeurs à l'Athénée Illustre d'Amsterdam; J. Roever-Kröber: Amsterdam, The Netherlands. p. 303.
25. Walker JA, Hedges DJ, Perodeau BP, Landry KE, Stoilova N, Laborde ME, Shewale J, Sinha SK, Batzer MA. 2005. Multiplex polymerase chain reaction for simultaneous quantitation of human nuclear,mitochondrial, and male Y-chromosome DNA: Application in human identification. Anal. Biochem. 337:89–97.
26. Shiang R, Thompson LM, Zhu YZ, Church DM, Fielder TJ, Bocian M, Winokur ST, Wasmuth JJ. 1994. Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. Cell. 78:335–342.
27. Boer LL, Morava E, Klein WM, Schepens-Franke AN, Oostra RJ. 2017. Sirenomelia: A Multi-systemic Polytopic Field Defect with Ongoing Controversies. Birth Defects Res. 109:791–804.
28. Oostra RJ, Baljet B, Schutgens RB, Hennekam RC. 1997. Smith-Lemli-Opitz syndrome diagnosed in a 130-year-old anatomical specimen. Am. J. Med. Genet. 31:257–259.
29. Hansen HB, Damgaard PB, Margaryan A, Stenderup J, Lynnerup N, Willerslev E, Allentoft ME. 2017. Comparing Ancient DNA Preservation in Petrous Bone and Tooth Cementum. PLoS ONE. 12, e0170940.
30. De Rooy L, van den Bogaard H. 2009. Forces of Form: The Vrolik Museum; University Press: Amsterdam, The Netherlands.
31. Hofreiter M. 2012. Nondestructive DNA Extraction from Museum Specimens. In Ancient DNA; Humana Press: New York, NY, USA. pp. 93–100.
32. Gomes C, Palomo-Díez S, Roig J, López-Parra AM, Baeza-Richer C, Esparza-Arroyo A, Gibaja J, Arroyo-Pardo E. 2015. Nondestructive extraction DNA method from bones or teeth, true or false? Forensic Sci. Int. Genet. Suppl. Ser. 5, e279–e282.
33. Bolnick DA, Bonine HM, Mata-Míguez J, Kemp BM, Snow MH, LeBlanc SA. 2012. Nondestructivesampling of human skeletal remains yields ancient nuclear and mitochondrial DNA. Am. J. Phys. Anthropol. 147:293–300.
34. Higgins D, Austin JJ. 2013. Teeth as a source of DNA for forensic identification of human remains: A Review. Sci. Justice. 53:433–441.
35. Adler CJ, Haak W, Donlon D, Cooper A. 2011. Survival and recovery of DNA from ancient teeth and bones. J. Archaeol. Sci. 38:956–964.

9 Supplementary discussion

In the previous chapters the findings and considerations of investigating Dutch teratological collections have been discussed extensively. In this chapter a supplementary general discussion will be given on a number of topics that have relevance for the content of this thesis in a broader sense. Limitations, ethical and societal considerations and future perspectives of researching teratological collections are given and finally a concluding remark of this thesis will be delineated.

The study of teratological collections

General issues in studying teratological collections

First, it is noteworthy that the initial collectors of teratological specimens and those who described these peculiar cases have (unconsciously) determined what we now can investigate. Many specimens and/or cases must have been neglected due to specific interest these early collectors had. The second cumbersome obstacle in the study of teratological collections is the "extent of museological value" that some caretakers strongly vindicate and append to their museological collections. [1] As most of these specimens are seen as priceless artifacts of great historical and heritological importance, it is inherent that most research performed—if at all—must be minimally invasive in order to maintain the object's integrity. [2] Although this premise is completely valid and defensible from a historical and preservational point of view, it restricts the options of additional investigations. An apparent inborn tendency to foster the integrity of the specimens is becoming increasingly present through time: the older the object the more precious it becomes—to the point that it is not even allowed to touch the specimen's jars (author's personal experience). Although this mindset could be (mandatorily) influenced by e.g. cultural aspects or institutional regulations, this *modus operandi* is not necessarily beneficial to explore the specimens' dormant potentials. When these museological norms persevere, the possibility rises that these specimens just remain tacit and nebulous museological objects with only the historical value that is proclaimed by their caretakers. This "hidden value" is however not necessarily seen by its visitors which potentially sees the subjectivity of the specimen: "a baby in a jar," no matter of its historical importance. Specimens should become more than just intriguing objects or fetuses for the fortuitous or inquisitive museum visitor. Lack of additional research could be due to hierarchical influenced institutional policy and the reason to stop maintaining or even discard the specimens: we don't use these old specimens so why should we keep them. Therefore, it is of utmost

importance to further explore these valuable collections. [3] Contemporary use of these specimens in e.g. etiological and pathogenetic issues could vitalize these collections and could potentially become matter for extended inquiry by e.g. physicians, researchers and historians. The inner stimulation to foster and maintain these collections grows if they are "used," not only for their caretakers but also for the institutes in which these collections reside. Besides maintaining the objects' integrity and consort with the institutes policy, a third limitation which is (mostly) inherently present in teratological collections is the manner in which these specimens are preserved: if not dried, they are often embalmed and kept on a farrago of aggressive preservatives such as alcohol or formalin based fluids. Although it is due to these substances that some specimens still exist after multiple decades and even centuries, they seriously affect the integrity and quality of various tissues and especially of their DNA. [4] This makes most specimens unsuitable for additional genetic investigations. On the other hand high-quality radiological images can be acquired due to the often quick fixation procedure of the specimens in the past. [5] Although institutional empathy and aggressive preservation fluids interfere, most teratological specimens can be excellently investigated by describing their external and internal (dys)morphological character. Interweave these descriptive findings to embryological and etiopathogenetic thoughts and a (differential) diagnoses can be made in many cases. Diagnosing teratological specimens purely based on descriptive analysis together with reasoning retrospectively to early embryological development is only feasible in the light of the severe nature of most teratological specimens. And it was exactly this premise which was the initial reason to collect these specimens—it is in these particular specimens that the answers may be found on difficult embryological and possible causative questions.

Societal and clinical implications of studying teratological collections

Seen from a broader perspective, by throwing light on the causes and mechanisms of rarely occurring birth defects—yet present in most teratological collections—these specimens could hypothetically contribute in a contemporary clinical approach. [6] Etiological and pathogenetically oriented research—based on teratological specimens—could potentially be the missing piece of the puzzle and the beginning of for instance a clinical attention or recognition. Historical, seemingly settled, etiopathogenetic thoughts should be re-evaluated and may subsequently come in the scope of current clinicians. In a social perspective, a survivable major congenital anomaly has adverse effects on the health status and/or the social acceptability of the affected individual.

[7] This makes individuals who survive charged with both a cosmetic, a social and a medical burden. [8] Moreover, growing global populations implicate that rarely occurring birth defects collectively affects millions of people worldwide, implying a massive socioeconomic burden. [9] Patients affected by rare diseases generally go through a very long and arduous diagnostic process known as the rare disease odyssey. Besides to—sometimes literally—"finding" a physician specialized in a particular disease, scientific advances crawl with little progress as it could be difficult to find a sufficient number of people affected by the same rare disease to fulfil successful research. Again, teratological collections could be used to stimulate or nourish this research.

Rare diseases were neglected for many years, however, the rise of so-called "European Reference Networks" (ERNs) underlines the social and scientific importance for rare diseases or congenital anomalies. [10] These virtual consolidating knowledge-sharing networks of care providers across Europe are set up to share and enhance current knowledge and resources used for treating rare diseases. Detailed Knowledge of all 6000 to 8000 rare diseases is impossible for any individual and lies on the cognitive barrier that prevents clinicians contemplating a rare disease. [11] Therefore these ERNs become increasingly active and important in recognizing and researching rare diseases. Studying teratological collections could contribute to these ERNs, as it is often the case that multiple specimens of the same type are present in these old collections. Moreover, many congenital anomalies show a spectrum between survivable and lethally affected. The latter could potentially give insights in etiopathogenetic answers and with that the possibilities and/or interventions in the milder affected new-borns seen in a clinical setting. Although abnormal morphogenetic pathways are not revealed by examining newborns, reasoning retrospectively to defects in embryogenesis could be seen as meaningful speculations. [12] Spranger *et al.* (1982) [13] stated: "although the exact cause and pathogenesis of a given anomaly may not be known, careful analysis of the history and physical findings of the patient and judicious inference from experimental data frequently give useful ideas about its probable cause and pathogenesis." It is exactly this premise which is still important in the contemporary study of teratological specimens. Indeed one cannot possibly survey all blastogenic or genetic pathways and subsequent alterations during embryogenesis if one looks at the pre- or full-term specimens. However, if substantiated perspectives from human embryology and experimental studies from developmental biology are included, it becomes possible to unravel the tip of the veil and the potential beginning of subsequent research ideas. Finally, the beginning of a possible paradigm shift could be initiated by fully exploiting and describing these historical specimens.

The ethics of exhibiting teratological collections

Although many anatomical museums exhibit their teratological collections to the general public, very rarely do they make explicit their ethical reasoning and considerations regarding the decision to display their teratological specimens. Most teratological specimens were collected during a period in which a number of moral aspects were approached and acted upon differently in comparison with modern times—in particular with respect to the handling of bodies of dead children, the doctor-patient relation and the necessity for consensus. In addition, there were hardly any options for therapeutic interventions, let alone for prevention of recurrence. Thus, different medical and societal morals, norms and values reigned during the time most teratological specimens were collected. Most teratological specimens residing in anatomical museums were obtained and anonymized in a way we now find unacceptable. [14] It is unknown in most (if not in all) cases if parents consented to the donation of their deceased child. As said before, the doctor-patient relation was completely different during the time these children were born; hierarchical and paternalistic relations preponderated. Therefore, it perhaps did not occur to doctors to ask parents for permission and it did not often occur to the parents to claim that right. The concept of (individual) autonomy and of decisional self-determination was virtually absent in those days. [15] It deemed sensible to remove the deceased child as soon as possible from the parents' sight. Moreover, different religion and spiritual traditions could have had its repercussions in the manner an anomalous child would have been perceived, handled and how one coped with it—in many religious denominations abortion as a response to fetal defects is still taboo. Some coping strategies invoked in the name of fait as for instance "God's will" could be seen as undermining factors for self-determination. In addition, many different rituals exist in the disposition of human remains, including a "hospital disposal" of lost pregnancies. [16] Finally, in the past, fetuses were seen as inert and impersonal entities. Today in the Western world they are increasingly depicted as active and personified entities. [17]

The aforementioned ethical issues and different time spirits in which most fetuses were collected, make teratological specimens prone for un-acceptance to the present beholder (authors personal experience). One could argue that parents of these children are bereaved form their grieving process and were unacceptably treated. It is difficult to place historically collected specimens—with archaic norms and values—in a rational and contemporary ethical perspective or framework. [18] From a modern perspective it seems quite remarkable to donate a child—let alone without

any permission or consent—to an anatomical museum. However, it rarely occurs that these collections are expanded with new clinical cases in which the parents have decided to endue their deceased child to donation programs (authors personal experience). In addition, most teratological collections originate from before the utilisation and ubiquitous availability of prenatal screening. Therefore, most specimens present in the anatomical collections are late-term fetuses or full-grown neonates. This premise even strengthens the observer's reactions to place these specimens in a present-day rationale. On the other hand, museums cannot undo this historical injustice which is inherently present in each teratological collection and hence an ethical dilemma arises for museums dealing with such collections. [3]

Few options are available and could be simplified in the following ways: 1) dispose and cremate the specimens, 2) maintain the specimens in storage without exhibiting them or 3) exhibit the specimens with or without information. Although for each option multiple arguments can be given, there is not a perfect solution as is the case in most ethical issues. The first option could be grounded by the feeling to finally give these children yet a respectful disposal but it would be devastating for these precious collections and specimens. The second option can be grounded on a more altruistic way in order to maintain this historical heritage for the future progeny or experts. The third choice—although the natural feeling of injustice may strongly be present—is to try to do as much as possible with these unique collections but with maintaining respect for the deceased children, their parents and their collectors. Even though most museums do not know why, how and when most specimens were collected, it is important to openly communicate certain (general) ethical considerations within the historical context if a museum chooses to exhibit their teratological collections. This information could generate public acceptation and empathy for the norms and values in earlier times. Unfortunately many exhibitions with teratological specimens—in which both the specimens and the exhibitions predominantly exist from a historical point of view—do little or nothing for their visitors to contribute to gaining insights in (failing) embryology and truly learning something about teratology or the developmental defects on display (authors personal experience). Teratological collections however confront these viewers with the imperfections of nature, the fragility of human existence and nourishes visual tactility. Exhibiting teratological specimens could create a valuable learning experience and potentially contribute to the social acceptance and awareness on developmental defects.

The public opinion of exhibiting teratological specimens

The publically accessibility and open informative attitude of the teratological exhibition in the Museum for Anatomy and Pathology in Nijmegen (See addendum) intrinsically led to the question what the museum visitor would perceive when being confronted with these teratological specimens. Therefore, the visitors were asked the following question: Do you consider it to be legit that we exhibit these children? The response on this question was to be filled in on to three colored papers which indicate: yes (light green), neutral (light yellow) or no (light blue) and could subsequently be posted on an "opinion board." In addition to giving their own opinion people could take notice of the opinions of other visitors—thereby creating some sort of ethical debate. Surprisingly, a multitude of opinions filled this board within a few weeks. Noteworthy is that in 95% of the cases a green card was chosen. Some of the more explicit comments are described below:

"I now realize that nature can make mistakes"

"It's very educational, I am happy that I am healthy"

"This shows how special development is"

"It leads to insights in human development and creation"

"Science can only become better by researching this. We as visitors are alerted that it is not obvious to have a healthy child"

"It's incomprehensible that one could collect these specimens, but we can now learn from this"

"Allowed me to gain a new perspective and an opportunity to learn a new respect for the medical field"

"I learned a lot trough this exhibition. It helped me broaden my horizon and I am really thankful for this museum"

"It's very special, in real life you hear about this although you cannot imagine it"

"Also as a mother I found it informative and impressive to see"

"My sister gave birth to a severely malformed child. It is correct to investigate this and increase its visibility. This is part of human life"

"These children have been given a place anyway, they did not come into this world for nothing. They are exhibited here with the greatest respect"

Of course the above-mentioned opinions show a large bias because most visitors are aware of what they are about to see and are apparently interested in anatomy as they visit an anatomical museum. In addition, the fact that almost all cards were green indicated that people who are interested and felt positive filled in a card—as is the case in many surveys; they yield an overall exaggerated positive feedback. On the other hand, everyone has opinions and in the world of digital technologies and innovations they are ventilated virtually everywhere. To epistemically exploit the aforementioned, the next step could be to effectuate a qualitative phenomenological study to answer the following research question: can we identify and explore the participant's opinion and experience after visiting the teratological exhibition? Virtually no literature is available with empirical evidence about how museum visitors experience and perceive exhibitions where teratological fetuses are shown. This is interesting because almost all medical museums in Europe publically exhibit full-term teratological fetuses to a broad audience. As a matter of fact, many of these museums are famous about their large teratological collections. A clear literature dearth is present and an empirical phenomenological study—using explorative and descriptive techniques in the form of in depth–interviews to explore, identify and understand the visitors' opinions and experiences—could be the next step to further explore this interface between science and ethics.

Future perspectives in the study of teratological collections

Besides novel qualitative studies, the future investigation of the specimens should focus on techniques with a minimally invasive approach. The relative new discipline of *post mortem* radiology is an expertise which is not common in a medical setting. [19] Now that its clinical implications become more clear, *post mortem* imaging appears to be complementary to standard autopsy. [20:21] Besides being clinically and forensically applicable and advantageous, *post mortem* radiology can be applied to investigate museological (e.g. teratological) specimens and hence contribute to the quest for etiopathogenetic clues. Moreover, they can additionally be used to educate the bio-medical student or resident in training for the recognition of congenital abnormalities. High resolution images of intricate internal structures can subsequently be used to create unique physical models to promote and increase learning immersion and engagement (see Fig. 1). Finally, acquiring radiological data from teratological fetuses results in a digital database which makes it easier to collect and exchange knowledge.

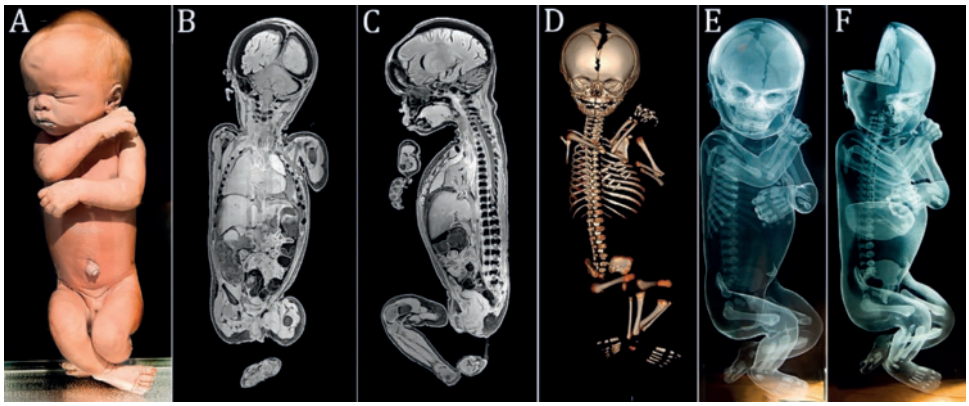


Fig. 1. [A] Photograph of a normal fetus of 35 weeks of gestation. [B] Single coronal T1-weighted image. [C] Single sagittal T1-weighted image. [D] Three-dimensional reconstruction based on CT data. [E] Photograph of 3D laser rotary engraved model of the fetus in a solid block of clear polymer. This model combines the outer contour and skeleton from the CT data. [F] Photograph of 3D laser rotary engraved model of the fetus in a solid block of clear polymer. This model combines MR data in which 3D volumes of the lung, pelvis and liver are manually segmented and digitally inserted. A single MR slice of the brain is positioned in the median plane. The outer contour and the skeleton is again based on CT data.

Besides producing actual models, the increasing release of technological innovations such as virtual and augmented reality could be beneficial to exploit the educational potential of teratological collections even further. [22] For instance, it becomes possible with augmented reality to enhance museological objects and improve the "dialogue" between the specimen and the observer. Moreover, the traditional museum could become more attractive. [22] Digital techniques could be complementarily and enhance student learning, engagement and performance and can be seen as supplementing content. [23] In addition to radiological imaging and technological innovations, the current rise of increasingly sensitive genetic diagnostic possibilities, it is expected that the problem of DNA decay in formalin fixed specimens can be conquered. [4] The rise of increasingly cheaper Next (or even Third) Generation Sequencing techniques could potentially open up an entire new perspective if DNA of fixed specimens becomes useable for additional diagnostics. [24:25] In addition, protein analysis—which is much less sensitive for formalin damage—could contribute in re-evaluating historically made diagnoses. An example of this method was described by Oostra *et al*. (1997) [26] who confirmed Smith-Lemli-Opitz syndrome in a 130-year-old specimen suggestive for this particular syndrome with cholesterol analysis from skin biopsies. Besides actual research on the specimens itself, extrapolating theoretically found etiopathogenetic mechanisms could be, in some cases, tested with experimental animal studies. One example could be e.g. testing the assumption that neurally conjoined

twins originate from two in close proximity located neural tubes within a single amniotic sac which secondarily fuse. This theoretical premise can be relatively easily tested with mechanical manipulations in e.g. *Xenopus* embryos during early gastrulation phases. Furthermore, describing complex congenital anomalies comprehensively could potentially give insight and answers in etiopathogenic issues as could be e.g. the case in describing concomitant concordant or discordant anomalies in conjoined twins. In addition, it is worth to extensively investigate teratological collections with additional radiological and/or genetic diagnostics and make this data publically accessible. These efforts could contribute to a worldwide available database which is findable, interoperable and reusable and may contribute to exploiting their scientific potentials beyond the limits of the currently present physical borders. In addition, it is important to seek expertise in different clinical and experimental fields—the puzzlement on teratology has many strands and it cannot be confined to a single discipline. It is because of this premise that a multitude of disciplines (e.g. developmental biology, epidemiology, embryology, anatomy and zoology) are all inter-related and could all contribute in researching teratological collections. The aforementioned gives a richness of content to teratology and an ever-unfolding newness and challenge that comprise its strengths. [8]

Concluding remarks of this thesis research

Overall, this thesis aims to show that it is possible to educationally and scientifically explore and exploit teratological collections with contemporary research output and potentials. Many European museums still house large teratological collections with specimens which could potentially be the missing link in an etiological or pathogenetic issue. Moreover, every teratological collection houses specimens which are so unknown, rare or even never seen before that it is worth to describe them comprehensively with the additional use of modern techniques. In that respect, a portal to future knowledge is still present and quietly awaits its awakening. The new generation of museum professionals who research and preserve these specimens will eventually determine what kind of past our future will have and *vice versa*. It is therefore that these specimens have to be treasured. The findings described in chapter 2 to 8 provide a "tip of the iceberg" of what can be done with institutionalized teratological collections. This approach will hopefully be an inspiration for future prospectors who are responsible for or intrigued by the many teratological specimens; not only in the Netherlands but also well beyond its borders.

References

1. Robbins N. 2017. Museum collection disposal: the role of museological value discussions in collection management. The Museum Review, Volume 2, Number 1.
2. Paluchowski P, Gulczyński J, Szarszewski A, Siek B, Halasz J, Iżycka-Świeszewska E. 2016. Insight into the history of anatomopathological museums - Part 1. From casual assemblages to scientific collections. Pol J Pathol. 67:207-215.
3. Gulczyński J, Paluchowski P, Halasz J, Szarszewski A, Bukowski M, Iżycka-Świeszewska E. 2018. An insight into the history of anatomopathological museums. Part 2. Pol J Pathol. 69:118-127.
4. Ruane S, Austin CC. 2017. Phylogenomics using formalin-fixed and 100+ year-old intractable natural history specimens. Mol Ecol Resour. 17:1003-1008.
5. Baljet B, Oostra RJ. 1999. Digital data and the 19th century teratology collection. J Audiov Media Med. 22:186-94.
6. Wilson J. 1977. Current status of teratology: general principles and mechanisms derived from animal studies. In: Wilson J, Fraser F, editors. Handbook of teratalogy. 4 vols. New York: Plenum Press. pp. 47-74.
7. Otake M, Schull WJ, Neel JV. 1990. Congenital malformations, stillbirths, and early mortality among the children of atomic bomb survivors: a reanalysis. Radiat Res. 122:1-11.
8. Kalter H. 2003. Teratology in the 20th century: environmental causes of congenital malformations in humans and how they were established. Neurotoxicol Teratol. 25:131-282.
9. Schieppati A, Henter JI, Daina E, Aperia A. 2008. Why rare diseases are an important medical and social issue. Lancet. 371:2039-41.
10. Russo FM, Cordier AG, De Catte L, Saada J, Benachi A, Deprest J. Workstream Prenatal Management, ERNICA European reference network. 2018. Proposal for standardized prenatal ultrasound assessment of the fetus with congenital diaphragmatic hernia by the European reference network on rare inherited and congenital anomalies (ERNICA). Prenat Diagn. 38:629-637.
11. Evans WR. 2018. Dare to think rare: diagnostic delay and rare diseases. Br J Gen Pract. 68:224-225.
12. Opitz JM, Herrmann J, Pettersen JC, Bersu ET, Colacino SC. 1979. Terminological, diagnostic, nosological, and anatomical-developmental aspects of developmental defects in man. Adv Hum Genet. 9:71-164.
13. Spranger J, Benirschke K, Hall JG, Lenz W, Lowry RB, Opitz JM, Pinsky L, Schwarzacher HG, Smith DW. 1982. Errors of morphogenesis: concepts and terms. Recommendations of an international working group. J Pediatr. 100:160-5.
14. Jones DG. 2015. Genetic privacy and the use of archival human material in genetic studies—current perspectives. Medicolegal & Bioethics 5:43–52.
15. Nelson-Marten P, Rich BA. 1999. A historical perspective of informed consent in clinical practice and research. Semin Oncol Nurs. 15:81-8.
16. Morgan LM. 2002. "Properly disposed of": a history of embryo disposal and the changing claims on fetal remains. Med Anthropol. 21:247-74.
17. Peelen J. 2011. Between birth and death. Rituals of pregnancy loss in the Netherlands. Thesis Radboud University. Nijmegen.

18. Jones DG, Gear R, Galvin KA. 2003. Stored human tissue: an ethical perspective on the fate of anonymous, archival material. Journal of Medical Ethics. 29:343-347.
19. Sonnemans LJP, Vester MEM, Kolsteren EEM, Erwich JJHM, Nikkels PGJ, Kint PAM, van Rijn RR, Klein WM, Dutch post-mortem imaging guideline group. 2018. Dutch guideline for clinical foetal-neonatal and paediatric post-mortem radiology, including a review of literature. Eur J Pediatr. 177:791-803.
20. Shelmerdine SC, Hutchinson JC, Sebire NJ, Jacques TS, Arthurs OJ. 2017. Post-mortem magnetic resonance (PMMR) imaging of the brain in fetuses and children with histopathological correlation. Clin Radiol. 72:1025-1037.
21. Rutty GN, Morgan B, Robinson C, Raj V, Pakkal M, Amoroso J, Visser T, Saunders S, Biggs M, Hollingbury F, McGregor A, West K, Richards C, Brown L, Harrison R, Hew R. 2017. Diagnostic accuracy of post-mortem CT with targeted coronary angiography versus autopsy for coroner-requested post-mortem investigations: a prospective, masked, comparison study. Lancet. 390:145-154.
22. Sugiura A, Kitama T, Toyoura M, Mao X. 2018. The Use of Augmented Reality Technology in Medical Specimen Museum Tours. Anat Sci Educ. 2018 Nov 19. [Epub ahead of print]
23. Moro C, Štromberga Z, Raikos A, Stirling A. 2017. The effectiveness of virtual and augmented reality in health sciences and medical anatomy. Anat Sci Educ. 10:549-559.
24. Christensen KD, Phillips KA, Green RC, Dukhovny D. 2018. Cost Analyses of Genomic Sequencing: Lessons Learned from the MedSeq Project. Value Health. 21:1054-1061.
25. Wordsworth S, Doble B, Payne K, Buchanan J, Marshall DA, McCabe C, Regier DA. 2018. Using “Big Data” in the Cost-Effectiveness Analysis of Next-Generation Sequencing Technologies: Challenges and Potential Solutions. Value Health. 21:1048-1053.
26. Oostra RJ, Baljet B, Schutgens RB, Hennekam RC. 1997. Smith-Lemli-Opitz syndrome diagnosed in a 130-year-old anatomical specimen. Am J Med Genet. 68:257-9.

10 English and Dutch summary

English summary

This thesis concerns past, present and future descriptive and explorative perspectives of Dutch teratological collections and starts with a general introduction (chapter 1). In this introduction the underlying concept of a museum and exhibitional considerations and choices are explained. Furthermore, the basics of teratology and the study of congenital anomalies are introduced after which a historical perspective in the study of congenital anomalies is given. In addition we shortly describe some general epidemiological and etiopathogenetic aspects of congenital anomalies and finally we switch from an examination of historical teratological specimens to contemporary teratological research.

This thesis is divided in three parts: past, present and future. In the first part, historical collections are extensively inventoried and described in detail. In the second part, contemporary approaches, insights and thoughts are applied to teratological specimens. In the last part, modern techniques are used to further examine teratological specimens. The different chapters are summarized separately below.

Part 1: Historical approach of Dutch teratological collections

In chapter 2 we provide an extensive overview of the teratological collection of the world-famous 17th century Dutch anatomist Frederik Ruysch currently housed in The Peter the Great Museum of Anthropology and Ethnography in Saint Petersburg (Russia). This collection was bought and shipped in 1717 by Czar Peter the Great, and currently still comprises more than 900 specimens. A modest number concerns specimens with congenital anomalies. We searched for teratological clues in the extant collection and in all Ruysch's descriptions and correspondences regarding specimens and cases he encountered during his career as *doctor anatomiae* and chief instructor of the surgeons and midwives in Amsterdam. We identified a total of 63 teratological specimens and case descriptions, including some exceedingly rare anomalies. As it turns out, we found that Ruysch was the first to describe several of the conditions we encountered, including intracranial teratoma, enchondromatosis, and Majewski syndrome. Although his comments pose an interesting view on how congenital anomalies were scientifically perceived in early 18th century Europe, Ruysch mostly refrained from explaining the causes of the conditions he encountered. Instead, he dedicated himself to careful descriptions of his specimens. Almost 300 years after his demise, Ruysch's legacy still impresses and inspires both scientists and lay men.

In Chapter 3, we redescribe, rediagnose and recategorize all present human teratological specimens from the teratological collection of the *Museum Anatomicum* in Leiden (The Netherlands) and match these with historical descriptions. The Leiden collection currently includes the oldest teratological specimens of The Netherlands. Throughout four centuries, hundreds of teratological specimens were acquired by more than a dozen collectors. Due to the rich history of this vast collection, teratological specimens can now be investigated in a unique retrospective sight. The entire 19th century collection was described in full detail by Eduard Sandifort (1742–1814) and his son Gerard Sandifort (1779–1848). We found a total of 642 human teratological specimens in the extant collection, including some exceptional conditions such as faciocranioschisis and conjoined twins discordant for cyclopia and sirenomelia. Both father and son Sandifort differed in their opinion regarding the cause of congenital anomalies. Their contemporaries Wouter Van Doeveren (1730–1783) and Andreas Bonn (1738–1817) both presented an interesting view on how congenital anomalies were perceived and explained during the 18th and 19th centuries; the golden age of descriptive teratology. This enormous collection is almost 400 years old and still impresses scientists, (bio)medical students, and laymen visiting and exploring the collections of the *Museum Anatomicum.*

Part 2: Contemporary approach of Dutch teratological collections

In chapter 4 we provide a critical appraisal about paleodysmorphology and paleoteratology in which we described the diagnoses and interpretation of congenital conditions of the skeleton in an anthropological context. Although most congenital conditions have a low prevalence, they collectively occur in a few percent of all live births. Congenital conditions are rarely encountered in anthropological studies, not least because many of them have no obvious effect on the skeleton. Two groups of congenital conditions specifically affect the skeleton, either qualitatively or quantitatively. Skeletal dysplasias or osteochondrodysplasias interfere with the formation, growth and maturation of skeletal tissues leading to diminished postural length. However, the building plan of the body is unaffected. Well-known skeletal dysplasias represented in the archeological record include osteogenesis imperfecta and achondroplasia. Dysostoses, in contrast, interfere with the building plan of the body, leading to e.g. missing or extra skeletal elements, but the architecture of the skeletal tissues is unaffected. Dysostoses can concern the extremities, the vertebral column, or the craniofacial region. Conditions pertaining to the cranial sutures, i.e., craniosynostoses, can be either skeletal dysplasias or dysostoses.

Congenital conditions that are not harmful to the individual are known as anatomical variations, several of which have a high and population-specific prevalence. In individual cases, specific congenital conditions could be determinative in establishing identity, provided that ante-mortem registration of those conditions was ensured.

In chapter 5 we focus on the rare congenital anomaly sirenomelia in which the most impressive phenotypical characteristic is the presence of a 180˚-rotated, axially positioned, single lower limb. Associated gastrointestinal and genitourinary anomalies are almost always present and become visible and obvious if both internal and external dysmorphological characteristics are properly described. This rare congenital anomaly is still subject of ongoing controversies concerning its nosology, pathogenesis and possible genetic etiology. We suggest that sirenomelia may be part of a syndromic continuum, overlapping with other complex conditions including caudal dysgenesis and VATER/VACTERL/VACTERL-H associations, which may all be part of a heterogeneous spectrum, and could originate from an early defect in blastogenesis. We review the contemporary hypotheses and conceptual approaches regarding the etiology and pathogenesis of sirenomelia, especially in the context of concomitant conditions. To expand on the latter we include the external and internal dysmorphology of one third trimester sirenomelic fetus from our anatomical museum collection, in which multiple concomitant but discordant anomalies are observed compared to classic sirenomelia. This case is diagnosed as VACTERL-H association with concomitant sirenomelia.

In chapter 6 we focus on the etiopathogenesis of conjoined twins which remains matter for ongoing debate and is currently cited as partial fission or secondary fusion. Both theories could potentially be extrapolated from embryological adjustments exclusively seen in conjoined twins. Adoption of these, seemingly factual, theoretical proposals has (unconsciously) resulted in crystallized patterns of verbal and graphic representations concerning their enigmatic genesis. Critical evaluating on their plausibility and solidity remains however largely absent. As it appears, both the fission and fusion theories cannot be applied to the full range of conjunction possibilities. We propose that initial duplication of axially located morphogenetic potent primordia could be the initiating factor in the genesis of ventrally, laterally and caudally conjoined twins. The mutual position of two primordia results in neo-axial orientation and/or interaction aplasia. Both these embryological adjustments result in conjunction patterns that may seemingly appear as being caused by fission or fusion. However, neither fission nor fusion are the

cause of most conjoined twinning types; rather what is interpreted as fission or fusion is actually the result of the twinning process itself. Furthermore, we will discuss the commonly assumed etiological correlation with monozygotic twinning. Finally, considerations are presented which indicate that the dorsal conjunction group is etiologically and pathogenetically different from other symmetric conjoined twins. A reason for the ongoing etiopathogenetic debate on the genesis of conjoined twins is because different types of conjoined twins are classically placed in one overarching receptacle—which has hindered the quest for answers.

Part 3: Modern approach of Dutch teratological collections

In chapter 7 we perform additional radiological imaging on 41 teratological specimens from the Museum for Anatomy and Pathology in Nijmegen (The Netherlands) by means of magnetic resonance imaging (MRI) and computed tomography (CT) to seek for advantages of radiological examination and explore the scientific and educational potential of these specimens. We show which technical parameters are appropriate to acquire high resolution images of fixed specimens. Out of the 41 scanned fetuses, some characteristic cases are described in detail, showing the added medical value of additional imaging. Radiological data can be used to re-evaluate historically given diagnoses and may give insight in very rarely occurring birth defects. Furthermore, this data can be complemented with contemporary dysmorphological insights and 3D models which may subsequently become implemented in teratological education of medical students and residents. Full-term teratological fetuses become increasingly rare and deserve a prominent place in every anatomical museum; they are suitable for contemporary teratological research and education. We conclude that radiological techniques markedly enhance their scientific and didactic value.

In chapter 8 we performed Sanger sequencing to find the pathognomonic mutation in a 180-year-old achondroplastic skeleton from the *Vrolik* Museum in Amsterdam (The Netherlands). Throughout the last four centuries, many anatomical museums across the world have collected teratological specimens that became precious objects. These can be regarded as spirits of the past which have captured the morphology of diseases through time. These valuable and irreplaceable specimens can be perfectly used in contemporary dysmorphological and/or genetic research. Unfortunately, due to the historical nature of these specimens and the regularly used aggressive preservation fluids, DNA degradation is often present. Furthermore, the use of material

for DNA extraction is restricted to preserve the appearance of these valuable museological specimens. The most challenging part in this perspective is to harvest sufficient DNA of good quality for further testing without damaging the specimens. Besides fixed specimens, most teratological collections contain dried skeletal and teeth materials which are an excellent source to extract DNA from. We here present a DNA-based method that enables genetic identification of the G1138A mutation of the *FGFR3* gene in a 180-year-old achondroplastic skeleton, confirming the previously morphologically determined disease.

In chapter 9 a supplementary discussion with limitations of the foregoing research efforts, ethical and societal considerations thereof and future perspectives of researching teratological collections is given.

Nederlandse samenvatting

Dit proefschrift omvat historische, hedendaagse en toekomstige bespiegelingen op Nederlandse teratologische collecties. In de algemene inleiding (hoofdstuk 1) wordt het onderliggende concept van een museum uitgelegd en worden overwegingen en keuzes toegelicht die in ogenschouw genomen moeten worden bij het creëren van een nieuwe museale expositie. Daarnaast komen de teratologie en de studie van congenitale anomalieën aan bod, gevolgd door een historisch overzicht van de studie van aangeboren aandoeningen. Verder beschrijven wij een aantal algemene aspecten in de epidemiologie en etiopathogenese van aangeboren aandoeningen. Ten slotte maken we de sprong van de beschrijving van historische teratologische preparaten naar hedendaags teratologisch onderzoek. Dit proefschrift bestaat uit drie delen: verleden, heden en toekomst. In het eerste deel worden historische collecties nader beschreven en uitgebreid geïnventariseerd. In het tweede deel worden hedendaagse benaderingen, gedachten en inzichten toegepast op teratologische preparaten. In het laatste deel worden moderne technieken gebruikt om oude preparaten nader te onderzoeken. De verschillende delen met bijbehorende hoofdstukken worden hieronder separaat samengevat.

Deel 1: Historische benadering van Nederlandse teratologische collecties

In hoofdstuk 2 geven we een uitgebreid overzicht van de teratologische collectie van de wereldberoemde 17e-eeuwse Nederlandse anatoom Frederik Ruysch. Deze collectie is gehuisvest in het Peter de Grote Museum voor Antropologie en Etnografie in Sint-Petersburg (Rusland). De verzameling werd in 1717 gekocht door Tsaar Peter de Grote en naar Rusland verscheept. Deze collectie omvat momenteel meer dan 900 preparaten. Een bescheiden deel hiervan bestaat uit preparaten met aangeboren aandoeningen. We hebben gezocht naar aanwijzingen voor teratologische aandoeningen in de bestaande verzameling en in alle beschrijvingen en correspondentie van Frederik Ruysch. Hij verzamelde en beschreef deze casus gedurende zijn loopbaan als *doctor anatomiae* en hoofdinstructeur van de chirurgijns en vroedvrouwenschool in Amsterdam. Er werden in totaal 63 teratologische preparaten en aan de teratologie gerelateerde casusbeschrijvingen gevonden, waaronder enkele buitengewoon zeldzame anomalieën. Het bleek dat Ruysch de eerste was die een aantal aandoeningen beschreef, zoals een intracranieel teratoom, enchondromatose en het syndroom van Majewski. Hoewel zijn opmerkingen een interessant beeld geven van hoe aangeboren aandoeningen wetenschappelijk werden gepercipieerd in de vroege 18e-eeuw

in Europa, onthield Ruysch zich er in de meeste gevallen van om de casus die hij tegenkwam te verklaren. In plaats daarvan wijdde hij zich aan het zorgvuldig beschrijven van zijn preparaten. Bijna 300 jaar na zijn overlijden maakt Ruysch's nalatenschap nog steeds indruk en inspireert zowel de wetenschapper als de leek.

In hoofdstuk 3 worden alle humane teratologische preparaten uit de teratologische collectie van het *Museum Anatomicum* in Leiden opnieuw gediagnosticeerd, gecategoriseerd en vergeleken met historische beschrijvingen. De Leidse collectie omvat momenteel de oudste teratologische preparaten van Nederland. Gedurende vier eeuwen zijn er honderden teratologische preparaten verzameld. Vanwege de rijke geschiedenis van deze uitgebreide collectie kunnen teratologische preparaten nu worden onderzocht met een unieke retrospectieve blik. Daarnaast is de gehele 19e-eeuwse collectie gedetailleerd beschreven door Eduard Sandifort (1742-1814) en zijn zoon Gerard Sandifort (1779-1848). In de huidige collectie vonden wij in totaal 642 menselijke teratologische preparaten, waaronder enkele uitzonderlijke gevallen zoals faciocranioschisis en Siamese tweelingen met concomitante cyclopie en sirenomelie. Vader en zoon Sandifort verschilden van mening over de oorzaken van aangeboren aandoeningen. Hun tijdsgenoten Wouter Van Doeveren (1730-1783) en Andreas Bonn (1738-1817) gaven beiden een interessant beeld van hoe congenitale anomalieën werden ervaren en verklaard in de 18e- en 19e-eeuw; de gouden eeuw van beschrijvende teratologie. Deze enorme, bijna 400 jaar oude collectie maakt nog steeds indruk op wetenschappers, (bio)medische studenten en leken die de collecties van het *Museum Anatomicum* bekijken.

Deel 2: Hedendaagse benadering van Nederlandse teratologische collecties

Hoofdstuk 4 is gewijd aan de paleodysmorfologie en paleoteratologie, waarbij we aangeboren aandoeningen van het skelet in een antropologische context plaatsen. Hoewel de meeste congenitale aandoeningen een lage prevalentie hebben, komen ze collectief relatief vaak voor (enkele procenten van alle levendgeborenen). Aangeboren aandoeningen komen zelden voor in antropologische studies, niet in de laatste plaats omdat veel van deze aandoeningen geen duidelijk effect op het skelet hebben. Twee groepen aangeboren aandoeningen treffen specifiek het skelet, zowel kwalitatief als kwantitatief: skeletdysplasieën en dysostosen. Skeletdysplasieën of osteochondrodysplasieën interfereren met de vorming, groei en rijping van skeletweefsel, uiteindelijk leidend tot een verminderde lichaamslengte.

Het bouwplan van het lichaam wordt echter niet beïnvloed. Bekende skeletdysplasieën die in de archeologie worden beschreven, omvatten osteogenesis imperfecta en achondroplasie. Dysostosen interfereren daarentegen met het bouwplan van het lichaam, wat uiteindelijk leidt tot bijvoorbeeld ontbrekende of extra skeletale elementen. De histologische architectuur van het skeletweefsel wordt echter niet beïnvloed. Dysostosen kunnen betrekking hebben op de ledematen, de wervelkolom of de craniofaciale regio. Aandoeningen die betrekking hebben op de craniale suturen, ofwel craniosynostosen, kunnen zowel skeletdysplasieën als dysostosen omvatten. Aangeboren aandoeningen die niet schadelijk zijn voor het individu staan bekend als anatomische variaties, waarvan er verschillenden een hoge en populatiespecifieke prevalentie hebben. In individuele gevallen kunnen specifieke aangeboren aandoeningen bepalend zijn voor het vaststellen van de identiteit, op voorwaarde dat dit *ante mortem* is vastgelegd.

In hoofdstuk 5 wordt de zeldzame aangeboren aandoening sirenomelie uitgebreid besproken. Sirenomelie wordt gekenmerkt door de aanwezigheid van een 180˚-geroteerde, axiaal gepositioneerde, enkel aangelegde onderste extremiteit. Geassocieerde gastro-intestinale en urogenitale aandoeningen zijn bijna altijd aanwezig en komen aan het licht wanneer de interne en externe dysmorfologische kenmerken uitgebreid in kaart worden gebracht. Sirenomelie blijft onderhevig aan voortdurende controverses betreffende de nosologie, pathogenese en mogelijke genetische etiologie. We suggereren dat sirenomelie deel kan uitmaken van een syndromaal continuüm en overlap laat zien met andere complexe aandoeningen zoals caudale dysgenese en VATER/VACTERL/VACTERL-H associaties, welke allemaal deel zouden kunnen uitmaken van een heterogeen spectrum en afkomstig kunnen zijn van een vroeg defect in de blastogenese. We geven een overzicht van de hedendaagse hypothesen en conceptuele benaderingen met betrekking tot de etiologie en pathogenese van sirenomelie, met specifieke aandacht voor concomitante aandoeningen. Om dit laatste uit te breiden, hebben we de externe en interne dysmorfologie van een derde trimester foetus met sirenomelie uit onze museumcollectie aan deze bespiegeling toegevoegd. In dit preparaat zijn meerdere concomitante maar discordante afwijkingen waargenomen, vergeleken met klassieke sirenomelie. Hierop werd de diagnose VACTERL-H met bijkomende sirenomelie is gesteld.

In hoofdstuk 6 focussen wij op de etiopathogenese van Siamese tweelingen. Het ontstaan van Siamese tweelingen blijft onderwerp van discussie en wordt in de medische literatuur alom verklaard met de concepten partiële splitsing

en/of secundaire fusie. Echter, beide theorieën zijn meer beschrijvingen van embryologische aanpassingen die te zien zijn in Siamese tweelingen, dan verklaringen ervan. Acceptatie van deze schijnbaar feitelijke theoretische modellen heeft (onbewust) geresulteerd in uitgekristalliseerde verbale en grafische patronen betreffende hun raadselachtige ontstaanswijze. Het kritisch beoordelen en evalueren van de plausibiliteit hiervan blijft echter grotendeels achterwege. Het lijkt erop dat zowel de partiële splitsing als de secundaire fusie niet toegepast kan worden op het volledige scala aan conjunctiemogelijkheden. Wij stellen voor dat initiële duplicatie van axiaal gelegen morfogenetische primordia de aansturende factor zou kunnen zijn bij het ontstaan van ventraal, lateraal en caudaal verbonden tweelingen. De onderlinge positie van deze primordia resulteert in neo-axiale oriëntatie en/of interactie-aplasie. Beide embryologische aanpassingen resulteren in samengestelde patronen die schijnbaar lijken te zijn veroorzaakt door partiële splitsing of secundaire fusie. Echter, noch splitsing noch fusie zijn de oorzaak van de meeste Siamese tweelingen. Wat wordt geïnterpreteerd als partiële splitsing of secundaire fusie is eigenlijk het resultaat van het tweelingmechanisme zelf. Daarnaast gaan we in op de algemeen veronderstelde etiologische correlatie met monozygote tweelingen. Tenslotte worden overwegingen gegeven die veronderstellen dat de dorsaal verbonden Siamese tweelingen zowel etiologisch als pathogenetisch verschillen van de ventraal, lateraal en caudaal verbonden tweelingen. De reden van het voortdurende etiopathogenetische debat over de ontstaanswijze van Siamese tweelingen zou te maken kunnen hebben met het feit dat verschillende typen Siamese tweelingen, wellicht ten onrechte, in één overkoepelende vergaarbak worden ondergebracht. Dit heeft de zoektocht naar antwoorden belemmerd.

Deel 3: Moderne benadering van Nederlandse teratologische collecties

In hoofdstuk 7 hebben we met radiologisch onderzoek 41 teratologische preparaten afkomstig van het Museum voor Anatomie en Pathologie bij het Radboudumc in Nijmegen (Nederland) met behulp van *magnetic resonance imaging* (MRI) en *computed tomography* (CT) nader in beeld gebracht om de meerwaarde van radiologische beoordeling in kaart te brengen en om de wetenschappelijke en educatieve potentie van deze preparaten te verkennen. We laten zien welke technische parameters geschikt zijn om radiologische beelden met hoge resolutie van gefixeerde preparaten te verkrijgen. Van de 41 gescande foetussen zijn enkele karakteristieke gevallen beschreven die weergeven wat aanvullende beeldvorming en beoordeling toevoegt. Radiologische gegevens zijn bruikbaar om historische diagnoses opnieuw te evalueren en inzicht te geven in zeer zeldzame congenitale aandoeningen.

Bovendien kunnen deze gegevens worden aangevuld met hedendaagse dysmorfologische inzichten en driedimensionale modellen die vervolgens geïmplementeerd kunnen worden in teratologieonderwijs aan medische studenten en artsen in opleiding. Voldragen teratologische foetussen worden steeds zeldzamer en verdienen een prominente plaats in ieder anatomisch museum; ze zijn geschikt voor het uitvoeren van hedendaags teratologisch onderzoek en onderwijs. We concluderen dat radiologische technieken de wetenschappelijke en didactische waarde van deze preparaten kunnen vergroten.

In hoofdstuk 8 hebben we met *Sanger sequencing* de pathognomonische mutatie in een 180 jaar oud achondroplastisch skelet van het Vrolik Museum in Amsterdam weten op te sporen. Gedurende de laatste vier eeuwen hebben veel anatomische musea over de hele wereld teratologische preparaten verzameld. Deze zijn door de tijd heen steeds waardevoller geworden. Deze preparaten kunnen worden beschouwd als objecten uit het verleden die de morfologie van ziekten door de tijd heen hebben vastgelegd. Deze waardevolle en onvervangbare preparaten kunnen uitstekend worden gebruikt in hedendaags dysmorfologisch of genetisch onderzoek. Helaas is door de historische aard en de regelmatig gebruikte agressieve conserveringsvloeistoffen sprake van DNA-afbraak. Bovendien is het gebruik van materiaal voor DNA-extractie beperkt om het uiterlijk van deze waardevolle museale preparaten te behouden. Het meest uitdagende onderdeel in dit perspectief is om voldoende DNA van goede kwaliteit te verzamelen en het preparaat tegelijkertijd zo min mogelijk te beschadigen. Naast gefixeerde preparaten bevatten de meeste teratologische collecties gedroogde skelet- en tandmaterialen die een uitstekende bron zijn voor het extraheren van DNA. In dit hoofdstuk presenteren we een op DNA gebaseerde methode die genetische identificatie mogelijk maakt van de G1138A-mutatie van het *FGFR3*-gen in een 180 jaar oud achondroplastisch skelet, hetgeen de eerdere morfologisch onderbouwde diagnose bevestigt.

In hoofdstuk 9 wordt een aanvullende discussie met beperkingen van de voorgaande onderzoeksinspanningen, ethische en maatschappelijke overwegingen hierbij en toekomstperspectieven van onderzoek naar teratologische collecties gegeven.

Appendices

Addendum

A novel teratological exhibition in Nijmegen (The Netherlands)

It was after seeing the teratological collection of the Museum for Anatomy and Pathology in Nijmegen (The Netherlands) when the initiative for researching (Dutch) teratological collections was born. This collection was predominantly collected between 1950 and 1980 by medical doctor Albert Verhofstad (†2008), affiliated as a staff member of the Department of Anatomy and subsequently of the Department of Pathology at the Radboudumc (The Netherlands). The teratological collection currently consists of 72—mostly pre- and full-term formalin fixed specimens with a variety of severe and predominantly lethal congenital anomalies. Before the novel teratological exhibition, approximately 20 teratological specimens were exhibited, albeit without any rationale. The small exposition only existed from a historical perspective; a clear defined storyline, information concerning the specimens was absent, the choice of specimens was unclear and a well thought groupment of the specimens was lacking. Observed reactions from visitors were often provocative and disrespectful: the collection of teratological fetuses was imbued with the analogy of a morbid cabinet (authors personal observations). To improve the visitors experience and ameliorate its learning output a novel and publically accessible teratological exhibition was created which officially opened in the autumn of 2017. However, some considerations were contemplated before creating this exhibition: 1) an appropriate place should be available which justifies the collection in an educational and esthetical point of view, 2) the collection should be publically accessible and suitable for every visitor, 3) the exhibition should include ethical aspects and it should be possible that visitors leave their opinion or feeling about the exhibition, 4) the exhibited specimens should promote wonder and curiosity and ideally provoke extended inquiry: the choice of exhibited specimens had to be clear and the "story" behind the specimens should be available, 5) visitors should learn something about teratology, the cause, consequence and possible treatment of multiple congenital anomalies and make a link with societal dilemmas, and finally, 6) the exhibition should be used in (bio)-medical curricula and post-academic education and should be part of a continuing academic working environment. In order to do justice to the deceased children and their parents, we felt obliged to exploit as much educational and scientific potentials and strived to exhibit these children as carefully, informatively, modestly, and aesthetically as possible. A serene and open place hidden from first sight was chosen. In addition, before entering the collection site a plaque is seen in which some essential considerations are given (See box).

THE TERATOLOGICAL COLLECTION

The collection of congenital anomalies was collected by Dr. A. Verhofstad between 1950 and 1980.

Many anatomical collections around the world contain similar specimens. Most museum's exhibit teratological collections for the general public. It is not always known whether permission was asked to the parents if their deceased child should be kept for education and research purposes.

However, at that time the medical-ethical way of thinking and legislation was completely different from today; it was the most natural thing in the world to preserve human preparations which showed rare anomalies. In today's society this is no longer imaginable. In view of the above reasons, this collection is unique and irreplaceable. Research and education have always served as a guideline to establish and preserve this collection, this is still the case up to present date.

It was decided to give this collection a prominent place in the museum. A place where education and research are paramount. A place that shows a sensitive subject, but also a place that, in a respectful way, shows a period of the past in which the ethical and legal aspects of the storage of fetal tissue were completely different.

PAY ATTENTION
It is forbidden to photograph. Respect the fetuses that can be seen here.
Consider whether you and/or your children wish to visit this part of the museum.

In this exhibition a total of 35 pre-defined teratological specimens are permanently exhibited and divided into eight general "anomaly groups." All fetuses were, both internally and externally redescribed and rediagnosed according to modern (dys)morphological aspects and insights. A touch screen provides holistic information of each exhibited fetus concerning their external and internal characteristics, it's possible pathogenesis, causes and prognoses. Furthermore, general teratological conversance and information about the origin of the collection are given. In addition, to increase its social context, a short documentary is presented which highlights the differences between the medical interventions of bladder exstrophy in the 1990's and its current clinical considerations. The novel exhibition is perceived as an academic working environment which is constantly monitored and changed if educational and or scientific results and/or literature changes. Finally, the exhibition is used in bio-medical curricula and post-graduate education besides being publically accessible which makes this valuable collection vivid and dynamic. The following page shows an overview of the exhibition.


Urogenitaal en bekken

Dankwoord

En dan is het zover: het schrijven van het dankwoord. Eén naam op de kaft, maar vele personen die een bijdrage hebben geleverd aan de totstandkoming van dit werk. Personen die niet alleen aan dit proefschrift hebben bijgedragen maar ook aan de vorming van mij als persoon. Met het schrijven van dit dankwoord leg ik de laatste hand aan mijn proefschrift waarvan de uitwerking nu voor u ligt.

In tegenstelling tot wat gebruikelijk is, wil ik beginnen om een drietal personen te noemen die niet direct hebben bijgedragen aan deze mijlpaal. Beste **Erwin van Zoelen** en **Theo Henskens**, vrienden. Onder jullie hoede heb ik mij het skeletteren eigen gemaakt. Ik heb van mijn hobby mijn werk kunnen maken en dat is mede dankzij jullie gelukt. Ik haal ontzettend veel inspiratie uit hetgeen ik met mijn handen maak. Beste **dr. Henry Dijkman**. Ik heb veel geleerd van de tijd bij de elektronenmicroscopie. Daarnaast heb jij mij laten zien dat geluk in je werk heel belangrijk is. Jij zag destijds iets in mij en zonder die kans stond ik niet waar ik nu sta.

Hooggeleerde promotor, **emeritus professor Ruiter**, beste Dirk. Ik ben je dankbaar dat jij mijn promotor in Nijmegen wilde zijn. Zonder jou geen proefschrift en geen openbare verdediging. Je altijd kritische houding en integriteit neem ik mee als voorbeeld in mijn verdere loopbaan. In het proefschrift van je laatste promovenda las ik dat ze dankbaar was dat zij jouw laatste promovendus mocht zijn. Zou dit boekje dan toch echt de allerlaatste zijn? De teller staat nu op 40! We weten het niet, je bent nog altijd even actief en daar heb ik enorm veel bewondering voor.

Hooggeleerde promotor, **professor Oostra**, beste Roelof-Jan, *amice*. Ook zonder jou geen proefschrift. Veel dank voor het vertrouwen dat je in mij had vanaf de eerste dag. De museumbezoeken in binnen- en buitenland waren altijd gezellig en leerzaam. De prachtige reis naar Rusland is een onvergetelijke ervaring. Ik bewonder je enthousiasme en je onuitputtelijke kennis over de teratologie in al haar facetten. Het in kaart hebben gebracht van de teratologische collectie in het Narrenturm is een eerste stap naar toekomstig onderzoek. Zoals je zelf altijd een mail afsluit: *Cheers*!

Weledelzeergeleerde co-promotor, **doctor Schepens-Franke,** beste Annelieke. Wij hebben vanaf de dag dat ik in het museum begon goed samen kunnen werken. Je vragen houden mij scherp. De vele complexe discussies over diepgaande embryologie waren altijd nuttig. We hebben samen het Nijmeegse teratologieonderwijs (opnieuw) op de kaart gezet en dat vind ik prachtig.

Weledelzeergeleerde co-promotor, **doctor Klein,** beste Willemijn. Je ongekende enthousiasme voor de *post mortem* radiologie werkt aanstekelijk. We hebben pas een paar foetussen digitaal beschreven in de vorm van een artikel maar er ligt nog voldoende data voor vele toekomstige stukken.

Hooggeleerde mentor, **professor Feitz**, beste Wout. Ik heb je leren kennen als een rustig, integer en kritisch persoon, zaken die ik belangrijk achtte als ik in de problemen zou komen. Gelukkig is dit niet gebeurd. Ik heb vooral een stok achter de deur gevoeld om een punt te zetten achter dit proefschrift na het schrijven van het 7e artikel.

Hooggeleerde leden van de manuscriptcommissie, **prof. Brunner, prof. Vandenbussche** en **prof. van Rijn**. Hartelijk dank voor het snel en kritisch beoordelen van dit proefschrift en uw aanwezigheid bij mijn openbare verdediging.

Prof. Kozicz, beste Tamás. Jij hebt altijd vertrouwen in mij, het museum en het teratologie onderzoek gehad. Je hebt me als leidinggevende gesteund en vrijgelaten in mijn doen en laten. Het bezoek aan de *Mayo Clinic,* nu jouw thuisbasis, is het begin van een nieuwe weg die we in zullen slaan als museum: patiënteducatie. Een uitdagende stip op de horizon.

Prof. Boerman, beste Otto. Begin 2018 kwam jij bij de afdeling als interim hoofd. Je enthousiasme voor het museum was direct voelbaar en dat heb ik als zeer prettig ervaren.

Dr. Kiliaan, **dr. Kooloos**, **drs. van Linge, dr. Vorstenbosch**, **dr. Capellen van Walsum** en **dr. Munneke**, beste Amanda, Jan, Albert, Marc, Anne-Marie en Moniek, beste anatomen. Bedankt dat jullie mij hebben opgenomen in het docentteam van de afdeling Anatomie en mij altijd hebben gesteund in zowel museum- als onderwijstaken. Ik haal veel energie uit het doceren.

Dr. van der Straaten, beste Joop, oud conservator en mijn leermeester in de anatomie. Jij hebt mij getoond hoe het prepareren van de mens in zijn werk gaat en hoe belangrijk het is om op details te letten in de humane anatomie. Jouw anatomiekennis was onuitputtelijk. Ook heb jij altijd geloofd dat ik jouw opvolger zou worden en dat is gelukt.

Beste **Marianne**. Wij zijn samen het kloppend hart van het museum. Je hebt al vele conservatoren langs zien komen en ik vergeet nooit je gezicht toen ik voor het eerst binnen kwam lopen. De rest is geschiedenis.

Beste **Theo Hafmans**, dank voor de mooie opmaak van dit boekje en dat je er vandaag bent om deze mijlpaal vast te leggen.

Overige collega's van de afdeling Anatomie. Te veel personen om separaat te bedanken, voel je niet gepasseerd. Bedankt dat ik op deze afdeling mijn plaats heb.

Beste **student-assistenten** die de afgelopen jaren in het museum hebben rondgelopen. Jullie doen veel kennis op bij de rondleidingen die jullie verzorgen. Het museum is een kweekvijver voor toekomstige specialisten: zonder jullie geen dynamisch museum. Eén student kan ik niet overslaan: **Tim Rijnhout**, vriend. Je hebt veel voor het museum betekend, als fotograaf van het jubileumboek en je hulp bij de vele plaatjes in dit boekje. Daarnaast sta je hier vandaag als **paranimf.**

Beste **bestuursleden** en **adviseurs** van de Stichting Vrienden van het Museum voor Anatomie en Pathologie. Bedankt voor jullie opbouwende en kritische adviezen gedurende mijn promotie en voor het mede mogelijk maken van dit boekje in deze vorm.

Drs. van Asten, beste Sjaak. Gedurende twee jaar hebben wij de woensdagavonden besteed aan het scannen van alle foetussen, altijd onder het genot van klassieke muziek. Jij bent degene die de prachtige radiologische beelden van de foetussen wist te maken.

Dr. de Rooy, beste Laurens, collega conservator van Museum Vrolik in Amsterdam. Het Amsterdamse museum en jullie prachtige preparaten komen regelmatig terug in dit boekje. Bedankt dat je daarmee een bijdrage hebt geleverd aan dit werk.

Dr. IJpma, **dr. Griffioen**, **dr. Driessen-van het Reve** en **Dhr. Mulders**, beste Frank, Cisca, Jozien en Wim. Naast Roelof-Jan zijn wij samen afgereisd naar Rusland. We hebben een prachtige tijd in Sint-Petersburg gehad om de Ruysch collectie te bezichtigen en opnieuw te beschrijven. Ik denk nog vaak terug aan deze bijzondere ervaring. Dit bezoek was voor mij een enorme drijfveer om de teratologische collecties in Nederland in kaart te brengen.

Lieve **schoonfamilie**, bedankt dat ik altijd welkom ben in de chaos van jullie gezin.

Lieve **Lydia** en **Edwin**. Jullie hebben iets verschrikkelijks meegemaakt en dat terwijl jullie het zo verdien(d)en om een gezond kindje te krijgen. **Mylena** was een geschenk maar het mocht toch niet zo zijn. Dit boekje heb ik voor haar geschreven, ook al weet ik dat dit verlies hierdoor op geen enkele manier verzacht wordt. De tijd heelt alle wonden, het litteken zal altijd achterblijven.

Lieve **papa** en **mama**. Ik ben blij dat jullie mij onvoorwaardelijk hebben gesteund en dat ik in mijn vroege tienerjaren al bezig mocht zijn met het ontleden en skeletteren van overleden dieren. Door deze vrijheid ben ik geworden hoe en wie ik nu ben en heb ik dit doel weten te bereiken.

Allerliefste **Elke**, lieve vriendin en vandaag ook **paranimf**. Jij bent heel speciaal voor mij en dat kan (en wil) ik niet in een paar woorden hier beschrijven. Het enige dat ik kwijt wil: ik hou enorm van jou!

Mocht ik nog mensen zijn vergeten, bij dezen: bedankt!

About the author

Lucas Leendert Boer was born on April 27th 1988 in Oss, The Netherlands. He graduated from secondary school in 2004 (RK Sint Jan's MAVO, Oss). Afterwards he obtained his MBO diploma (Clinical chemistry) and subsequently graduated from the university of applied sciences in Nijmegen (Cytohistopathology). Since 2012 he was appointed in the Museum for Anatomy and Pathology from the Radboud university medical center as a dissector. During this period he was trained as a curator by dr. J. van der Straaten and em. prof. D.J. Ruiter. In 2015 he was appointed curator and junior lecturer at the Department of Anatomy under the auspices of prof. T. Kozicz. In addition to his curatorship and research activities, he currently contributes as a lecturer to the anatomy, embryology and teratology education for the (bio) medical curricula of the Radboud university medical centre and the Radboud University and is co-author of the book "*Ontleed in verwondering*" that he published together with the former curator and his first thesis supervisor. In addition, he has been engaged for more than 15 years in the professional preparation of skeletons of mammals, birds and fossils, for both individuals and (inter)national museums and research institutions.

List of publications

Articles

2016:

Rita. M. Kappel, Lucas L. Boer, and Henry Dijkman. 2016. Gel bleed and rupture of silicone breast implants investigated by light-, electron microscopy and energy dispersive X-ray analysis of internal organs and nervous tissue. Clinical Medical Reviews and Case Reports, vol. 3, no. 6, article 087.

Roelof-Jan Oostra, Lucas L. Boer L and Lia E. van der Merwe. 2016. Paleodysmorphology and paleoteratology: Diagnosing and interpreting congenital conditions of the skeleton in anthropological contexts. Clin Anat. 29:878-91.

2017:

Lucas L. Boer, Anna B. Radziun and Roelof-Jan Oostra. 2017. Frederik Ruysch (1638–1731): Historical perspective and contemporary analysis of his teratological legacy.
Am J Med Genet A. 173:16-41.

Lucas L. Boer, Annelieke N. Schepens-Franke, Jack. J.A. van Asten, Dennis G.H. Bosboom, Karin Kamphuis-van Ulzen, Tamas L. Kozicz, Dirk J. Ruiter, Roelof-Jan Oostra and Willemijn M. Klein. 2017. A pictorial review. Radiological imaging of teratological fetuses: what can we learn? Insights Imaging. 8:301-310.

Lucas L. Boer, Jana Naue, Laurens de Rooy and Roelof-Jan Oostra. 2017. Detection of G1138A Mutation of the FGFR3 Gene in Tooth Material from a 180-Year-Old Museological Achondroplastic Skeleton. Genes (Basel). 8:214.

Lucas L. Boer, Eva Morava, Willemijn M. Klein, Annelieke N. Schepens-Franke and Roelof-Jan Oostra. 2017. Sirenomelia: a multi-systemic polytopic field defect with ongoing controversies. Birth Defects Res. 109:791-804.

2018:

Lucas L. Boer, Peter Boek, Andries J. van Dam and Roelof-Jan Oostra. 2018. History and Highlights of the Teratological Specimens in the Museum Anatomicum of the Leiden University, The Netherlands. Am J Med Genet A. 176:618-637.

2019:

Lucas L. Boer, Annelieke N. Schepens-Franke and Roelof-Jan Oostra. 2019. Two is a crowd: on the enigmatic etiopathogenesis of conjoined twinning. Clin Anat. 32:722-741.

Book

2017:

Joop van der Straaten, Lucas Boer and Dirk Ruiter. “Ontleed in Verwondering” ISBN:9789081835626

PhD portfolio

Name PhD student: Lucas L. Boer
Department: Anatomy
PhD period: 1-04-2014 – 31-12-2018
Promoter(s): Prof. D.J. Ruiter and Prof. R-J. Oostra
Co-promotor(s): Dr. A.N Schepens-Franke and Dr. W.M. Klein
Graduate school: Radboud Institute for Health Sciences (RIHS)

Training activities

		Year(s)	ECTS
a)	Courses & workshops		
-	RIHS Introduction Course for PhD candidates	2017	0.75
-	Scientific Integrity for PhD candidates	2017	1.0
b)	Symposia & congresses		
-	Eurodysmorpho (3 days) European conference, Strasbourg Oral presentation	2014	1.25
-	Dutch Anatomical Society (2 days) Annual Dutch conference, Lunteren	2014-17	2.0
-	Anatomical Society Winter Meeting (3 days) European conference, London	2016	0.75
-	Dutch Association of Medical education (NVMO) (2days) Annual Dutch conference, Egmond aan Zee Poster presentation	2017	0.75
-	Anatomical Society Winter Meeting (3 days) European conference, Dundee Poster presentation	2017	1.25
-	Dutch Anatomical Society (2 days) Annual Dutch conference, Lunteren Poster presentation	2018	0.75
-	Dutch Anatomical Society (2 days) Annual Dutch conference, Lunteren Oral presentation	2019	0.75

Teaching activities

c) Lecturing

Throughout the years of this PhD tract, I have contributed intensively in the development and execution of anatomical, embryological and teratological oriented education within the Department of Anatomy affiliated with the Radboud university medical center and included, among other, educational programs:

- Initial bachelor education for medicine, biomedical and dentistry students in the following courses: Elective course Anatomy, KMP5, MED-B3Me2t, Q1MAN2/3, 1MBE3, Q2MNAT1/3, Q3MSV2, Q5MRL1/2/3, Q6MSS, 9B1T2, B3 and Minor5/19/28. Total calculated EC's for 256.5 contact hours = 25.65
- Optional anatomy education medical curricula (KANA1/2/4). Total calculated EC's for 68 contact hours = 6.8
- Post academic teratology education for gynaecologists, geneticists, ethicists, pathologists (in training). Total calculated EC's for 14 contact hours = 1.4

Total EC's during this PhD tract = 43.1

Radboud University Radboudumc university medical center

Institute for Health Sciences
Radboudumc