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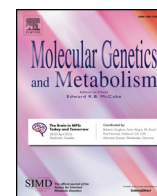
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Antibodies against recombinant alpha-galactosidase A in Fabry disease: Subclass analysis and impact on response to treatment

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ABSTRACT

Background: Treatment of Fabry disease (FD) with recombinant alpha-galactosidase A (r-αGAL A) is complicated by the formation of anti-drug antibodies in the majority of male patients with the classical disease phenotype. Detailed information regarding antibody subtypes, onset and persistence of antibody development and their effect on treatment efficacy is sparse.

Methods: A retrospective study was carried out in 39 male patients with classical FD, treated with either agalsidase-alfa or agalsidase-beta (mean follow up of 10 years). With six to twelve months intervals plasma-induced *in vitro* inhibition of enzyme activity, lysoglobotriaosylsphingosine (lysoGb3) levels and renal function were assessed. In a subset of 12 patients, additionally anti- r-αGAL A IgM, IgA and IgG1, 2, 3 and 4 levels were analyzed.

Results: In 23 out of 39 patients, plasma-induced *in vitro* inhibition of r-αGAL A activity was observed (inhibition-positive). The inhibition titer was strongly negatively correlated to the decrease in lysoGb3: agalsidase-alfa ($FE_{\log 10(\text{inhibition})} = -10.3$, $P \leq .001$), agalsidase-beta ($FE_{\log 10(\text{inhibition})} = -4.7$, $P \leq .001$). Inhibition-positive patients had an accelerated decline in renal function ($FE = 1.21$, $p = .042$). During treatment IgG1 anti-r-αGAL A levels increased only in inhibition-positive patients ($p = .0045$). IgG4 anti-r-αGAL A antibodies developed in 7 out of 9 inhibition-positive patients. Other antibody subclasses were either not present or too low to quantify. **Conclusion:** Development of inhibiting antibodies against r-αGAL A negatively affects the biochemical response to ERT and resulted in an accelerated decline in renal function. The presence of IgG1 and IgG4 anti-r-αGAL A antibodies is associated with *in vitro* αGAL A activity inhibition.

1. Introduction

Fabry disease (OMIM 301500) is an X-linked lysosomal storage disorder resulting from a deficiency of the enzyme alpha-galactosidase A (αGAL A, EC 3.2.1.22). The resulting failure to hydrolyze the terminal alpha-galactosyl moiety from globotriaosylceramide (Gb3) causes accumulation of Gb3 in lysosomes and elsewhere in the cell. Early characteristic clinical manifestations include severe neuropathic pain (acroparesthesia), skin lesions (angiokeratomas) and ocular signs (cornea verticillata). Later in life, cardiac, renal and cerebrovascular

complications are responsible for severe morbidity and a shortened lifespan [1]. The phenotypic spectrum of Fabry disease is broad. Mutations in the GLA gene that result in complete absence of αGAL A activity generally results in classical, more severe disease, especially in male patients. Less severe mutations, predominantly missense mutations, result in non-classical disease phenotypes, with later onset and variable disease progression. Because of the X-linked inheritance, women have residual enzyme activity and disease manifestations are usually less extensive and develop later in life compared to male patients [2].

Abbreviations: ACEi, Angiotensin-converting-enzyme inhibitor; ARB, angiotensinreceptor blockers; ADAs, anti-drug antibodies; anti-r-αGALA, antibodies against recombinant alpha-Galactosidase A; ERT, Enzyme replacement therapy; FD, every other week (eow)Fabry disease; Gb3, Globotriaosylceramide; LysoGb3, lysoglobotriaosylsphingosine; LSD, Lysosomal storage diseases; r-αGALA, recombinant alpha- Galactosidase A

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At present, two recombinant preparations of alpha-galactosidase A are available for treatment, agalsidase-alfa (Replagal), manufactured by Shire in human fibroblasts and registered at a dose of 0.2 mg/kg/eow and agalsidase-beta (Fabrazyme), manufactured by Sanofi-Genzyme in Chinese Hamster ovarian (CHO) cells and registered at a dose of 1 mg/kg/eow. Treatment with enzyme-replacement therapy (ERT) results in a notable reduction of Gb3 and its deacylated form lysoglobotriaosyl-sphingosine (lysoGb3) in plasma and urine [3,4] as well as morphological clearance of storage material in endothelial cells and, to a lesser extent, podocytes [5,6]. In male patients with classical Fabry disease, treatment with ERT delays the occurrence of complications, especially when treatment is initiated before the onset of irreversible organ damage [7,8]. However, more than half of classically affected male patients treated with ERT develop anti-drug-antibodies (ADAs) [4,9,10]. In female patients and patients with a non-classical disease phenotype, antibody formation against the administered recombinant enzyme is rarely observed [4,9,10]. In addition to hypersensitivity reactions, ADAs can cause inhibition of α GAL A activity. The impact of ADAs on ERT effectiveness has been addressed previously and a clear effect on (lyso)Gb3 clearance has been described by several groups [9,11–13]. Recently, the influence of ADAs on clinical outcomes was investigated in 41 male Fabry patients [9]. The ADA positive group had a lower eGFR and higher MSSI- and disease severity score, compared to the ADA negative group. Although analyses were corrected for mutation type (nonsense or missense), drawing conclusions regarding the effect of ADAs based on this data is difficult, since there was no stratification for classical *versus* non-classical phenotype. Correcting for mutation type does not solve this problem, since missense-mutations can result in both classical and non-classical disease. Given the negative effect of the presence of ADAs on clearance of storage materials from endothelial cells [14], a deleterious influence of inhibiting ADAs on therapy efficacy is likely. The aim of this longitudinal retrospective cohort study is to characterize the antibody response in Fabry disease and determine its effect on both biochemical and clinical response to treatment in male Fabry patients with a classical disease phenotype.

2. Methods

2.1. Patients

This study was conducted in accordance with the principles of the Helsinki Declaration, as revised in 2000. Informed consent was obtained from all included patients.

From the total Fabry patient population followed at the Amsterdam Lysosome Center (SPHINX), only male patients with a classical phenotype who were treated with enzyme replacement therapy ($n = 39$) were included in this study. Classification of patients as having classical or non-classical Fabry disease was based on the residual enzymatic activity and the presence or absence of characteristic symptoms, as described by Arends et al. [2].

During follow up, clinical data, as well as plasma samples, were collected at baseline and at every six months during treatment. *In vitro* plasma-induced enzyme activity inhibition (inhibition titer), lysoGb3 levels and renal function were determined as part of routine care in our hospital.

Samples were centrifuged and plasma aliquoted and stored at -80°C . Data and samples from an average treatment duration of 9.7 years (range 1.5 to 16.6 years) were available. 6 patients were treated with agalsidase-alfa only, 15 with agalsidase-beta only and 18 alternated between agalsidase-beta and agalsidase-alfa.

To avoid potential influence of the different enzyme preparations on antibody formation due to switching, only patients who started treatment with agalsidase-beta and stayed on this treatment for at least 4 years (mean treatment duration of 7 years) were studied for the presence and titers of the different Ig subclasses. Twelve out of 39 patients fulfilled these criteria.

2.2. Biochemistry and in vitro inhibition

LysoGb3 (nmol/l) was analyzed as previously described [15,16]. Samples collected before august 2015 were analyzed with isotope-labeled lysoGb3 as a standard. Subsequent analyses were performed with glycine-labeled lysoGb3 as a standard. Results of both methods correlate closely [2]. Biochemical response to treatment at any given time was determined as follows: $\text{decrease LysoGb3}_X = (\text{LysoGb3}_X / \text{LysoGb3}_{\text{baseline}}) * 100$, in which X stands for an individual time point. *In vitro* plasma-induced inhibition of r- α GAL A activity was measured as previously described by Linthorst et al. [13]. In short, inhibition titers represent the dilution factor of plasma resulting in 50% inhibition of the r- α GAL A activity. Patients are considered inhibition-positive if they had an inhibition titer > 6 at any point during treatment.

2.3. Renal function and albuminuria

Creatinine values were obtained from electronic medical records of patients and used to estimate glomerular filtration rate using the CKD-EPI formula.

Albuminuria was categorized into A1, A2 and A3 according to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines [17].

2.4. Anti- α GAL A immunoglobulin ELISA's

Plasma samples from different time points of 10 anti r- α GAL A antibody positive patients were pooled and used as a reference sample to quantify levels of immunoglobulin subclasses in arbitrary units (AU). 96-wells microtiter plates (Nunc/Maxisorp) were coated overnight at 4°C with $100\ \mu\text{l}$ $1\ \mu\text{g/ml}$ alpha-galactosidase beta (Fabrazyme, Genzyme) in sodium bicarbonate buffer (pH 9.8). Plates were washed five times and incubated for 1.5 h at 37°C with $200\ \mu\text{l}$ blocking buffer composed of PBS containing 2% (w/v) BSA fraction V (Merck). Subsequently, plates were washed five times. Plasma samples were diluted in PBS containing 0.1% (v/v) Tween-20 and 2% (w/v) BSA fraction V (Dilution buffer). $100\ \mu\text{l}$ of each dilution was incubated for 1.5 h at 37°C after which plates were washed five times. Next, plates were incubated with horseradish peroxidase (HRP) labeled anti-human IgG, IgG1, IgG2, IgG3, IgG4, IgA and IgM antibodies, respectively (Sanquin Reagents) diluted $2500\times$ in dilution buffer. Plates were washed five times again before $100\ \mu\text{l}$ TMB-substrate was added and plates were incubated 5 min at room temperature before the reaction was stopped using $1\ \text{M H}_2\text{SO}_4$. Absorbance was measured using a microtiter plate reader (Spectramax plus 384) at 450 nm using 540 nm as a reference for background absorption. A plasma pool of healthy donors (Sanquin, Amsterdam, the Netherlands) was used as a negative control. Patient samples were measured in duplicates per plate and on two different plates. All wash steps were done with PBS 0.1% (v/v) Tween-20.

2.5. Statistical analysis

For statistical analysis R (version 3.4.3) was used. Distributions were tested visually as well as by using Shapiro-Wilk test of normality and homogeneity of variances was tested using Bartlett test. Depending on the distribution, differences in baseline characteristics were tested using Mann-Whitney-Wilcoxon test or unpaired *t*-test for continuous variables. Fisher exact test was used for categorical variables. Baseline IgG1 of patients was compared to IgG1 level of a plasma pool of healthy blood donors using a one sample *t*-test. Fisher exact test was used to compare the number of patients with a rise in IgG1 during treatment in inhibition-positive and -negative patients. Correlation between immunoglobulin (Ig) subclasses and inhibition titer were assessed using non-parametric correlation analyses (Spearman's Rho). A linear mixed effect model (package lme4) was used to determine the effect of inhibition titer on decrease in lysoGb3 from baseline (start of treatment)

in each patient. Inhibition titer was transformed to log10 to optimize fit. Analyses were performed separately on data from patients treated with agalsidase-alfa 0.2 mg/kg/eow and those treated with agalsidase-beta 1 mg/kg/eow. Samples collected on doses other than the recommended doses and samples measured within 1 year after start of treatment or within one year after any dose switch were excluded from analysis. This was done since the nadir of the plasma lysoGb3 concentration is reached within the first year of treatment, and on stable dose of ERT, lysoGb3 concentrations remain stable thereafter (see supplemental material B, Fig. 2). In supplemental material B, Fig. 1, the repeated measurements within the patients are depicted and measurements before and after the first dose or treatment switch are depicted differently. To correct for repeated measurements, patient number was used as a random effect. The model was corrected for age of the patients at the start of ERT. The effect of inhibition status on renal function was assessed using a linear mixed model (package lme4) [18]. Random intercept and random slope were added to correct for repeated measurements. The model was corrected for the cumulative dose of ERT received at each time point as well as age, eGFR and the grade of proteinuria at start treatment. *P* values < .05 were considered statistically significant. Full model specifications and R syntax for models and visualization are added as supplemental material A.

3. Results

3.1. Patient characteristics

Patient characteristics of the 39 included patients are outlined in Table 1. Inhibition-positive patients (*n* = 23) more often had a nonsense or frameshift mutation, whereas missense mutations were more prevalent in the inhibition-negative group (*n* = 16). There was also a significant difference in treatment type and dose: inhibition-positive patients were more often treated with agalsidase-beta only and inhibition-negative patients more often with agalsidase-alfa only, resulting in a higher mean and cumulative dose in the inhibition-positive group. Baseline lysoGb3, enzyme activity, age at start of ERT, albuminuria, smoking and hypertension status were not significantly different between these two groups. However, there was a trend for older age and lower eGFR at baseline in the inhibition-positive group.

Table 1

Characteristics of 39 male patients with classic Fabry disease.

	Inhibition-positive	Inhibition-negative	P-value
Nr of patients	23 (59%)	16 (41%)	–
Nonsense/frameshift mutations	14 (61%)	3 (19%)	0.02
Missense/other mutations	9 (39%)	13 (81%)	
LysoGb3 at baseline	115.5 (53–178)	95.3 (61–149)	0.14
Enzyme activity (% of mean reference)	1.1% (0–3)	0.3% (0–5)	0.35 *
Age at start ERT (years)	35.4 (9–58)	20.1 (13–52)	0.07
Agalsidase alfa only	0 (0%)	6 (15%)	> 0.001
Agalsidase beta only	13 (33%)	2 (5%)	
Switched	10 (26%)	8 (21%)	
Cumulative dose received at end of follow up (mg/kg)	121 (29–360)	79 (37–308)	0.07*
Mean dose per infusion (mg/kg/eow)	0.7 (0.2–1)	0.3 (0.2–1)	0.004*
eGFR (ml/min/1.73 ²) at baseline	108.5 (24–172)	132.1 (38–158)	0.09
Baseline albuminuria			0.47
No albuminuria (< 30 mg/24 h)	8 (35%)	9 (56%)	
Mild albuminuria (30–300 mg/24 h)	10 (43%)	5 (31%)	
Severe albuminuria (> 300 mg/24 h)	5 (22%)	2 (13%)	
Treated with ACEi/ARB**	11/23 (48%)	7/16 (44%)	1
Smoker	4/13 (31%)	4/8 (50%)	0.46
Hypertension	3/22 (14%)	2/12 (17%)	1

Continuous variables are depicted as median (range), categorical variables are depicted as number (percentage). Missing values: lysoGb3 (*n* = 1), smoker (*n* = 18), hypertension (*n* = 5), enzyme activity (*n* = 6). Fisher exact was performed on categorical variables, continuous variables were analyzed using Mann-Whitney-Wilcoxon test or unpaired two-tailed *t*-test, depending on distribution. * Exact *p*-value could not be computed due to ties. ** For at least one year during treatment with ERT.

3.2. Relationship between in vitro inhibition and biochemical response to treatment

Twenty three out of 39 patients were inhibition-positive at any point during treatment (59%). Ten out of those 23 patients became inhibition-negative or alternated between inhibition-positive and inhibition-negative status during treatment. In general, these were patients with low inhibition titers. In patients with a persistent antibody response the highest titer in each individual patient ranged from 130 to 15,000 (mean 2188), while in patient with a fluctuating antibody response the highest measured titer ranged from 8 to 375 (mean 107).

There was a clear negative correlation between inhibition titer and the decrease in lysoGb3 in response to treatment. This relation was most pronounced in patients treated with agalsidase-alfa (0.2 mg/kg/eow). With each tenfold increase in inhibition titer there is an estimated 10% less decrease in lysoGb3 in patients treated with agalsidase-alfa ($FE_{\log_{10}(\text{inhibition})} = -10.3$, $SE = 1.9$, $P \leq .001$) (Fig. 1B). In patients treated with agalsidase-beta this was 5% ($FE_{\log_{10}(\text{inhibition})} = -4.7$, $SE = 0.9$, $P \leq .001$) (Fig. 1A).

3.3. Relationship between in vitro inhibition and decline in renal function during ERT

Using a linear mixed effect model correcting for the cumulative ERT dose received at each time point as well as age, eGFR and the category of proteinuria at baseline, we found an accelerated decline in renal function in inhibition-positive patients of approximately 1.2 ml/min/1.73m² per year while on treatment compared to patients that were inhibition-negative ($FE = 1.21$, $SE = 0.59$, $p = .042$) (Fig. 2). Adding treatment with ACEi or ARB or mutation type as covariates to the model did not result in a better fit or different results.

3.4. Total anti r-αGALA immunoglobulin and Ig subclass titers and their relation to in vitro enzyme activity inhibition

Of the 39 Fabry patients, 12 patients were solely treated with agalsidase-beta and stayed on this treatment for at least 4 years. Before start of ERT, all 12 patients had low titers of anti r-αGAL A IgG1 antibodies, mean titers were higher in treatment naïve patients compared to the background measured in healthy donor plasma pool (39 vs 10 au,

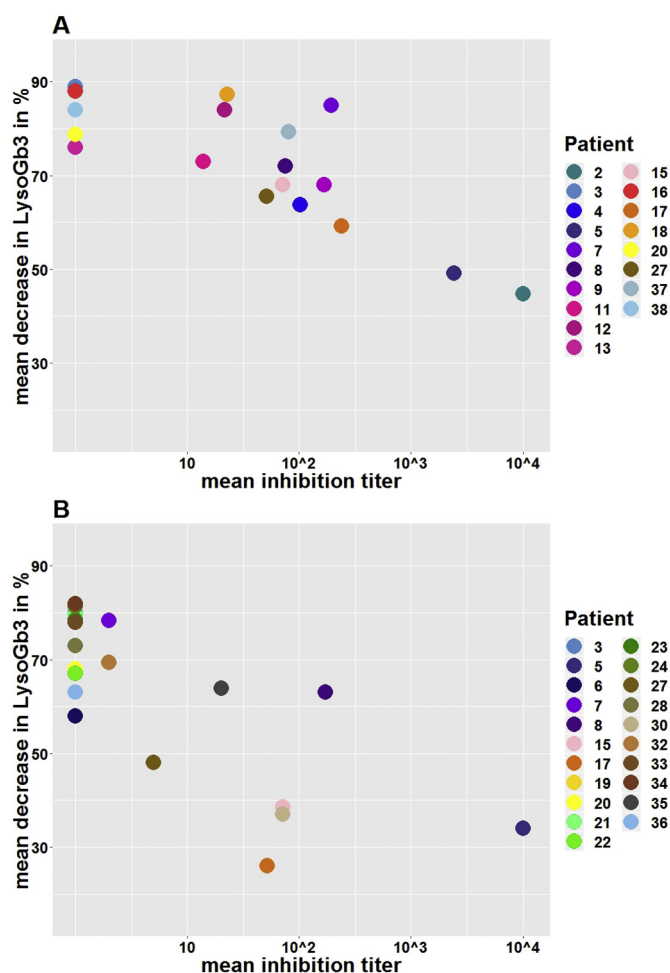


Fig. 1. Effect of *in vitro* inhibition on biochemical response to treatment with ERT. Main *in vitro* inhibition and mean decrease in lysoGb3 from baseline are depicted per patient under treatment with agalsidase-beta 1.0 mg/kg/eow (A) or agalsidase-alfa 0.2 mg/kg/eow (B). Time points within 1 year after start treatment as well as time points within 1 year after any dose switch were excluded from analyses. Samples from 31 patients remained. Seven out of these 31 patients appear in both graphs. Each color represents an individual patient. All measured time points are depicted in supplemental material B, Fig. 1.

$p = .0032$). During treatment, IgG1 anti r- α GAL A levels increased during treatment in all 9 inhibition-positive patients (range: 3–24-fold increase from baseline), but not in the 3 inhibition-negative patients ($p = .0045$, Fig. 3). IgG4 anti r- α GAL A antibodies developed in 7 out of 9 inhibition-positive patients (range 3–2082 au) and none of the 3 inhibition negative patients. Only 2 patients had a sustained IgG4 response, these patients also showed the highest levels of *in vitro* inhibition of r- α GAL A activity (Fig. 3). In one of these patients ERT was discontinued because of pronounced disease progression during treatment with ERT (Fig. 3D), the other patient died at the age of 57 due to complications of a myocardial infarction (Fig. 3K). A different patient underwent renal transplantation during follow up at a time point at which a significant IgG1 r- α GAL A antibody and inhibition titer was present. After transplantation, the IgG1 r- α GAL A antibody and inhibition titers went down to pretreatment level (Fig. 3E).

Levels of anti r- α GAL A IgG2 and IgA were present in some of the inhibition-positive patients but were too low to reliably quantify. Anti r- α GAL A IgM and IgG3 were not detectable in any of the plasma samples. Total anti r- α GAL A IgG, as well as anti r- α GAL A IgG1 and IgG4 correlated well with *in vitro* measured plasma inhibition of r- α GAL A enzyme activity ($\rho = 0.71$, 0.60 and 0.67 respectively).

4. Discussion

In this study, *in vitro* inhibition of r- α GAL A activity by ADAs in plasma of Fabry patients was clearly associated with a less robust reduction in lysoGb3 in response to treatment with ERT. In this study we showed for the first time that this effect was titer dependent. Higher inhibition titers led to an inferior biochemical response. This effect was most pronounced during treatment with agalsidase-alfa and most likely caused by the difference in dose between agalsidase-alfa and -beta, as previously described [11]. The proposed explanation is that a higher proportion of the lower concentration of agalsidase-alfa (dose 0.2 mg/kg/eow in contrast to 1.0 mg/kg/eow for agalsidase-beta) of enzyme is inhibited when antibodies are present.

LysoGb3 is thought to be directly involved in the development of glomerular injury [19], induction of fibrosis [20] as well as neuropathic pain [21]. Despite the known effect of ADAs on the biochemical response to treatment, investigations showing a clinical meaningful effect of ADAs in Fabry disease are scarce. In the current study we assessed the effect of inhibiting antibodies on renal function with a linear mixed effect analysis correcting for cumulative ERT dose at each time point as well as age, renal function and proteinuria at treatment initiation. Inhibition-positive patients had an accelerated decline in renal function of 1.2 ml/min/1.73m² per year compared to inhibition-negative patients. Although the observed effect was on average modest, it is clinically highly relevant as a more rapid loss of renal function implies an earlier need for dialysis or renal transplantation. Confirmation of the negative effect of antibodies on renal function, correcting for the above-mentioned factors, in a second patient cohort would strengthen our observation. Previously Lenders et al. reported higher lysoGb3 levels, greater left ventricular mass and worse renal function in inhibition-positive compared to inhibition-negative patients [9]. However, the fact that classical and non-classical patients were studied as one cohort hampers the interpretation of the results, since there are significant differences in disease course between these patient groups [2]. More recently a prospective French study reported no clinical difference between inhibition-positive and inhibition-negative patients [12]. Although, due to the relative slow disease progression and small patient group (29 treated classical males), no meaningful changes could have been expected during the 2 year follow up. To overcome these limitations, the current study was carried out in a relatively large cohort of male patients with the classical disease phenotype during a mean follow up duration of 10 years. However, our study still has some limitations. In the inhibition-positive group more patients had a nonsense or frameshift mutation compared to the inhibition-negative group, in which missense mutations were more prevalent. This can have two effects: 1. the nature of nonsense and frameshift mutation (leading to truncated protein) may make the patients more prone to ADA development 2. nonsense and frameshift mutation may cause more severe disease, leading to an overestimation of the effect of the ADAs on treatment outcome. The latter was not confirmed, since adding mutation type to the model of the effect of ADAs on renal function did not improve the model or change the outcome. Moreover, all 39 patients were classical male Fabry patients and no statistical differences were found in lysoGb3 or enzyme activity at baseline, thus the unfavorable effect of ADAs on disease outcome is likely to be caused by the development of inhibiting antibodies. Another limitation was that the cohort was too small to be able to study the effects of ADAs on clinical events (e.g. myocardial infarction or cerebrovascular event) due to the clinically relevant trend for younger age and the accompanying very low rate of events in the inhibition-negative patients.

What stands out is the highly relevant effect on lysoGb3 and the relation with (persisting) high titers of ADA. We argue that a biochemical response is of importance and relates to clinical responses. The fact that there is not always a relationship between reductions in lysoGb3 and clinical effects has to do with the slow progressive nature and the different stages of the disease: in patients with advanced

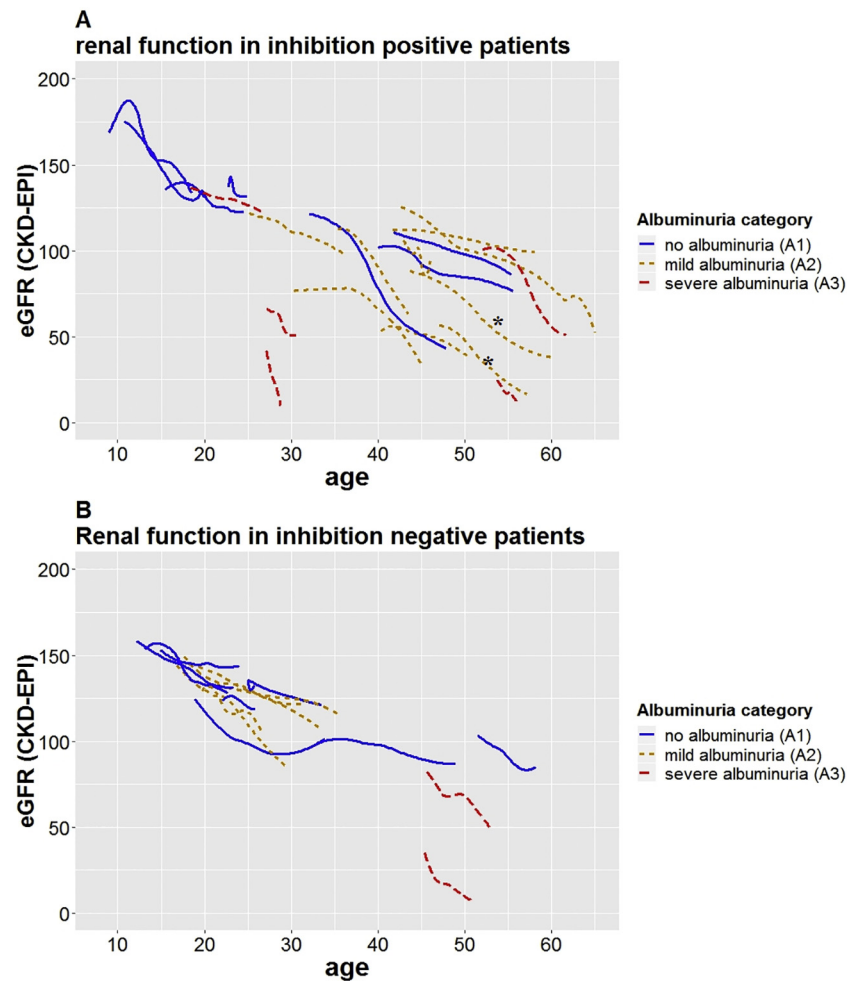


Fig. 2. Effect of *in vitro* inhibition on renal function (GFR estimated by CKD-EPI in ml/min/1.73m²) during treatment with ERT in classical male Fabry patients. Each line represents an individual patient. Panel A depicts the slope of renal function in inhibition-positive patients, panel B depicts inhibition-negative patients. Differences in treatment are depicted in Table 1. Color and line type represent the stage of proteinuria before start with ERT. * The 2 patients in Fig. 2A who were found to have a sustained IgG4 response were marked with an asterisk.

disease at start of ERT, treatment may not sufficiently influence the disease course, even if a robust decline in lysoGb3 is achieved. *Vice versa*, in those in whom progression can still be halted, a decline in lysoGb3 supports a beneficial effect of therapy. As such, reduction in lysoGb3 is a prerequisite for any clinical effect [8] and interfering ADAs, blunting the lysoGb3 decline, are thus of clinical importance.

Longitudinal analysis of immunoglobulin subclasses against recombinant α GAL A showed that anti-r- α GAL A antibodies in our studied sub-cohort were mainly of the IgG1 and IgG4 subclasses. This is in accordance with the findings of Mauhin et al. [12]. Low levels of IgG1 were also found in the plasma pool of healthy controls. Interestingly, baseline levels of anti- α GAL A IgG1, *i.e.* before any exposure to exogenous administered enzyme, were higher in all 12 measured patients compared to healthy control subjects. However, no relation between baseline IgG1 titer and the development of *in vitro* inhibition during treatment was found. Presence of anti-drug antibodies in protein replacement therapies prior to start of treatment have been described in for example hemophilia A [22,23] and mucopolysaccharidosis IVA [24]. Suggested mechanisms of development of these antibodies include early antigen exposure (*e.g.* from the maternal circulation during birth), exposure to mutated protein and antibody producing B cell clone maturing independent from antigen exposure [22,25].

Previously, Lenders et al. demonstrated that IgG4 isolated from patient plasma was capable of inhibiting enzymatic activity [26]. Furthermore, they found that the inhibitory capacity per microgram total

IgG differed per patient indicating that total IgG levels *per se* may not be indicative of the effect of ADAs on disease course. In our study, the occurrence of *in vitro* inhibition of enzyme activity during treatment was associated with an increase in IgG1 or both IgG1 and IgG4 anti- α GAL A, other antibody subclasses were not detected in significant amounts. The two patients that developed a sustained IgG4 response were also the two patients with the highest inhibition titers. This is consistent with finding in hemophilia A, where low-titer inhibition patients had primarily IgG1 anti-FVIII antibodies, whereas IgG4 antibodies were more prominent in patients with high inhibition titers [27,28]. From an immunological point of view this distribution makes sense. While antibody responses to soluble proteins primarily induce a IgG1 response, repeated exposure to antigens in non-infectious settings are known to induce IgG4 formation [29]. IgG4 is often referred to as a ‘blocking’ antibody because they bind the epitope, but do not initiate a pathogenic immunological response due to its lack of binding to C1q and poor binding to Fc γ receptors [29]. In this manner they may prevent the negative immunological effect of other immunoglobulins by competing for epitope binding. In addition, autoimmune disease associated with IgG4 subclasses are attributed to the inappropriate activation or blockage of endogenous enzymes or receptors by IgG4 antibodies, for example in muscle-specific kinase myasthenia gravis [30,31]. In the same manner, IgG ADAs in Fabry could inhibit r- α GAL A function. However, inhibition of enzymatic activity is not the only way ADAs can influence treatment efficacy, pharmacokinetics and

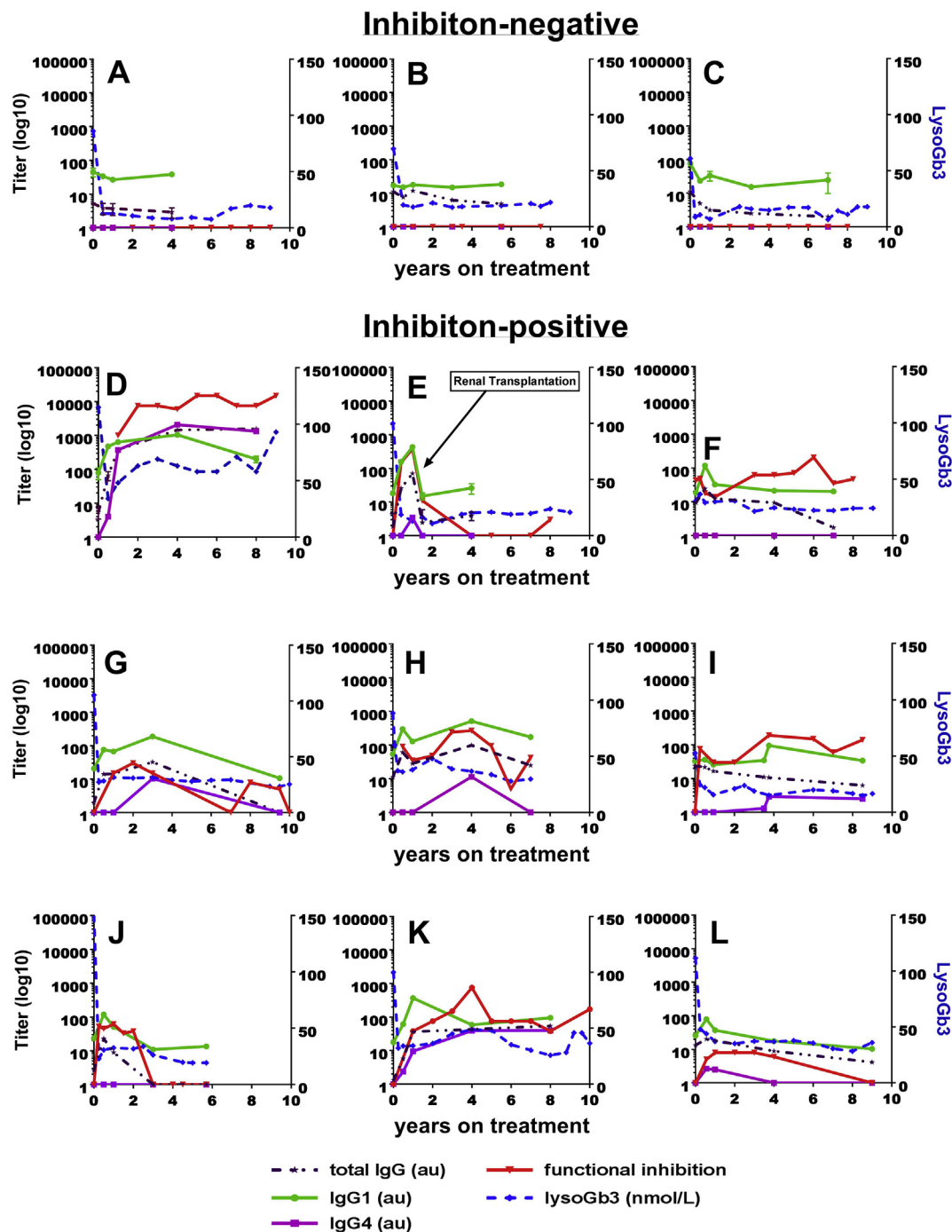


Fig. 3. Titers of *in vitro* inhibition, IgG1, IgG4, total IgG (arbitrary units) and lysoGb3 (nmol/L). Patients A–C are inhibition-negative, patients D–L are inhibition-positive. In patients G, J and L inhibition disappeared spontaneously during treatment. In Patient E inhibition disappeared after renal transplantation and treatment with immunosuppressive therapy. Values of antibodies and inhibition are depicted on the left y-axis in Log (10). LysoGb3 (nmol/L) is depicted on the right y-axis. X axis depicts the years since treatment initiation.

uptake in target cells may also be altered. A protein coated with antibodies is cleared more rapidly from the circulation by phagocytotic cells resulting in decreased availability for other cell types [32,33]. In Pompe disease, patients with high ADA titers had a 50% increase in clearance rate of Myozyme [34]. Complex formation and increased clearance of r- α GAL A when ADAs are present have also been demonstrated in Fabry disease [13]. We hypothesize that enzyme uptake by target cells in Fabry disease (e.g. cardiomyocytes, podocytes and endothelial cells) is also negatively affected by anti r- α GAL A antibodies.

Ways forward could include development of strategies to reduce or prevent the occurrence of ADAs. Immunomodulation before treatment

initiation may be considered to prevent antibody formation. However, given the potential side effects, precise prediction of which patients are at risk for ADA development is necessary. The finding that patients that started ERT treatment after renal transplantation (and were thus treated with immunosuppressive drugs) did not develop antibodies against ERT suggests this approach holds promise. In the same study, patients transplanted while already on ERT had an initial reduction in inhibition titer, but in some patients the titer rose again after tapering of the immunosuppressive medication [35]. Therefore, immunomodulation before start of ERT in patients most at risk for clinically significant antibody development might be the best way forward.

Declarations

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jymgme.2018.11.008>.

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