## Enzyme replacement therapy in Fabry disease, towards individualized treatment

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## Agalsidase alfa versus agalsidase beta for the treatment of Fabry disease: an international cohort study

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*Submitted*

## Abstract

*Background:* Two recombinant enzymes (agalsidase alfa 0.2 mg/kg/every other week and agalsidase beta 1.0 mg/kg/every other week) have been registered for the treatment of Fabry disease (FD), at equal high costs. An independent international initiative compared clinical and biochemical outcomes of the two enzymes.

*Methods:* In this multicenter retrospective cohort study, clinical event rate, left ventricular mass index (LVMI), eGFR, antibody formation and globotriaosylsphingosine (lysoGb3) levels were compared between FD patients treated with agalsidase alfa and beta at their registered dose after correction for phenotype and sex.

*Results:* 387 patients (192 women, 248 patients received agalsidase alfa) were included. Mean age at start of ERT was 46 (±15) years. The decrease in plasma lysoGb3 was more robust following treatment with agalsidase beta, specifically in men with classical FD (β:-18 nmol/l, p<0.001), persisting in the presence of antibodies. The risk to develop antibodies was higher for patients treated with agalsidase beta (OR:2.8, p=0.04). LVMI decreased in a higher proportion following the first year of agalsidase beta treatment (OR:2.27, p = 0.03). The event rate and eGFR slopes (103 patients with one or more events) were similar for both enzymes..

*Conclusions:* Treatment with agalsidase beta at higher dose compared to agalsidase alfa results in a greater biochemical response, also in the presence of antibodies, and better reduction in left ventricular mass. Clinical events and deterioration in kidney function happened despite treatment, especially in those with more advanced disease: no difference for these outcomes was established between the two enzymes.

# Introduction

Fabry disease (FD) (OMIM 301500) is an X-linked lysosomal storage disorder characterized by the accumulation of globotriaosylceramide (Gb3) in several cell types due to deficiency of the enzyme alpha galactosidase A (aGAL) (enzyme commission number: 3.2.1.22).[1] The disease is associated with potentially life threatening complications such as renal failure, cardiac rhythm disturbances, heart failure and stroke. Circulating levels of plasma globotriaosylsphingosine (lysoGb3) can be used to differentiate between the severe classical phenotype and the more attenuated non-classical phenotype and as a biochemical marker to monitor treatment effects.[2-4] Men with classical FD often have childhood onset of symptoms, and usually have one or more characteristic FD signs or symptoms such as cornea verticillata, neuropathic pain and clustered angiokeratoma[5] while non-classical FD generally has a later onset with more limited disease, often primarily affecting the heart. Despite the X-linked inheritance pattern, women often have signs and symptoms of FD, but they are in general less severely affected.[6] Classically affected women generally show a disease course that is similar to that of men with non-classical FD.[7]




Enzyme replacement therapy (ERT) with recombinant alpha galactosidase was the first available specific treatment for FD. In Europe and Canada, two ERTs have received marketing authorization: agalsidase alfa (Replagal, Shire) and agalsidase beta (Fabrazyme, Sanofi Genzyme), while in the United States of America only agalsidase beta is licensed. Although the preparations are biochemically and structurally very similar,[8-10] there is a fivefold difference in recommended dose (agalsidase alfa 0.2 mg/kg/every other week (EOW)[11]; agalsidase beta 1.0 mg/kg/EOW[12]). Studies have shown that treatment with both enzymes can delay some of the clinical complications of the disease.[13-15] However, only two clinical trials have directly compared the two agents: a small randomized controlled trial comparing both agents at a 0.2 mg/kg/EOW dose which showed no clinically relevant differences,[16] except for a dose-dependent decline of plasma lysoGb3 in a follow-up study[17] and the Canadian Fabry Disease Initiative (CFDI), which showed no difference in event rate after a mean of 50 months of follow-up in 92 patients randomized to receive either agalsidase alfa or beta at licensed doses.[18]

Indirect comparisons of non-randomized observational studies using agalsidase alfa or beta are hampered by differences in inclusion criteria, endpoints definition and an absence of stratification for phenotype, which is an important predictor of the disease course.[7] Also, development of neutralizing antibodies directly against the enzymes has been associated with a smaller decrease of lysoGb3[19] while their effect on the occurrence of clinical manifestations has not been fully elucidated.[19,20]

The current study aims to compare clinical and biochemical outcomes of agalasidase alfa versus beta as part of a large international, collaborative project including three European centers of excellence combined with data from the CFDI.

# Methods

### Patients

Retrospective data from three European FD centers of excellence (Academic Medical Center (AMC), The Netherlands; Royal Free London NHS Foundation Trust, United Kingdom; and the University Hospital Wuerzburg, Germany) were merged into one database. For the current analysis, these data were combined with the prospectively collected 8 years follow-up data from patients who newly started ERT (cohort 1b) in the CFDI.[21] Data included diagnostic data, clinical, biochemical and imaging outcomes, comorbidities and medication use.



Patients were included who had a definite FD diagnosis according to previously developed criteria,[22] were treatment naïve and treated with either agalsidase alfa at a dose of 0.2 mg/kg/EOW or agalsidase beta at a dose of 1.0 mg/kg/EOW for at least 9 months.Baseline was defined as start of ERT. Follow up ended at switch to the other enzyme preparation or to a different dose, discontinuation of ERT, or the last recorded clinic visit.

Medication with angiotensin-converting-enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARBs), antiplatelet therapy and antihypertensive therapy was applied according to the best practices at that time.

### Phenotype

Patients were categorized as classical or non-classical on the basis of enzyme activity and the presence or absence of characteristic FD symptoms (neuropathic pain, clustered angiokeratoma and/or cornea verticillata.)[5] A detailed description of the classification method has been published previously and can be found in supplemental material A.[7] The CFDI database did not always capture the same criteria for phenotype, thus pedigree analysis was added for some uncertain cases.

### Clinical outcomes

We assessed the clinical event rate from start of therapy until first event or end of follow-up. Clinical and laboratory measurements were longitudinally analyzed.

*Clinical events*

Clinical events were defined as follows:

- Renal events: CKD (Chronic Kidney Disease) category G5 (eGFR <15ml/min/1.73m$^2$), renal transplantation or dialysis

- Cardiac events: atrial fibrillation, admission for any rhythm disturbance, admission for congestive heart failure, implantation of an implantable cardiac defibrillator (ICD) or pacemaker (PM), myocardial infarction, coronary artery bypass graft surgery or a percutaneous transluminal angioplasty intervention
- Cerebral events: stroke or transient ischemic attack (TIA) diagnosed by a neurologist
- Death from any cause

*Renal function*

Renal function was evaluated using the estimated glomerular filtration rate (eGFR) and the amount of protein excretion in urine. The eGFR was calculated using the CKD-EPI in adults and the Schwartz formula in children up to 18 years of age.[23,24] The eGFR of patients who had received a renal transplant or were undergoing dialysis was set at 10 ml/min/1.73m$^2$. Albuminuria and proteinuria excretion was categorized according to Kidney Disease Improving Global Outcomes (KDIGO) guidelines.[24]





*Cardiac involvement*

Cardiac involvement was assessed by echocardiography. Left ventricular mass index (LVMI) was calculated using the Devereux formula and was corrected for height (m$^{2.7}$).[25] The upper reference limit for men and women is 48 and 44 gram/m$^{2.7}$ respectively.[25] Analysis of cardiac MRI data was not feasible due to the small number of patients with baseline and follow up MRIs.

*LysoGb3 and antibodies*

Plasma lysoGb3 levels were measured at the AMC with tandem mass spectrometry using glycine or isotope labeled lysoGb3 as internal standard.[26,27] Results from both internal standards correlated very well.[7] Antibodies were measured as previously described.[28] A titer of ≥6 was considered as antibody positive. Patients were considered antibody positive if all antibody measurements after treatment initiation were positive. Since antibody development in women is rare,[19] antibodies were measured in men only. No lysoGb3 and antibody data were obtained from the CFDI.

**Statistical analysis**

R (version 3.1.5) was used for statistical analysis. Data are presented as mean and standard deviation (SD) or median and range where appropriate. A Cox proportional hazard model was used to assess the clinical event rate defined as first event (renal, cardiac or cerebral), or death. ERT type (*i.e.* agalsidase alfa or beta), baseline eGFR, sex, phenotype and the interaction between sex and phenotype were included as covariates (full model specifications: supplemental material B). Inclusion of baseline LVMI and a history of an event before initiation of ERT (stroke, dialysis, transplantation and/or ICD/PM implantation) did not improve the model. Patients were censored when an event occurred or at end of follow up. The proportional hazard assumption was visually tested by using Schoenfeld residuals. In addition, we applied

propensity score matching in a 1:1 ratio (package: MatchIt) in order to assess if an uneven distribution of covariates could bias the results, by using calipers of width equal to 0.1 SD of the estimated propensity score. Propensity scores were based on sex, phenotype, baseline LVMI measured on echocardiography, baseline eGFR, events before ERT and age at initiation of ERT. Subsequently, we performed a Cox proportional hazard model on the matched data.

The proportion of patients with a decrease in LVMI one year after initiation of ERT was analyzed by logistic regression. Mixed effect models (package: nlme) were used to analyze the eGFR, LVMI and lysoGb3 over time. Only adult patients were included in the analysis of the eGFR and LVMI. Both LVMI and lysoGb3 values showed a decrease in the first year followed by constant levels in the following years. In order to account for this non-linear relation between time and LVMI/lysoGb3, we used the change from baseline in LVMI and lysoGb3 in the respective models. A random slope and random intercept were included when appropriate. Time on ERT, ERT type, age at start of ERT, sex, phenotype, ACEi/ARBs, baseline eGFR, baseline LVMI and/or baseline lysoGb3 were included as covariates when appropriate (full model specifications: supplemental material B). Models were selected in a stepwise manner, and the Akaike Information Criterion (AIC) was used to evaluate the goodness of fit. Furthermore, eGFR analyses were stratified for low or high eGFR at baseline (eGFR <60 and ≥60 ml/min/1.73m$^2$, respectively). In the ΔLVMI analyses results were stratified by the presence or absence of LVH at baseline. Differences in the prevalence of antibodies were assessed with the Fisher exact test. The relation between antibody formation and the change in lysoGb3 over time was evaluated in by a linear mixed effect model. Mixed effect model assumptions were visually tested by diagnostic plots. Variance inflation factor was used to explore potential multicollinearity. P-values <0.05 were considered statistically significant. Where appropriate, 95% confidence intervals (95% CI) are given. Results are reported in accordance to the 'The Strengthening the Reporting of Observational Studies in Epidemiology' (STROBE) statement.[29]



**Ethics statement**

According to Dutch law, and after review of the AMC ethics committee, no approval of the study protocol was needed because of the observational nature of the study. All data were obtained from medical records. Patient records were anonymized and de-identified prior to analysis. All patients have provided consent for the use of their medical data and samples in accordance with local ethics requirements.

# Results

### Patients

In total, 283 European and 104 Canadian patients (54% females) were included in the analysis (table 1). Mean age at start of ERT was 46 (±15) years. Treatment consisted of agalsidase alfa in 248 and agalsidase beta in 139 patients with a median follow-up time of 4.9 (0.8-14.4) years. In general, patients treated with agalsidase beta were more likely to have classical disease, to have received a renal transplant or dialysis before start of therapy, and to have higher lysoGb3 and lower eGFR at baseline (all p<0.05). Patient characteristics stratified for sex, phenotype and ERT type can be found in supplemental material C. Discontinuation of treatment (*n=15*) due to patient preferences or treatment failure, change in dose (*n=48*) or switch of ERT (*n=37*) occurred in 100 patients (26%), which resulted in censoring. The shortage of agalsidase beta was the main reason to reduce dose (*n=47*) or switch to agalsidase alfa (*n=17*).



### Clinical events

One or more events occurred in 103 patients (27%). In the agalsidase alfa group 65/248 (26%) patients developed a clinical event compared to 38/139 (27%) patients receiving agalsidase beta. Cardiac events (*n=54*) were most common as first event after initiation of ERT, cerebral events (*n=25*) and renal events (*n=10*) were less frequent. Ten patients died without experiencing any other event during treatment. Causes of death in these patients were congestive heart failure *(n=2),* sudden cardiac death (*n=2*), secondary complications of end stage renal disease (*n=2*), stroke (*n=1*), hepatic encephalopathy (*n=1*), meningitis (*n=1*) and ovarian cancer (*n=1*).

The event rate of patients treated with agalsidase alfa or beta was similar when stratified for sex and phenotype and adjusted for age at initiation of ERT and baseline eGFR ($HR_{alfa\ vs\ beta}$: 0.96, 95% CI: 0.59 – 1.57, p=0.87). A sensitivity analysis with addition of a decrease in eGFR of ≥33% and an increase in LVMI of ≥20% to the definition of clinical events revealed similar results ($HR_{alfa\ vs\ beta}$: 0.84, 95% CI: 0.55 – 1.29, p=0.44). Likewise, neither the inclusion of LVMI (*n=314*) as covariate to the original analysis ($HR_{alfa\ vs\ beta}$: 0.94, 95% CI: 0.55 – 1.59, p=0.81), nor the exclusion of patients with a renal event before treatment initiation (*n=20*) ($HR_{alfa\ vs\ beta}$: 0.84, 95% CI: 0.50 – 1.40, p=0.50) changed the results. With propensity scores, 188 patients were matched in a 1:1 ratio. The subsequent Cox regression analysis showed similar results ($HR_{alfa\ vs\ beta}$: 0.98, 95% CI: 0.55 – 1.77, p=0.95) as the unmatched analyses (figure 1).

**Table 1** Patient characteristics at start of ERT

| | Agalsidase alfa (0.2 mg/kg) | Agalsidase beta (1.0 mg/kg) | p-value |
|---|---|---|---|
| **Patients** | 248 | 139 | |
| **Men, classical** | 69 (28%) | 71 (51%) | <0.001 |
| **Men, non-classical** | 47 (19%) | 7 (5%) | 0.22 |
| **Women, classical** | 95 (38%) | 42 (30%) | 0.14 |
| **Women, non-classical** | 37 (15%) | 18 (13%) | 0.86 |
| **Age at start ERT (years)** | 45 (±16) | 44 (±13) | 0.63 |
| **ERT start <18 years of age** | 15 (6%) | 3 (2%) | 0.13 |
| **Follow up time (years)** | 5.2 (0.8-14.4) | 3.8 (0.8-12.1) | <0.001 |
| **Events before initiation of ERT** | | | |
| ▪ **Dialysis/renal transplant** | 8 (3%) | 12 (9%) | 0.007 |
| ▪ **PM/ICD** | 21 (8%) | 9 (7%) | 0.87 |
| ▪ **Stroke** | 22 (9%) | 17 (12%) | 0.09 |
| ▪ **Any of the above** | 46 (19%) | 31 (22%) | 0.08 |
| **LysoGb3 (nmol/l)** | 10 (0.7-146) | 80 (2.0-178) | <0.001 |
| **eGFR (ml/min/1.73m$^2$)** | 89 (10-159) | 86 (10-140) | 0.009 |
| **CKD category A3** | 44/195 (23%) | 42/113 (37%) | 0.008 |
| **LVMI (gram/m$^{2.7}$)** | 49 (15-117) | 52 (20-148) | 0.14 |
| **Use of ACEi/ARBs** | 89/248 (36%) | 52/139 (37%) | 0.83 |
| **Hypertension** | 109/236 (39%) | 62/137 (45%) | 0.23 |
| **BMI (kg/m$^2$)** | 26 (±4.9) | 25 (±5.6) | 0.30 |
| **HDL cholesterol (mmol/l)** | 1.5 (±0.4) | 1.5 (±0.4) | 0.92 |
| **LDL cholesterol (mmol/l)** | 2.7 (±0.9) | 2.7 (±0.8) | 0.76 |
| **Total cholesterol (mmol/l)** | 4.8 (±1.1) | 4.7 (±1.0) | 0.51 |
| **Triglycerides (mmol/l)** | 1.2 (0.2-5.9) | 1.2 (0.3-3.6) | 0.18 |

*Continuous variables are presented as mean (±SD) or median (range). Missing values (percentage): lysoGb3 (54%), eGFR (5%), LVMI (18%), BMI (5%), HDL cholesterol (28%), LDL cholesterol (18%), total cholesterol (17%), triglycerides (17%).For baseline characteristics per sex and phenotype see supplemental material C, for detailed genotype-phenotype information see supplemental material D.*

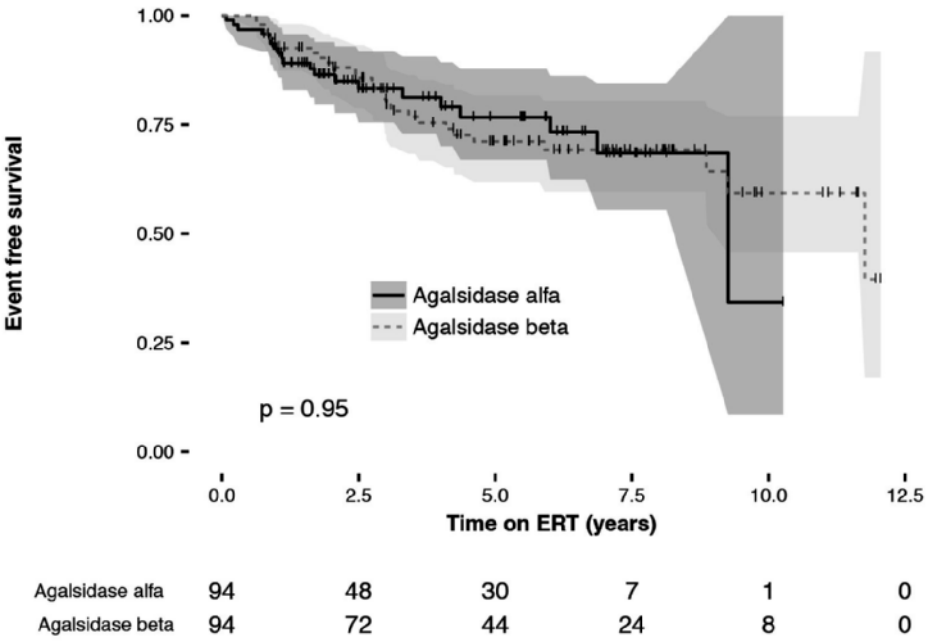


**Figure 1** Kaplan Meier curve for any first event (renal, cardiac or cerebral event, or death) after propensity score matching.

**Renal function**

Longitudinal data on eGFR was available for 337 adult patients (supplemental material E). Adjusted for sex and phenotype, there was no difference in the slope of eGFR between agalsidase alfa and beta in patients with a baseline eGFR ≥60 ($\beta_{slope\ alfa\ -\ beta}$: -0.12 ml/min/1.73m$^2$/year, 95% CI: -0.76 – 0.51, p=0.70). Also, in patients with an eGFR <60 there was no difference in the rate of decline ($\beta_{slope\ alfa\ -\ beta}$: -0.85 ml/min/1.73m$^2$/year, 95% CI: -2.31 – 0.62, p=0.26). Adding the use of ACEi/ARBs and/or the presence of proteinuria at baseline as covariates to the model did not result in a better fit or different results.

**Left ventricular mass**

Two hundred and seventy-eight adult patients were included in the longitudinal analysis of LVMI. In patients *without* LVH at baseline (*n=110*) there was no change in LVMI after one year of treatment. In patients *with* LVH (*n=168*) there was a decrease after one year of treatment. The magnitude of the decrease depended on the LVMI at baseline (p<0.001) and was independent of sex and phenotype (figure 2). Patients with an LVMI above the reference value but <75 gram/m$^{2.7}$, that were treated with agalsidase beta showed a larger but non-significant decrease of LVMI over the first year compared with alfa ($\beta_{alfa\ -\ beta}$: -3.31 gram/m$^{2.7}$, 95% CI: -6.84 – 0.23, p=0.07), but no difference for the entire group was found ($\beta_{alfa\ -\ beta}$: -2.26 gram/m$^{2.7}$, 95% CI: -5.39 – 0.87, p=0.15). The decrease over the first year was followed by stabilization of LVMI in the following years ($\beta_{time\ on\ ERT}$: 0.22, p=0.33). Hence, the observed difference between agalsidase alfa and beta over the first year persisted during the following years.

The analysis on the number of patients that showed a decrease in LVMI after one year of treatment revealed that treatment with agalsidase beta resulted in a higher proportion of patients with a decrease in LVMI compared with agalsidase alfa (79% vs 62%) (OR: 2.27, 95% CI: 1.11 – 4.86, p=0.03), adjusted for the LVMI at baseline.

**LysoGb3**

Longitudinal data on lysoGb3 was available for 153 patients (figure 3). After initiation of ERT, lysoGb3 concentrations rapidly decreased, followed by stabilization in all subgroups (men with classical FD: β: 0.83 nmol/l/year, p=0.08; men with non-classical FD and women: β: 0.03 nmol/l/year, p=0.94). After adjustment for baseline lysoGb3 concentration, sex and phenotype, the decrease in lysoGb3 (ΔlysoGb3) in men with classical FD was more pronounced in those treated with agalsidase beta ($\beta_{alfa\ -\ beta}$: -18.06 nmol/l, 95% CI: -25.81 – -10.03, p<0.001). For example, in a classically affected man with a baseline lysoGb3 value of 100 nmol/l, the lysoGb3 concentration will be on average 45 nmol/l following 1 year of treatment with agalsidase alfa and 27 nmol/l after 1 year of treatment with agalsidase beta. In the other patients (women and non-classical men) this difference was also significant but smaller ($\beta_{alfa\ -\ beta}$: -1.07 nmol/l, 95% CI -2.04 – -0.11, p=0.03).

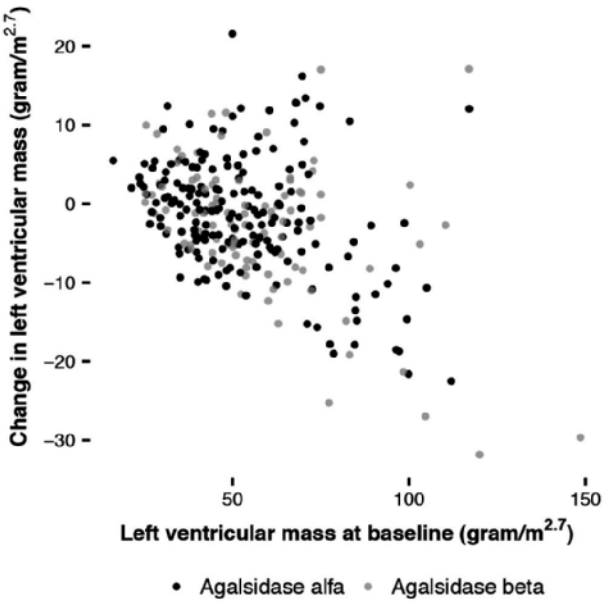


**Figure 2** Estimates of the change in LVMI from baseline after 1 year per patient, results from the linear mixed model of the change in LVMI.

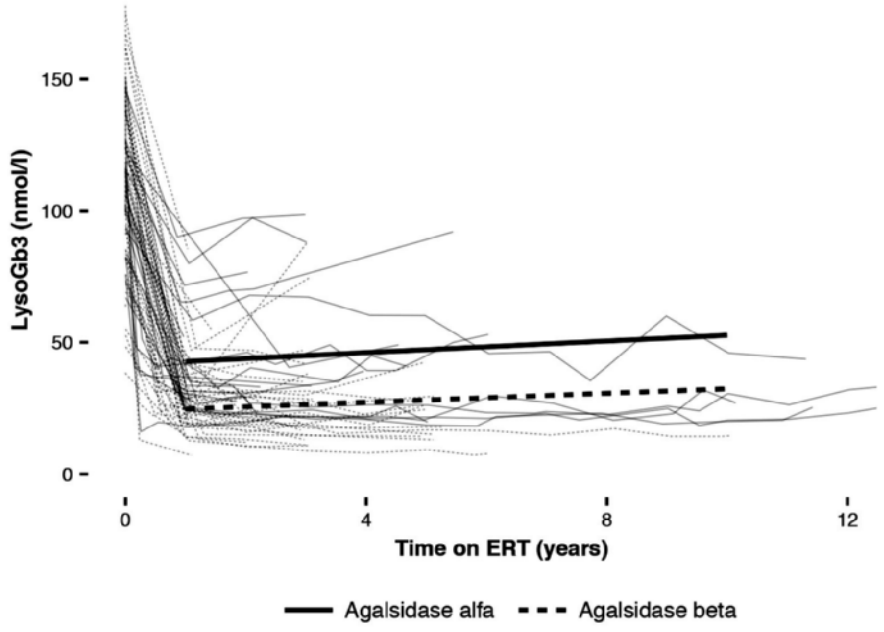


**Figure 3** Linear mixed model of lysoGb3 adjusted for lysoGb3 at baseline in men with classical FD. The figure presents data of men with the classical phenotype. The larger lines represent the predicted values at group level, the smaller lines represent the raw individual patient data.

**Antibodies**

One or more antibody assays were performed for 124 men. From 32 non-classical men (agalsidase alfa: *n=27*, agalsidase beta: *n=5*) only one developed transient antibodies. Antibody measurements were available for 92 classical men. Patients with a mixed antibody response over time (negative as well as positive antibody measurements) were excluded (*n=11*). Of the remaining 81 patients, persisting antibodies were established in 33 men, of whom 11 were treated with agalsidase alfa (11/39, 28%) and 22 with agalsidase beta (22/42, 52%) resulting in an odds ratio of 2.8 (95% CI: 1.02 – 7.88, p=0.041). Results did not change substantially if the first antibody measurements of patients with a mixed antibody response were included (OR: 3.14, p=0.011). Antibody titers remained stable over time (supplemental material F).





A comparison of the clinical event rate, LVMI and eGFR between patients treated with agalsidase alfa and agalsidase beta with or without antibodies was hampered by the uneven distribution of disease severity among groups. The limited number of patients (*n=81*) and events in these groups (*n=29*) makes extensive correction for disease severity variables impossible.

Analysis of the influence of antibodies on the decrease in lysoGb3 in men with classical FD treated with agalsidase alfa (*n=21*) or beta (*n=35*) revealed the following: in patients treated with agalsidase alfa the presence of antibodies was associated with a less prominent decrease in lysoGb3 following ERT, resulting in 34.77 nmol/l (95% CI: 23.65 – 45.88, p<0.001, adjusted for baseline lysoGb3 concentrations) higher lysoGb3 concentrations in the antibody positive group compared to the antibody negative group. In patients receiving agalsidase beta, the decrease in lysoGb3 after ERT initiation was minimally affected by the presence or absence of antibodies ($\beta_{AB+ \text{ vs } AB-}$: 6.72 nmol/l, 95% CI: -1.43 – 14.87, p=0.10) (figure 4).

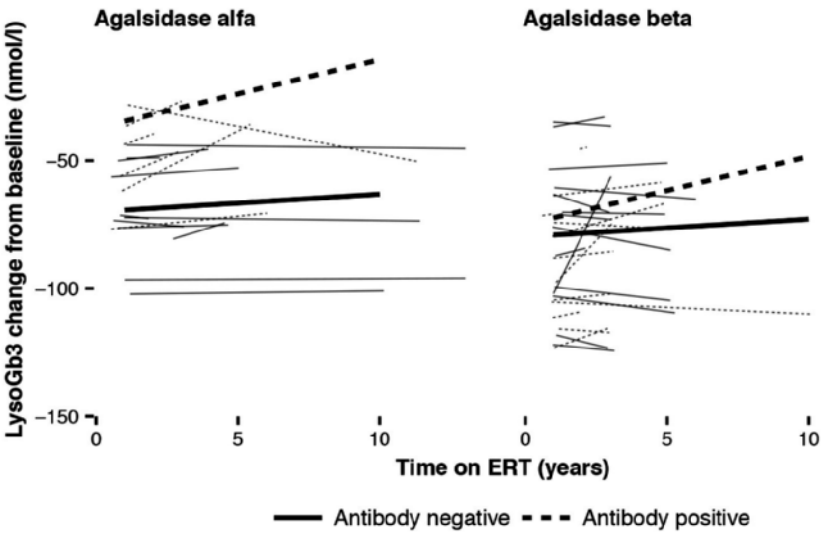


**Figure 4** Effect of antibody formation on lysoGb3 in men with classical FD. Linear mixed model of the change in lysoGb3 adjusted for lysoGb3 at baseline, stratified for ERT type and antibody status. The larger lines represent the predicted values at group level (at the mean lysoGb3 concentration of 105 nmol/l in these patients), the smaller lines represent the predicted values at individual patient level.

# Discussion

In this study, we systematically compared clinical outcomes and biochemical response in a large cohort of almost 400 Fabry patients treated with either agalsidase alfa or agalsidase beta at authorized dose. There is no difference in clinical event rate between both enzymes, but treatment with agalsidase beta results in a larger decrease in lysoGb3 concentrations compared to agalsidase alfa. In addition, treatment with agalsidase beta has a better effect on left ventricular mass, in patients with a LVMI <75 gram/m$^{2.7}$. Fewer patients had an immunological response to agalsidase alfa as compared to beta. There were considerable baseline differences between both treatment groups, which could be explained by differences in proportions of patients with classical FD as well as prescription behavior between centers. In order to account for these differences, all analyses were adjusted for phenotype and disease severity.

In line with our findings, a small observational study showed that treatment with agalsidase beta resulted in a more pronounced decrease in lysoGb3 compared to agalsidase alfa.[17] Furthermore, a slight increase in lysoGb3 was observed in men with classical FD who received a lower dose or switched to agalsidase alfa during the shortage of agalsidase beta.[30] Also, a correlation between cumulative dose and podocyte Gb3 clearance has been reported.[31] A dose effect on biochemical markers is further supported by the observations from this study

on the influence of antibodies. We confirmed the higher prevalence of antibodies in patients treated with agalsidase beta.[11,12,19] This may be related to the dose or the manufacturing method of the product (*i.e.* human vs CHO cell line and the possible differences in glycosylation).[8-10] The presence of antibodies in patients treated with agalsidase alfa is associated with a less prominent decrease in lysoGb3, while the decrease in lysoGb3 is almost unaffected by antibody formation during treatment with agalsidase beta, which has also been found in an earlier study.[17] This is most likely caused by the fivefold higher dose, which overcomes the negative effects of antibody formation. In other lysosomal storage disorders, such as Gaucher disease, biochemical markers including glucosylsphingosine, correlate well with clinical disease parameters and can be used to monitor the effect of therapeutic intervention.[4,32] In FD, previous studies have shown that plasma lysoGb3 is derived from the storage material and is related to phenotype and disease severity.[3,7,33] In addition, lysoGb3 levels are associated with MSSI scores and LVMI in men and the presence of WML in women.[34] Although most previous studies were unable to show differences in clinical outcomes between antibody negative and antibody positive patients,[19,35,36] a more recent study showed larger LVMI and worse renal function in patients with antibodies.[20] In that study, however, no differentiation was made by phenotype, and results were only adjusted for having a nonsense mutation. This has probably led to a confounding effect of phenotype, since the most severely affected patients (*i.e.* classical patients) are most likely to develop antibodies. Correction for phenotype in the current study limits the risk of this bias but also made it impossible to analyze differences in clinical outcome between those with and without antibodies.




The observed dose effect on lysoGb3 may not always be followed by clinically relevant outcomes, as irreversible damage to organs such as the kidneys will not be reversed. Left ventricular hypertrophy has been shown to be reversible to some extent. Indeed, this study shows that a better effect of agalsidase beta was established during the first year of treatment. However, no effect on eGFR or clinical event rate could be established. It is possible that the current study is still underpowered to detect a significant difference in event rate. The CFDI investigators previously calculated that almost 300 patients per group (*i.e.* 600 patients in total) would be required to detect a 10% difference[18] which is more than the nearly 400 patients we were able to include, even after combining data from three European referral centers and the CFDI. Likewise, none of the studies that evaluated the effects of switching or dose reduction during the shortage of agalsidase beta was able to show a change in clinical event rate, since the cohorts were often small and had relatively short follow-up.[30,37-39] Nonetheless, the results of one of the largest studies suggested a steeper decline in eGFR and higher disease severity scores in patients who had been switched or received a lower dose.[39]

In conclusion, although we were unable to show a difference in event rate between patients treated with agalsidase alfa and beta, our results suggest an improved treatment response on cardiac mass and lysoGb3 with agalsidase beta. The decrease in lysoGb3 is negatively

influenced by the presence of antibodies in men with classical FD treated with agalsidase alfa but not in those receiving agalsidase beta. However, agalsidase beta is associated with a higher risk to develop antibodies. In view of these outcomes, individual choices for treatment need to be made.

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# Supplemental material A

*Phenotypic classification adapted from Arends et al (2017) with permission*[7]

Patients were classified as classical or non-classical FD on the basis of their enzyme activity (men only) and the presence or absence of characteristic symptoms.[22] Men were considered to have a classical phenotype when they met the following criteria: 1) a *GLA* mutation, 2) enzyme activity ≤5% of the mean reference range, 3) ≥1 characteristic FD symptoms (*i.e.* Fabry neuropathic pain, angiokeratoma and/or cornea verticillata, for definitions see [5]). Men not fulfilling these criteria were categorized as non-classical FD.
Women with a *GLA* mutation and ≥1 characteristic FD symptoms (*i.e.* Fabry neuropathic pain, angiokeratoma and/or cornea verticillata[5]) were classified as having a classical phenotype. Women without these characteristic FD symptoms were classified as non-classical FD.





**Supplemental table A1** Criteria for phenotypic classification

| **Classical FD** | |
|---|---|
| **Men** | **Women** |
| ▪ A mutation in the *GLA* gene* | ▪ A mutation in the *GLA* gene |
| ▪ ≥ 1 of the following characteristic Fabry disease symptoms: Fabry neuropathic pain, angiokeratoma and/or cornea verticillata | ▪ ≥ 1 of the following characteristic Fabry disease symptoms: Fabry neuropathic pain, angiokeratoma and/or cornea verticillata |
| ▪ Severely decreased or absent leukocyte AGAL activity (<5% of the normal mean) | |
| **Non-classical FD** | |
| ▪ A mutation in the *GLA* gene, and not fulfilling the criteria for classical FD | |

**The following genetic variants were considered no FD (neutral variants): A143T, P60L, D313Y, R118C, T385A, IVS0-10 C>T, the complex haplotype: IVS0-10 C>T/IVS4-16A>G/IVS6-22C>T. In patients in whom classification on the basis of these criteria was not feasible, the final judgement was made by the treating physician.*

Classification on the basis of phenotypic features and residual enzyme activity was challenging in two groups of patients. It was decided that in these cases a final judgement was made by the treating physician. These groups were:

1) Patients with the N215S mutation: this group is especially prevalent in the UK. According to literature and physician experience, patients exhibit a non-classical (mostly cardiac) phenotype, but exceptions may occur. In this group of 49 patients, 6 had a characteristic symptom, but without confirmatory deficiency of *GLA* activity in leucocytes in men (*n=3).* Furthermore, one of the N215S patients presented with renal disease at young age (with no other cause). Renal disease was observed in his family (not included in our cohort). In addition one other N215S patient had an absent enzyme activity and cornea verticillata. According to the judgement of the treating physician these patients were classified as classical FD while the other N215S patients were all classified as non-classical FD. Similarly, three patients with characteristic symptoms and the P389A mutation (one man, one woman) or R112H (one woman)

mutation were discussed with the treating physician. These patients all had a late onset presentation, only minimal cornea verticillata (no other characteristic FD symptoms) and a family history of non-classical FD. Consequently they were classified as non-classical FD.

2) Men with slightly higher than 5% enzyme activity in the presence of 1 or more characteristic symptoms (*n=15*). Residual enzyme activity ranged from 6% to 23% in leucocytes (*n=12)*, and from 6% to 20% in plasma (*n=3*). All had at least one characteristic FD symptom and the majority had a relative with classical FD and consequently were considered having classical FD.

Furthermore, we included two patients (one men, one women, all from the same family) with the A143T mutation. They were classified as having classical FD based on the combination of characteristic deposits on renal biopsy or post mortem biopsy, the presence of one or more characteristic FD symptoms, low enzyme activity (3,9%, 21% respectively) and high plasma lysoGb3 concentrations (man 1: 35-50 nmol/l while receiving ERT; woman 1: 16 nmol/l while receiving ERT). In these cases, a combination of the A143T mutation and an unknown mutation and/or other (genetic) disease modifiers may have caused the classical FD presentation.





**Supplemental table A2** Characteristics of patients with the A143T or N215S mutation who were classified as having classical FD

| | Sex | Mutation | Enzyme activity | Characteristic symptoms | LysoGb3 | Clinical features | Comments |
|---|---|---|---|---|---|---|---|
| **1*** | Female | A143T | 32% | Acroparesthesia | 16*** | Mild LVH | On the basis of the presence of classical FD in a relative (pt 2) |
| **2*** | Male | A143T | 5%** | Angiokeratoma, acroparesthesia | 50*** | LVH, WML | Nearly absent enzyme activity, presence of characteristic FD symptoms, high lysoGb3. Probably due to a yet unknown secondary mutation or other disease modifying factors |
| **3** | Male | N215S | 0%** | Possible acroparesthesia | Not available | ESRD | Absent enzyme activity, ESRD at the age of 24 with no other identifiable cause |
| **4** | Male | N215S | 0% | Cornea Verticillata | Not available | LVH | Absent enzyme activity, cornea verticillata |

** relatives, ** plasma enzyme activity, *** while receiving ERT.*

# Supplemental material B

**Survival analysis**

*Type of analysis*

- *Cox regression analysis*
- Left truncated (adjusted for age)

*Covariates included:*

- ERT type, eGFR at baseline, Sex, Phenotype, Sex * phenotype
- Left truncated (adjusted for age)

*R syntax:*

- coxph(Surv([Age at start of ERT], [Age at censoring], [status]) ~ [ERT type] + [eGFR at baseline] + strata([sex] + [phenotype] + [sex:phenotype])



**eGFR**

*Type of analysis*

- *Linear mixed model*

*Covariates included*

- Time on ERT, eGFR baseline class, ERT type, Sex, Phenotype and interactions

*Definitions of covariates*

- eGFR baseline class: 0 = baseline eGFR ≥ 60 ml/min/1.73m$^2$; 1 = baseline eGFR < 60 ml/min/1.73m$^2$

*R syntax*

- LME(eGFR ~ [Time on ERT] * ([eGFR baseline class] * [ERT type] + [sex] * [phenotype]), random = ~ [Time on ERT] | [patient], control = "optim")

**Proportion of patients with a decrease in LVMI one year after ERT**

Response = A decrease or stable LVMI (ΔLVMI ≤0) of the measurement closest to the one year time point after ERT

*Type of analysis*

- *Logistic regression*

*Covariates included*

- LVMI baseline class, LVMI at baseline, ERT type and interactions
- Including age, sex or phenotype as covariates did not led to an improvement of the model

*Definitions of covariates*

- LVMI baseline class: 0 = baseline LVMI < 49 gram/$m^{2.7}$ (men) and LVMI < 44 gram/$m^{2.7}$ (men); 1 = baseline LVMI ≥ 49 gram/$m^{2.7}$ (women) and LVMI ≥ 44 gram/$m^{2.7}$ (women)

*R syntax*

- GLM(response ~ [baseline LVMI class] * ([ERT type] + [baseline LVMI]), random = ~ [Time on ERT] | [patient], control = "optim")

**Change in LVMI**

ΔLVMI = the change in LVMI from baseline





*Type of analysis*

- *Linear mixed model*

*Covariates included*

- Time on ERT, LVMI baseline class, LVMI at baseline, ERT type, and interactions
- Including age, sex or phenotype as covariates did not led to an improvement of the model

*Definitions of covariates*

- LVMI baseline class: 0 = baseline LVMI < 49 gram/$m^{2.7}$ (men) and LVMI < 44 gram/$m^{2.7}$ (men); 1 = baseline LVMI ≥ 49 gram/$m^{2.7}$ (women) and LVMI ≥ 44 gram/$m^{2.7}$ (women)

*R syntax*

- LME(ΔLVMI ~ [baseline LVMI class] * ([ERT type] + [baseline LVMI]), random = ~ [Time on ERT] | [patient], control = "optim")

**Change in LysoGb3**

No differences were observed between non-classical men, classical women and non-classical women. Therefore they were considered as one group. Two separate analysis were performed for men with classical FD and the other patients because of the large differences in the range of lysoGb3 values between these groups

ΔLysoGb3 = the change in lysoGb3 from baseline

*Type of analysis*

- *Linear mixed model*

*Covariates included*

- Time on ERT,  ERT type, lysoGb3 at baseline and interactions

*R syntax*

- LME(ΔLysoGb3 ~ ( [Time on ERT] * [ERT type] + [Baseline lysoGb3]), random = ~ [Time on ERT] | [patient], control = "optim")

**Change in LysoGb3 in relation to antibody status**

Only men with classical FD were included since antibody development was limited to this group.

ΔLysoGb3 = the change in lysoGb3 from baseline.

*Type of analysis*

- *Linear mixed model*

*Covariates included*

- Time on ERT, ERT type, lysoGb3 at baseline, antibody status and interactions

Antibody status 0 = no antibodies present, 1 = antibodies present
R syntax:

- LME(ΔLysoGb3 ~ [Time on ERT] * [ERT type] * [Antibody status] + [Baseline lysoGb3] , random = ~ [Time on ERT] | [patient], control = "optim")

# Supplemental material C

**Supplemental table C1** Patient characteristics at start of ERT Agalsidase alfa (0.2 mg/kg)

| | Men | | Women | |
|---|---|---|---|---|
| | **Classical** | **Non-classical** | **Classical** | **Non-classical** |
| **Patients** | 69 | 47 | 95 | 37 |
| **Age at start ERT (years)** | 35 (±12) | 52 (±16) | 46 (±16) | 51 (±15) |
| **Follow up time (years)** | 6.7 (0.8-14.1) | 3 (0.8-14.4) | 5.3 (1.0-13.3) | 4.6 (1.0-13.5) |
| **Events before initiation of ERT** | | | | |
| ▪ **Dialysis/renal transplant** | 5 (9%) | 2 (4%) | 1 (1%) | 0 |
| ▪ **PM/ICD** | 1 (1%) | 14 (30%) | 6 (6%) | 0 |
| ▪ **Stroke** | 7 (10%) | 1 (2%) | 13 (14%) | 1 (3%) |
| ▪ **Any of the above** | 11 (16%) | 15 (32%) | 16 (17%) | 1 (3%) |
| **LysoGb3 (nmol/l)** | 104 (62-146) | 8.0 (4.1-36.2) | 10.3 (2.7-22.6) | 4.7 (0.7-19.5) |
| **eGFR (ml/min/1.73m$^2$)** | 106 (10-139) | 80 (10-136) | 89 (10-160) | 86 (32-126) |
| **CKD category A3** | 12/52 (23%) | 9/29 (31%) | 21/82 (26%) | 2/32 (6%) |
| **LVMI (gram /m$^{2.7}$)** | 52 (24-112) | 59 (16-117) | 44 (23-97) | 51 (16-96) |
| **Use of ACEi/ARBs** | 15/69 (22%) | 21/47 (45%) | 37/95 (39%) | 16/37 (43%) |
| **Hypertension** | 14/65 (22%) | 21/41 (49%) | 38/93 (59%) | 18/37 (51%) |
| **BMI (kg/m$^2$)** | 22 (16-35) | 27 (20-41) | 25 (18-45) | 25 (18-42) |
| **HDL cholesterol (mmol/l)** | 1.2 (0.6-2.6) | 1.3 (0.7-2.4) | 1.5 (0.8-2.8) | 1.6 (0.7-2.9) |
| **LDL cholesterol (mmol/l)** | 2.2 (1.1-4.8) | 2.6 (0.7-4.8) | 2.6 (1.4-5.1) | 2.8 (1.2-5.3) |
| **Total cholesterol (mmol/l)** | 4.2 (2.4-6.2) | 4.8 (2.4-7.4) | 4.9 (2.8-8.1) | 5.1 (3.5-7.4) |
| **Triglycerides (mmol/l)** | 1.1 (0.5-3.5) | 1.4 (0.6-5.6) | 1.1 (0.3-4.4) | 1.3 (0.5-5.9) |

*Continuous variables are presented as mean (±SD) or median (range).*

**Supplemental table C2** Patient characteristics at start of ERT Agalsidase beta (1.0 mg/kg)

| | Men | | Women | |
|---|---|---|---|---|
| | **Classical** | **Non-classical** | **Classical** | **Non-classical** |
| **Patients** | 71 | 7 | 43 | 18 |
| **Age at start ERT (years)** | 38 (±10) | 55 (±13) | 51 (±10) | 53 (±16) |
| **Follow up time (years)** | 3.8 (1.0-12.1) | 3.7 (1.4-7.3) | 4.0 (0.8-7.8) | 4.2 (0.8-7.2) |
| **Events before initiation of ERT** | | | | |
| ▪ **Dialysis/renal transplant** | 12 (16%) | 0 (0%) | 0 (%) | 0 (0%) |
| ▪ **PM/ICD** | 4 (6%) | 1 (14%) | 3 (7%) | 1 (6%) |
| ▪ **Stroke** | 8 (11%) | 1 (14%) | 7 (16%) | 1 (6%) |
| ▪ **Any of the above** | 19 (27%) | 1 (14%) | 9 (21%) | 2 (11%) |
| **LysoGb3 (nmol/l)** | 116 (38-178) | 9.5 (2.0-39.7) | 9.9 (5.1-23.5) | 8.3 (3.2-20.0) |
| **eGFR (ml/min/1.73m$^2$)** | 85 (10-137) | 45 (10-95) | 87 (42-140) | 90 (40-118) |
| **CKD category A3** | 25/54 (46%) | 5/7 (71%) | 9/40 (23%) | 3/13 (23%) |
| **LVMI (gram /m$^{2.7}$)** | 51 (20-149) | 75 (42-100) | 58 (25-120) | 48 (25-70) |
| **Use of ACEi/ARBs** | 23/71 (32%) | 5/7 (71%) | 18/43 (42%) | 6/18 (33%) |
| **Hypertension** | 32/70 (46%) | 5/7 (71%) | 18/42 (43%) | 7/18 (39%) |
| **BMI (kg/m$^2$)** | 23 (15-34) | 26 (23-38) | 27 (19-45) | 22 (18-38) |
| **HDL cholesterol (mmol/l)** | 1.3 (0.8-1.9) | 1.3 (1.0-1.7) | 1.5 (0.8-2.8) | 1.6 (1.1-2.3) |
| **LDL cholesterol (mmol/l)** | 2.6 (1.3-4.2) | 3.5 (1.1-4.3) | 2.7 (1.4-4.5) | 3.6 (1.2-1.6) |
| **Total cholesterol (mmol/l)** | 4.4 (2.8-6.7) | 5.4 (3.7-6.6) | 4.9 (3.1-7.2) | 5.8 (3.5-7.1) |
| **Triglycerides (mmol/l)** | 1.3 (0.4-3.4) | 2.1 (1.2-2.4) | 1.1 (0.4-3.4) | 1.4 (0.6-3.6) |

*Continuous variables are presented as mean (±SD) or median (range).*

# Supplemental material D

**Supplemental table D** Mutations and phenotypes

| | | Men | | Women | |
|---|---|---|---|---|---|
| Mutations | Total | classical | non-classical | classical | non-classical |
| c.35_47del13 | 1 | 1 | 0 | 0 | 0 |
| c.358-6 | 1 | 1 | 0 | 0 | 0 |
| c.369+5G>T | 1 | 0 | 1 | 0 | 0 |
| c.370-533_c.1277 del4.5kb | 2 | 0 | 0 | 2 | 0 |
| c.548-1G>A | 1 | 0 | 0 | 1 | 0 |
| c.639+919 G>A | 2 | 0 | 2 | 0 | 0 |
| c.640-2A>C | 2 | 0 | 0 | 2 | 0 |
| c.748_IVS6+8del62 | 1 | 1 | 0 | 0 | 0 |
| c.802-3_802-2delCA | 1 | 1 | 0 | 0 | 0 |
| c.1000-2 A>G | 2 | 1 | 0 | 1 | 0 |
| duplication exon 3+4 | 1 | 1 | 0 | 0 | 0 |
| exon 1 deletion | 3 | 2 | 0 | 1 | 0 |
| IVS2+1G>A | 1 | 1 | 0 | 0 | 0 |
| IVS3+1G>A | 4 | 3 | 0 | 0 | 1 |
| IVS4+1G>A | 1 | 0 | 0 | 1 | 0 |
| IVS5-3_2delCA | 1 | 0 | 0 | 1 | 0 |
| IVS6-10G>A | 1 | 1 | 0 | 0 | 0 |
| IVS6-2A>T | 1 | 0 | 0 | 1 | 0 |
| p.Ala135Val | 2 | 1 | 0 | 1 | 0 |
| p.Ala143Pro | 26 | 7 | 0 | 19 | 0 |
| p.Ala143Thr | 2 | 1 | 0 | 1 | 0 |
| p.Ala156ProFS*9 | 2 | 1 | 0 | 0 | 1 |
| p.Ala156Thr | 1 | 0 | 0 | 1 | 0 |
| p.Ala257Ser | 1 | 1 | 0 | 0 | 0 |
| p.Ala285Asp | 5 | 0 | 2 | 3 | 0 |
| p.Ala309Pro | 2 | 0 | 0 | 1 | 1 |
| p.Ala31Val | 1 | 0 | 0 | 1 | 0 |
| p.Ala348Pro | 2 | 1 | 0 | 1 | 0 |
| p.Arg112Cys | 5 | 5 | 0 | 0 | 0 |
| p.Arg112His | 4 | 0 | 3 | 0 | 1 |
| p.Arg220* | 10 | 5 | 0 | 5 | 0 |
| p.Arg227* | 18 | 8 | 0 | 8 | 2 |
| p.Arg227Gln | 2 | 2 | 0 | 0 | 0 |
| p.Arg301* | 4 | 2 | 0 | 2 | 0 |
| p.Arg301Gln | 4 | 0 | 2 | 0 | 2 |
| p.Arg301Pro | 1 | 1 | 0 | 0 | 0 |
| p.Arg332Lys Fs*7 | 4 | 2 | 0 | 2 | 0 |
| p.Arg342* | 8 | 4 | 0 | 4 | 0 |
| p.Arg342Gln | 13 | 6 | 0 | 7 | 0 |
| p.Arg342Leu | 4 | 2 | 0 | 2 | 0 |
| p.Arg363Cys | 1 | 0 | 1 | 0 | 0 |
| p.Arg49Cys | 1 | 1 | 0 | 0 | 0 |
| p.Arg49Leu | 2 | 2 | 0 | 0 | 0 |
| p.Asn215Ser | 49 | 2 | 33 | 0 | 14 |
| p.Asn224Gly | 1 | 0 | 0 | 1 | 0 |
| p.Asn298Ser | 1 | 0 | 1 | 0 | 0 |

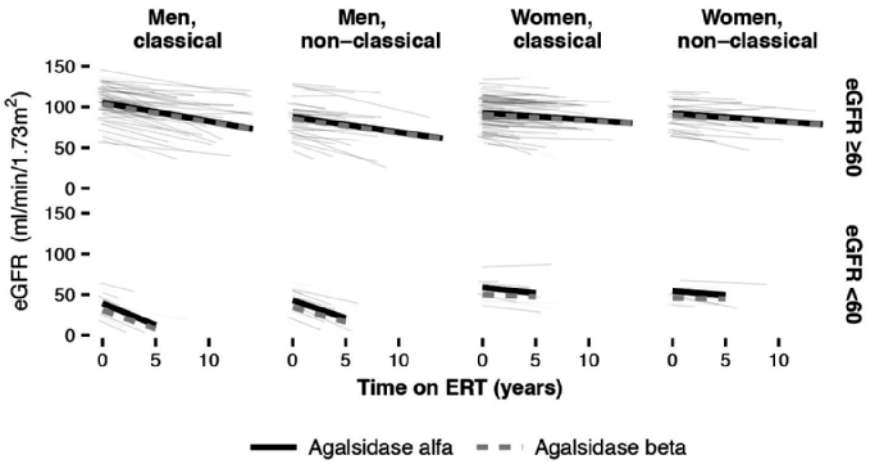
**Supplemental table D** Mutations and phenotypes (Continued)

| | | Men | | Women | |
|---|---|---|---|---|---|
| **Mutations** | **Total** | **classical** | **non-classical** | **classical** | **non-classical** |
| **p.Asn33Asp** | 1 | 1 | 0 | 0 | 0 |
| **p.Asn34Lys fs*22** | 1 | 0 | 0 | 1 | 0 |
| **p.Asn355_Ile359del** | 1 | 0 | 0 | 1 | 0 |
| **p.Asn408Ile fs*10** | 4 | 1 | 0 | 1 | 2 |
| **p.Asn53Leu fs*57** | 2 | 1 | 0 | 1 | 0 |
| **p.Asp136Glu** | 2 | 1 | 0 | 0 | 1 |
| **p.Asp136Tyr** | 7 | 4 | 0 | 2 | 1 |
| **p.Asp165Val** | 1 | 1 | 0 | 0 | 0 |
| **p.Asp170Asn** | 1 | 1 | 0 | 0 | 0 |
| **p.Asp234del** | 1 | 0 | 0 | 1 | 0 |
| **p.Asp299Glu** | 1 | 0 | 1 | 0 | 0 |
| **p.Asp55Thr fs*66** | 1 | 1 | 0 | 0 | 0 |
| **p.Cys174Val fs*4** | 6 | 1 | 0 | 5 | 0 |
| **p.Cys202Trp** | 1 | 0 | 0 | 1 | 0 |
| **p.Cys63Ala** | 1 | 1 | 0 | 0 | 0 |
| **p.Gin386*** | 3 | 0 | 0 | 3 | 0 |
| **p.Gln107*** | 1 | 0 | 0 | 0 | 1 |
| **p.Gln280His** | 2 | 1 | 0 | 0 | 1 |
| **p.Gln312*** | 1 | 0 | 0 | 1 | 0 |
| **p.Gln321_Asp322 DelInsHisAsn** | 1 | 1 | 0 | 0 | 0 |
| **p.Gln321Leu** | 2 | 0 | 1 | 1 | 0 |
| **p.Gln386*** | 2 | 1 | 0 | 1 | 0 |
| **p.Gln416*** | 2 | 1 | 0 | 0 | 1 |
| **p.Glu338Lys** | 3 | 0 | 0 | 1 | 2 |
| **p.Glu341Lys** | 2 | 2 | 0 | 0 | 0 |
| **p.Glu358Asp fs*16** | 3 | 0 | 0 | 2 | 1 |
| **p.Gly132Glu** | 1 | 1 | 0 | 0 | 0 |
| **p.Gly183Asp** | 2 | 1 | 0 | 0 | 1 |
| **p.Gly260Glu** | 1 | 1 | 0 | 0 | 0 |
| **p.Gly261Val** | 1 | 1 | 0 | 0 | 0 |
| **p.Gly325Ala fs*23** | 2 | 1 | 0 | 1 | 0 |
| **p.Gly325Ser** | 2 | 0 | 1 | 0 | 1 |
| **p.Gly35Arg** | 2 | 1 | 0 | 0 | 1 |
| **p.Gly361Arg** | 4 | 3 | 0 | 1 | 0 |
| **p.Gly373Asp** | 2 | 1 | 0 | 1 | 0 |
| **p.Gly375Glu fs** | 2 | 2 | 0 | 0 | 0 |
| **p.Gly43Val** | 1 | 0 | 0 | 1 | 0 |
| **p.Ile232Thr** | 1 | 0 | 1 | 0 | 0 |
| **p.Ile253Leu fs*16** | 1 | 1 | 0 | 0 | 0 |
| **p.Ile317Thr** | 7 | 2 | 0 | 3 | 2 |
| **p.Ile319Leu fs*10** | 3 | 2 | 0 | 1 | 0 |
| **p.Ile319Thr** | 1 | 0 | 1 | 0 | 0 |
| **p.Ile91Thr** | 1 | 0 | 0 | 0 | 1 |
| **p.Leu129Pro** | 5 | 3 | 0 | 1 | 1 |
| **p.Leu166Pro** | 1 | 0 | 1 | 0 | 0 |
| **p.Leu166Ser** | 1 | 0 | 0 | 1 | 0 |
| **p.Leu21Pro** | 2 | 0 | 0 | 1 | 1 |
| **p.Leu268Ser** | 1 | 1 | 0 | 0 | 0 |
| **p.Leu311Val** | 1 | 1 | 0 | 0 | 0 |

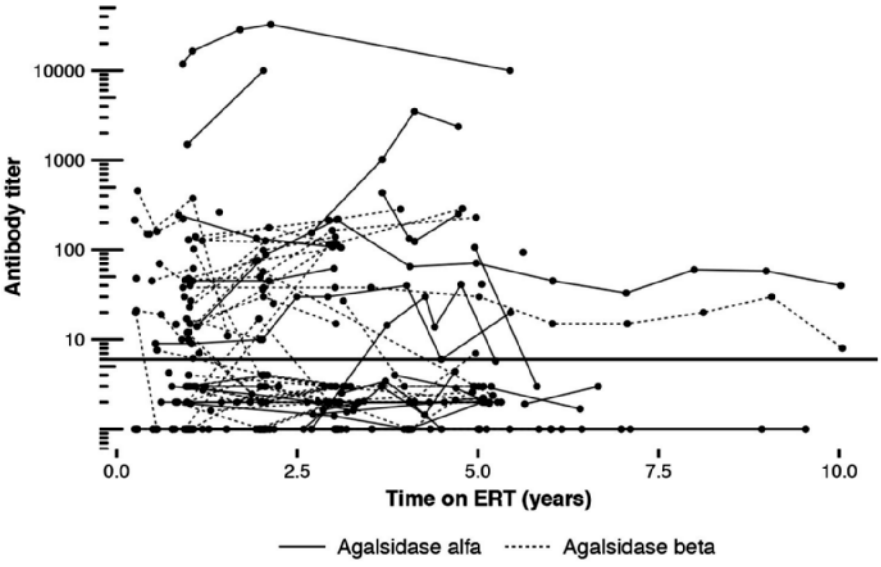
**Supplemental table D** Mutations and phenotypes (Continued)

| | | Men | | Women | |
|---|---|---|---|---|---|
| **Mutations** | **Total** | **classical** | **non-classical** | **classical** | **non-classical** |
| **p.Leu347Phe fs*28** | 2 | 0 | 0 | 1 | 1 |
| **p.Leu372Pro** | 2 | 1 | 0 | 1 | 0 |
| **p.Leu403*** | 2 | 2 | 0 | 0 | 0 |
| **p.Leu414Ser** | 1 | 0 | 0 | 1 | 0 |
| **p.Leu54Pro** | 1 | 0 | 1 | 0 | 0 |
| **p.Lys240Gly FS*9** | 4 | 2 | 0 | 2 | 0 |
| **p.Met187Ser FS*5** | 1 | 1 | 0 | 0 | 0 |
| **p.Met187Val** | 1 | 0 | 0 | 0 | 1 |
| **p.Met1Thr** | 2 | 2 | 0 | 0 | 0 |
| **p.Met42Val** | 2 | 1 | 0 | 1 | 0 |
| **p.Met72Arg** | 1 | 1 | 0 | 0 | 0 |
| **p.Phe169Ser** | 1 | 0 | 0 | 1 | 0 |
| **p.Phe18Ser** | 5 | 0 | 0 | 4 | 1 |
| **p.Pro205Thr** | 10 | 3 | 0 | 5 | 2 |
| **p.Pro293His** | 1 | 0 | 0 | 0 | 1 |
| **p.Pro293Leu** | 2 | 1 | 0 | 0 | 1 |
| **p.Pro389Ala** | 4 | 0 | 2 | 0 | 2 |
| **p.Pro409Thr** | 2 | 0 | 0 | 0 | 2 |
| **p.Ser126Gly** | 1 | 0 | 0 | 0 | 1 |
| **p.Ser345Arg fs*29** | 1 | 1 | 0 | 0 | 0 |
| **p.Ser345Pro** | 6 | 1 | 1 | 4 | 0 |
| **p.Thr282Ile** | 2 | 2 | 0 | 0 | 0 |
| **p.Thr410Ile** | 2 | 0 | 0 | 2 | 0 |
| **p.Trp204*** | 1 | 1 | 0 | 0 | 0 |
| **p.Trp204Cys** | 1 | 0 | 0 | 0 | 1 |
| **p.Trp209*** | 1 | 1 | 0 | 0 | 0 |
| **p.Trp226*** | 3 | 2 | 0 | 1 | 0 |
| **p.Trp236Cys** | 2 | 1 | 0 | 1 | 0 |
| **p.Trp236Leu** | 1 | 0 | 0 | 1 | 0 |
| **p.Trp277*** | 1 | 1 | 0 | 0 | 0 |
| **p.Trp349*** | 5 | 2 | 0 | 2 | 1 |
| **p.Trp399*** | 2 | 2 | 0 | 0 | 0 |
| **p.Trp47Gly** | 1 | 0 | 0 | 1 | 0 |
| **p.Trp81Ser** | 1 | 1 | 0 | 0 | 0 |
| **p.Tyr134fs*1** | 1 | 0 | 0 | 1 | 0 |
| **p.Tyr134Ser** | 1 | 0 | 0 | 1 | 0 |
| **p.Tyr184*** | 2 | 0 | 0 | 2 | 0 |
| **p.Tyr207fs** | 1 | 0 | 0 | 1 | 0 |
| **p.Val269Ala** | 3 | 1 | 0 | 2 | 0 |
| **p.Val316Glu** | 2 | 1 | 0 | 0 | 1 |

# Supplemental material E




**Supplemental figure E** Linear mixed model of eGFR adjusted for sex and phenotype, stratified for baseline eGFR <60 and ≥60 ml/min/1.73m$^2$. The larger lines represent the predicted values at group level, the smaller lines represent the predicted values at individual patient level.




**Supplemental figure F** Antibody titers per patient. The solid dark line represent the cut-off, at an antibody titer of 6.