Molecular Genetics and Metabolism 137 (2022) 328–341

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Review article

Clinical relevance of globotriaosylceramide accumulation in Fabry disease and the effect of agalsidase beta in affected tissues

Camilla Tøndel [a,b,*], Beth L. Thurberg [c,1], Pronabesh DasMahapatra [d], Nicole Lyn [d], Manish Maski [d], Julie L. Batista [d], Kelly George [e], Hiren Patel [d], Ali Hariri [d,2]

[a] Department of Pediatrics, Haukeland University Hospital, Bergen, Norway
[b] Department of Clinical Science, University of Bergen, Bergen, Norway
[c] Department of Pathology, Sanofi, Framingham, MA, USA
[d] Sanofi, Cambridge, MA, USA
[e] Metabolic and Lysosomal Storage Disease Research, Rare and Neurological Diseases Therapeutic Area, Sanofi, Cambridge, MA, USA

ARTICLE INFO

Article history:
Received 26 August 2022
Received in revised form 18 October 2022
Accepted 20 October 2022
Available online 29 October 2022

Keywords:
Fabry disease
Lysosomal storage disorders
Globotriaosylceramide
Pathophysiology
Agalsidase beta
Tissue toxicity

ABSTRACT

Fabry disease (FD) is a rare lysosomal storage disorder, characterized by a reduction in α-galactosidase A enzyme activity and the progressive accumulation of globotriaosylceramide (GL3) and its metabolites in the cells of various organs. Agalsidase beta, an enzyme replacement therapy (ERT), is approved for use in patients with FD in Europe, Canada, Australia, South America, and Asia, and is the only ERT approved for use in the United States. In this review, we discuss the clinical relevance of GL3 accumulation, the effect of agalsidase beta on GL3 in target tissues, and the association between treatment-related tissue GL3 clearance and long-term structure, function, or clinical outcomes. Accumulation of GL3 in the kidney, heart, vasculature, neurons, skin, gastrointestinal tract and auditory system correlates to cellular damage and irreversible organ damage, as a result of sclerosis, fibrosis, apoptosis, inflammation, and endothelial dysfunction. Damage leads to renal dysfunction and end-stage renal disease; myocardial hypertrophy with heart failure and arrhythmias; ischemic stroke; neuropathic pain; skin lesions; intestinal ischemia and dysmotility; and hearing loss. Treatment with agalsidase beta is effective in substantially clearing GL3 in a range of cells from the tissues affected by FD. Agalsidase beta has also been shown to slow renal decline and lower the overall risk of clinical progression, demonstrating an indirect link between treatment-related GL3 clearance and stabilization of FD.

Contents

Abbreviations: 3NT, 3-nitrotyrosine; ABR, auditory brainstem response; AKT, protein kinase B; COX-2, cyclooxygenase-2; DBPC, double-blind placebo controlled; DCT, distal convoluted tubule; DRG, dorsal root ganglia; EC, endothelial cells; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; eNOS, endothelial nitric oxide synthase; ERT, enzyme replacement therapy; FD, Fabry disease; FDA, United States Food and Drug Administration; FPW, foot process width; GFR, glomerular filtration rate; GL3, globotriaosylceramide; GLA, α-galactosidase A; iNOS, inducible nitric oxide synthase; IV, intravenous; LC3-II, microtubule-associated protein 1 light chain 3; Lyso-GL3, globotriaosylsphingosine; mTOR, mammalian target of rapamycin; PBMC, peripheral blood mononuclear cells; OLE, open-label extension; SD, standard deviation; shRNA, small hairpin ribonucleic acid; siRNA, small interfering ribonucleic acid; TGF-β1, transforming growth factor beta 1; UPCR, urine protein to creatinine ratio.

* Corresponding author at: University of Bergen/Haukeland University Hospital, Bergen, Norway.
E-mail address: Camilla.tondel@helse-bergen.no (C. Tøndel).
[1] Beth Thurberg Orphan Science Consulting LLC, 56 Bennington street, Newton, MA, USA, 02458
[2] Eloxx Pharmaceuticals, 480 Arsenal Way, Watertown, MA, USA, 02472

https://doi.org/10.1016/j.ymgme.2022.10.005

1. Introduction to Fabry disease

Fabry disease (FD; OMIM #301500) is a rare, X-linked, genetic disorder which results in markedly reduced or absent activity in the enzyme α-galactosidase A (GLA; EC 3.2.1.22) [1]. The disease affects both males and females, with an estimated prevalence of 4.4 to 25.9 per 100,000 from newborn screening studies [2–6]. Progressive lysosomal accumulation of glycosphingolipids, primarily globotriaosylceramide (GL3) and its deacylated metabolite, globotriaosylsphingosine (lyso-GL3), in tissues and blood is associated with damage in a range of cell types and eventually irreversible damage in multiple organ systems [1,7–9].

FD has a spectrum of disease phenotypes ranging from an early-onset severe (classic) form to a later-onset (non-classic) phenotype. Patients with FD remain mainly asymptomatic throughout infancy, although pathologic GL3 accumulation begins in utero, with the first overt clinical symptoms typically emerging by early childhood [1,10]. In the classic phenotype of FD, early symptoms include neuropathic pain, heat and cold intolerance, gastrointestinal pain, corneal opacity, hearing loss, angiokeratoma, and bradycardia [1,11,12]. Pain is reported by most patients with classic FD and is characterized by burning pain in hands and feet, often following fever, stress, or exercise [1,11,13]. As patients age, increasing accumulation of GL3 and progressive tissue damage is followed by organ failure and vascular events. Later-onset FD is characterized by a more variable disease progression and clinical dysfunction may be limited to one organ [1,14].

Treatments approved for use in patients with FD include the intravenous enzyme replacement therapies (ERTs) agalsidase alfa (Replagal®, Takeda) and agalsidase beta (Fabrazyme®, Sanofi) [15]. Both agalsidase alfa and beta have received approval for use in patients with FD in Europe [16,17], Canada, Australia, South America, and Asia. However, agalsidase beta is the only ERT approved by the United States Food and Drug Administration (FDA) for patients with FD aged 2 years and older [18,19]. In addition, migalastat (Galafold®, Amicus Therapeutics), a pharmacological oral chaperone therapy, is approved in the United States and Canada for use in a subset of patients with FD aged 18 years or older with an amenable *GLA* variant, as well as in Europe, Japan, and Australia for adult and adolescent patients with an amenable *GLA* variant [20]. Highlights of prescribing information (Fabrazyme), FDA, 2003.

In the current review, we summarize the available published literature to discuss (1) the clinical relevance of GL3 accumulation as tissue-toxic in FD, (2) the effect of agalsidase beta on GL3 in target tissues, and (3) the association between treatment-related tissue GL3 clearance and long-term structure, function, or clinical outcomes.

2. GL3 as a hallmark of Fabry disease

GL3 is naturally present in all cells of the body, and lack of its efficient degradation in the lysosome leads to the primary pathology observed in FD. Accumulation of GL3, central in FD progression, is measured in the form of circulating biomarkers in plasma and urine or histological assessment of tissues. The utility of urinary and plasma GL3 and plasma lyso-GL3 as biomarkers of disease progression and response to treatment in FD has been investigated [21–27]. A notable limitation of plasma and urinary GL3 is the lack of sensitivity to distinguish between female heterozygotes and controls, as some females with FD may have normal plasma and urine concentrations of GL3 [27,28]. The metabolite lyso-GL3 plasma level has a better predictive value for identifying male and female patients with FD versus controls, can be used to distinguish between phenotypes (classic vs later-onset) and clinically relevant mutations, correlates with the severity of disease, and is useful for the diagnosis and therapeutic monitoring of patients with classic FD [29–34]. However, in some females with FD lyso-GL3 plasma levels can be normal [35], therefore, genetic testing is still needed. Histopathological assessment of tissue GL3 accumulation in the kidney, heart and skin is indicative of target organ involvement in FD. Accumulated tissue GL3 in biopsied renal peritubular capillaries was used as a surrogate biomarker endpoint in a pivotal Phase 3 study, assessing the safety and efficacy of biweekly infusions of agalsidase beta in patients with FD [36–38].

3. Toxicity of accumulated GL3 in affected tissues in FD

The intracellular accumulation of GL3 is used clinically for the diagnosis of FD and is associated with structural damage and functional loss in disease-related tissues as shown in preclinical and clinical studies (Table 1); additional details on these studies are included in Supplementary Table 1 and Supplementary Table 2.

3.1. Renal damage in Fabry disease

In the natural history of FD, most male patients, and approximately 15 to 20% of female patients, develop Fabry nephropathy, which can

Table 1
Studies reporting GL3 accumulation in tissues where FD causes structural damage and functional loss.

Study	Study design and population	Key results
Renal		
Gubler et al. 1978 [39]	Cross-sectional study of renal biopsies from 12 patients (9 males, 3 females) with FD	• In male patients, GL3 accumulation was distributed homogenously in glomerular, vascular, and interstitial cells; glycolipid storage in tubules was irregular and mainly in the loop of Henle and the distal tubules. • In female patients, glycolipid storage had the same morphological features but was found irregularly. • Age-related degenerative renal changes were present in the majority of patients.
Thurberg et al. 2002 [37]	Multicenter, double-blind, randomized, placebo-controlled, Phase 3 trial of agalsidase beta in 58 patients with FD	• At baseline, GL3 was present in nearly all renal cell types, with particularly dense accumulations in podocytes and distal tubular epithelial cells. • After 11 months of agalsidase beta, there was complete clearance of glycolipid from the endothelium of all vasculature, as well as from the mesangial cells of the glomerulus and interstitial cells of the cortex. • Moderate clearance was noted from the smooth muscle cells of arterioles and small arteries; podocytes and distal tubular epithelium also demonstrated evidence for decreased GL3.
Tøndel et al. 2008 [40]	Cross-sectional study of renal biopsies from nine children (7 males, 2 females) with FD	• Light microscopy showed glomerular, interstitial, or vascular changes in nearly all patients, either alone or in combination: glomerular sclerosis (three of nine patients); interstitial fibrosis (three of nine patients); arteriopathy (four of nine patients); and glomerular hyaline (two of nine patients), and early signs of focal segmental glomerulosclerosis were seen in one patient. • Electron microscopy showed podocyte inclusions, segmental foot-process effacement, and distal tubular cell inclusions in all patients. Mesangial cell and glomerular endothelial cell inclusions were found in all patients except two who were treated with ERT before renal biopsy.
Lubanda et al. 2009 [41]	Open-label, single-arm study of agalsidase beta in 21 ERT-naïve adult male patients with FD	• GL3 clearance of the endothelial cells of the interstitial capillaries was achieved in all patients with 1.0 mg/kg and maintained in 90% with 0.3 mg/kg. • GL3 reduction or clearance was maintained with 0.3 mg/kg in approximately 70% of patients in other renal cell types (glomerular endothelial, mesangial, non-capillary endothelial, interstitial, distal tubule/collecting duct, smooth muscle cells, podocytes) and superficial dermal capillary endothelium.
Fogo et al. 2010 [42]	Cross-sectional study of renal biopsies from 59 patients (35 males, 24 female) with mostly clinically mild FD nephropathy	• Males had greater podocyte vacuolization (corresponding to extracted GL3 deposits) on light microscopy and glycosphingolipid inclusions than females. • Males also had significantly more proximal tubule, peritubular capillary and vascular intimal inclusions compared with females. • Arteriolar hyalinosis was similar, but females had significantly more arterial hyalinosis.
Najafian et al. 2011 [43]	Cross-sectional, observational study of 14 ERT-naïve young patients with FD. Nine healthy, living kidney donors as controls	• Podocyte GL3 inclusion volume density increased progressively with age ($r = 0.77$, p = 0.001) in parallel with podocyte foot process width (nm; $r = 0.65$, $p = 0.01$). • Foot process width (nm) and podocyte GL3 inclusion volume density both correlated directly with proteinuria (UPCR): $r = 0.65$, $p = 0.01$ and $r = 0.68$, $p = 0.008$, respectively. • Fenestration of glomerular endothelial cells was reduced in patients with FD vs controls ($p = 0.01$).
Liebau et al. 2013 [44]	Human podocyte cell line with knocked-down GLA via shRNA	• α-galactosidase A deficient podocytes showed a marked upregulation (>2-fold) of the autophagic marker LC3-II ($p < 0.01$), suggesting activation of autophagic cellular machinery. • Markedly increased podocyte LC3-II was visualized via immunofluorescence staining demonstrating an increased number of autophagosomes in GL3-laden podocytes. • mTOR and AKT activities were reduced in GL3-laden podocytes (4-fold and 2-fold reductions, respectively; $p < 0.001$ and $p < 0.01$, respectively), indicating defective mTOR and AKT signaling.
Tøndel et al. 2013 [45]	Longitudinal, interventional study of paired renal biopsies from 12 young patients with FD pre-ERT and after 5 years of ERT (agalsidase alfa or agalsidase beta)	• Regression analysis showed significant correlation between podocyte GL3 inclusion clearance and reduction in albumin-to-creatinine ratio ($r = 0.837$, $p = 0.001$). • The GL3 clearance was found to be dose dependent.
Tøndel et al. 2015 [46]	Cross-sectional study of pre-ERT kidney biopsies from eight children with classic FD	• All children displayed significant GL3 accumulation in several types of kidney cells, with high amounts of GL3 in podocytes. • Six of eight patients demonstrated segmental foot process effacement, a sign of podocyte cell injury, on baseline kidney biopsy.

Table 1 (*continued*)

Study	Study design and population	Key results
Wijburg et al. 2015 [47]	Baseline data from a randomized, open-label, parallel-group, Phase 3B trial of 31 treatment-naïve male pediatric patients with FD	• GL3 accumulation was reported in superficial skin capillary endothelial cells (23/31 patients) and deep vessel endothelial cells (23/29 patients). • Renal biopsy revealed GL3 accumulation in all glomerular cell types (podocytes and parietal, endothelial, and mesangial cells), as well as in peritubular capillary and noncapillary endothelial, interstitial, vascular smooth muscle, and distal tubules/collecting duct. • Lesions indicative of early arteriopathy and foot process effacement were found in all patients ($n = 6/6$).
Fall et al. 2016 [48]	Cross-sectional urinalysis of samples from 39 patients with FD and 24 healthy controls	• Number of podocytes per gram of urine creatinine was 3.6-fold greater in patients with FD than healthy people. • Patients with FD with normoalbuminuria and normoproteinuria had over 2-fold greater number of podocytes per gram of urine creatinine than healthy subjects ($p = 0.048$). • Number of podocytes per gram of urine creatinine was inversely related to eGFR in male patients ($r = -0.69$, $p = 0.003$), and directly related UPCR ($r = 0.33$; $p = 0.04$) in all patients with FD.
Najafian et al. 2016 [49]	Longitudinal study of pre- and post-ERT paired kidney biopsies from five adult male patients with classic FD from Phase 3 agalsidase beta study and its OLE [37,50], and one additional adult male with classic FD from outside these trials	• FPW (nm) after ERT was significantly inversely correlated with the magnitude of the decrease in podocyte GL3 content from baseline to 11–12 months of ERT ($r = -0.85$, $p = 0.03$).
Skrunes et al. 2017A [51]	Case series of treatment-naïve patients with classical FD who had been treated with agalsidase beta	• Complete clearance of GL3 from mesangial and endothelial cells and partly cleared podocytes after agalsidase beta. • Re-accumulation of podocyte GL3 was seen by shortage-related dose reduction in all three patients.
Skrunes et al. 2017B [52]	Observational, single-center cohort study of 20 patients (12 males, 8 females) with classic FD starting ERT (low or high dose agalsidase alfa or beta)	• No statistical differences were found between group for baseline or final GFR or albuminuria. • Kidney biopsies showed significant reduction of podocyte GL3 in both the lower fixed dose group (-1.39 [SD = 1.04]; $p = 0.004$) and the higher dose group (-3.16 [SD = 2.39]; $p = 0.002$). • Arterial/arteriolar intima GL3 cleared significantly in the higher dose group, all seven patients with baseline intimal GL3 cleared the intima, one patient gained intimal GL3 inclusions ($p = 0.03$), and medial GL3 did not change statistically in either group.
Svarstad et al. 2018 [53]	Cross-sectional, prospective study of renal biopsies from 31 consecutive enzyme-treated or treatment-naïve patients with FD (14 males, 17 females)	• Significant correlations ($p < 0.001$) were found between the stereomicroscopic scoring of glomerular characteristic white storage material and the amount of podocyte GL3 deposits scored by standardized light microscopy.
Politei et al. 2018 [54]	Retrospective chart study of clinical, biochemical and histological assessments in 14 children with classic FD before ERT initiation	• Lyso-GL3 levels were above the normal range in all patients; podocyturia was documented in all patients. • Using light microscopy, kidney biopsy revealed glomerular sclerosis (2/14), interstitial fibrosis (3/14), arteriopathy (11/14), and tubular inclusions (12/14) in nearly all patients. • Electron microscopy showed podocyte inclusions in all patients, and foot process effacement was present in 12/14 patients.
Najafian et al. 2020 [55]	Cross-sectional study of kidney biopsies from 55 males with classic FD and seven healthy, living kidney donors as controls	• GL3 accumulation was associated with podocyte injury and loss, as evidenced by increased FPW ($r = 0.40$, $p = 0.004$) and by decreased podocyte number density per glomerular volume ($r = -0.47$, $p = 0.033$). • Increasing podocyte GL3 volume fraction and FPW were associated with increasing urinary protein excretion ($r = 0.44$, $p = 0.003$ and $r = 0.41$, $p = 0.007$, respectively) as well as with decreasing GFR.
Rozenfeld et al. 2020 [56]	Cross-sectional study of kidney biopsies from 15 adult ERT-naive patients with FD	• GL3 was present in epithelial tubular cells, epithelial Bowman cells, endothelial glomerular cells, podocytes, mesangial cells, and peritubular capillary endothelial cells from FD samples. • Positive staining for TGF-β1 was found in proximal tubular epithelial cells in all FD biopsies. • Active caspase 3 staining revealed induction of apoptosis in tubules from all FD biopsies and in the mesangial glomerular cells in 80% of them.
Vascular		
Ferrans et al. 1969 [57]	Autopsy case report of a female with FD	• Extensive glycolipid deposits in the cardiac muscle fibers, vascular smooth muscle, endothelium, and connective tissue cells of the mitral valve.
Namdar et al. 2012 [58]	Human microvascular cardiac ECs	• GL3 inhibited total eNOS expression in human microvascular cardiac ECs both unstimulated and stimulated (by TNF-alpha) by 28% and 43%, respectively ($p = 0.002$ and $p = 0.001$, respectively). • GL3 increased iNOS expression in unstimulated human microvascular cardiac ECs by 1.7-fold ($p = 0.0014$). • GL3 increased COX-2 expression by 1.5-fold in unstimulated human microvascular cardiac ECs.

(*continued on next page*)

Table 1 (*continued*)

Study	Study design and population	Key results
Shu et al. 2014 [59]	Cell culture studies using a EA.hy926 hybrid cell line. Plasma measurements of 3NT from GLA knockout mice and biobanked plasma from 13 patients with classic FD compared with age- and gender-matched controls ($n = 11$)	• GL3 accumulation was associated with >60% reduction in eNOS activity in cell culture experiments. • 3NT (marker for reactive nitrogen species) levels increased 40- to 120-fold without corresponding changes in other oxidized amino acids, consistent with eNOS-derived reactive nitrogen species as the source. • eNOS uncoupling was confirmed by the observed increase in free plasma and protein-bound aortic 3NT levels in GLA knockout mice. • 3NT levels were over six-fold elevated in plasma FD samples as compared with age- and gender-matched controls ($p < 0.01$).
Neurological		
Gadoth et al. 1983 [60]	Autopsy case report of 37-year-old male with FD	• Presence of swollen neurons in dorsal root ganglia containing periodic acid-Schiff-positive granules.
Kahn et al. 1973 [61]	Autopsy case series of three males with FD	• GL3 storage was observed in pigmented cells of the substantia nigra and in anterior horn cells; and there was degeneration of nerve fibers in the dorsal root entry zone and substantia gelatinosa of the spinal cord.
Germain et al. 2002 [62]	Observational study 22 male patients with classic FD pre-ERT	• 12 patients (54.5%) were found to have abnormal audition; five patients had progressive hearing loss and seven patients (32%) experienced sudden deafness. • Hearing loss on high-tone frequencies was found in seven out of the 10 remaining patients without clinical impairment, despite their young age at time of examination.
Schachern et al. 1989 [63]	Otologic histopathology described from autopsy case report findings of two patients with FD	• GL3 deposition in spiral ganglia and vestibular structures described from autopsy of two patients in the literature.
Dermatological		
Üçeyler et al. 2016 [64]	Cohort study of 84 patients with FD (38 males, 46 females) and 27 healthy controls	• Males ($p < 0.01$) and females ($p < 0.05$) with a classic FD phenotype had higher distal skin GL3 load than healthy controls. • Males with impaired renal function had more GL3 deposits in distal skin than healthy controls ($p < 0.05$). • Patients with FD with small fiber neuropathy had more GL3 deposits in distal skin than controls ($p < 0.05$).
Ramaswami et al. 2019 [65]	Randomized, open-label, parallel-group, Phase 3b study of two low-dose agalsidase beta regimens in 31 ERT-naïve male pediatric patients with classic FD (without clinically evident kidney, heart, or brain involvement)	• There was complete, sustained GL3 clearance from the superficial skin capillary endothelium in the majority of patients in both dosing regimens. • In the overall cohort, change from non-0 to 0-scores for superficial skin capillary endothelium GL3 were significant at years 1, 3, and 5. • Clearance was variable in patients with anti-agalsidase IgG antibody peak titers >3200. • Renal tissue ($n = 7$) showed GL3 accumulation and vasculopathy, and a potential of GL3 reduction by 5 years of ERT compared with baseline kidney biopsies was found.
Other		
Cox-Brinkman et al. 2007 [66]	Observational study of 42 patients with FD (20 male, 22 females). Control images were selected from an existing collection of healthy subjects (80 males, 80 females) without FD	• Pattern recognition techniques achieved a discrimination accuracy of up to 85% for male patients compared with healthy controls; the discrimination accuracy in female patients achieved only 67%. • Facial dysmorphism is likely attributed to GL3 accumulation in growing facial bones and developing facial connective tissues.
Francesco et al. 2013 [67]	Analysis of peripheral blood samples from 29 patients with FD (15 males, 14 females) and 15 healthy controls	• Cultured PBMC from patients with FD present a higher proinflammatory cytokine expression and production. • Among PBMC, dendritic cells and monocytes present a basal proinflammatory cytokine production profile, which is further exacerbated with an inflammatory stimulus. • Normal, monocyte-derived dendritic cells and macrophages display the same proinflammatory profile when cultured in the presence of GL3 and an inhibitor of α-Gal; this effect can be abolished using a TLR4 blocking antibody, indicating that TLR4 is necessary in the process.
Politei et al. 2017 [68]	Two case reports of patients with classic FD (one female, one male)	• GL3 accumulation was present in the ganglion cells of Auerbach's plexus present between the two muscular layers of the muscularis propria and within Meissner's plexus of the submucosa. • Accumulation was also present in the smooth muscle cells of small arteries present in the submucosa and within smooth muscle myocytes of the muscularis propria.

3NT, 3-nitrotyrosine; AKT, protein kinase B; EC, endothelial cells; eNOS, endothelial nitric oxide synthase; ERT, enzyme replacement therapy; FD, Fabry disease; FPW, foot process width; GL3, globotriaosylceramide; GLA, α-galactosidase A; GFR, glomerular filtration rate; iNOS, inducible nitric oxide synthase; LC3-II, microtubule-associated protein 1 light chain 3; lyso-GL3, globotriaosylsphingosine; mTOR, mammalian target of rapamycin; OLE, open-label extension; PBMC, peripheral blood mononuclear cells; SD, standard deviation; shRNA, small hairpin ribonucleic acid; TGF-β1, transforming growth factor beta 1; UPCR, urine protein to creatinine ratio.

progress to end-stage renal disease [43]. Nephropathy is a major contributor to the morbidity and mortality associated with FD and progression to end-stage renal failure is one of the primary causes of death in male patients [1]. Although the pathogenesis of nephropathy in FD is likely to be multifactorial, the increased deposition of GL3 has been shown to cause cellular and histological dysfunction. As a result of the pathophysiological implications of renal damage in FD, kidney function is monitored frequently in patients using serum creatinine to estimate glomerular filtration rate (eGFR), total urinary protein, albumin in urine, and urinary sodium excretion [1]. Many patients with Fabry nephropathy will go on to require dialysis and/or kidney transplant.

3.1.1. Preclinical

In a human podocyte cell line in which *GLA* function was impaired by 93%, GL3-laden podocytes were shown to have increased levels of an autophagic marker, microtubule-associated protein 1 light chain 3, indicating the increased presence of autophagosomes [44]. Dysregulation of autophagy was suggested to be as a result of reduced mammalian target of rapamycin (mTOR) kinase activity, which was four-fold lower in *GLA*-deficient cells compared with control [44].

3.1.2. Clinical

Histologic analysis of renal biopsies can be an important method of renal disease evaluation, particularly at the early stages of disease [69]. A number of studies have evaluated renal biopsies taken from children and adults with FD. These show that in treatment-naïve patients, there is GL3 accumulation in a range of renal cell types, including podocytes, vascular endothelial cells, vascular smooth muscle cells, glomerular endothelial and parietal cells, mesangial cells, interstitial cells, tubular epithelial cells, peritubular capillary endothelial cells and epithelial Bowman cells [37,39,40,42,46,47,56]. In studies of children and adolescents, particularly with classic FD, evaluation of renal biopsy samples revealed glomerular, interstitial or vascular changes in almost all patients, including glomerular sclerosis, glomerular hyalinosis, interstitial fibrosis and arteriopathy [39,40,43,45–47,53–55]. Stereomicroscopy of fresh renal biopsies from patients with untreated FD shows white glomeruli, representing the severe podocyte GL3 accumulation observed in FD (Fig. 1) [53]. This cascade of events leads to loss of renal function that may progress to end-stage renal disease if left untreated.

An analysis of renal biopsies from male and heterozygous female patients with FD showed diffuse glycolipid accumulation in all glomerular, vascular, and interstitial cells in biopsies from male patients [39]. In female patients, although the glycolipid storage had the same morphological features, its distribution was irregular, with some normal cells and others showing substantial accumulation [39]. Associated degenerative renal changes were present in all patients (except one heterozygous female). The variability observed in female patients is likely due to variations in residual enzyme activity and random X-chromosome inactivation patterns [7,8]. In another analysis of renal biopsies in 59 patients with Fabry nephropathy, male patients with FD had significantly more proximal tubule, peritubular capillary and vascular intimal inclusions than female patients, as well as greater podocyte vacuolization [42].

Renal manifestations and end-stage renal disease are more common in males than females in FD, likely owing to the fact that in female heterozygous patients inactivation of the X-chromosome with the *GLA* pathogenic variant in a fraction of tissues often results in a milder phenotype than observed in males [1,70]. However, a recent study has shown that there was no difference in affected podocyte GL3 volume or volume fraction between aged-matched males and females with classic FD, suggesting affected podocyte GL3 accumulation in females is similar to that of males [71]. Histopathological studies show clear evidence of disease manifestations in females with FD, although the degree of involvement is variable and lesser than males consistent with clinical findings.

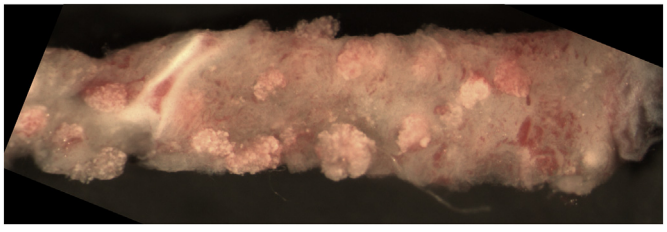

Fig. 1. Stereomicroscopy evaluation of renal biopsy from male patient with classic FD showing typical 'white glomeruli'.
Figure used with permission from Svarstad E, et al. Bedside Stereomicroscopy of Fabry Kidney Biopsies: An Easily Available Method for Diagnosis and Assessment of Sphingolipid Deposits. *Nephron*. 2018;138(1):13–21 [53]. (C) S. Karger AG, Basel.
FD, Fabry disease.

Podocytes, as long-living cells, accumulate GL3 inclusions more than other renal cell types in patients with FD [43,48]. The resultant podocyte damage, which causes podocyte detachment and excretion in the urine (podocyturia), has been linked to early structural and functional damage of the kidneys [43,48]. The accumulation of GL3 in renal podocytes is associated with increased foot process width [49,55], and segmental foot process effacement [40,46] – signs of podocyte injury – as well as loss of podocyte density [55]. Furthermore, worsening of these podocyte structural parameters has been associated with increased urinary protein excretion and decreased GFR [55]. It has also been shown that the degree of podocyte GL3 accumulation correlates directly with urinary protein excretion, which is considered the first clinical manifestation of nephropathy in FD. Patients with more severe proteinuria often advance more rapidly to end-stage renal failure, and as kidney failure progresses, patients may experience increased blood pressure that can further contribute to the decline of kidney function [72–74]. GL3 inclusion volume in podocytes and podocyte foot process width both progressively increase with age, and collectively, these data suggest that podocyte injury is critical in the development of renal damage in FD [43]. Additionally, compared with healthy volunteers, podocyturia is 3.6-fold greater in patients with FD [48]. There is a significant inverse correlation between podocyturia and eGFR in male patients with FD, and a significant direct correlation with urinary protein creatinine ratio in all patients with FD [48]. Podocytes have a limited capacity to regenerate, therefore, the loss of podocytes in FD and subsequent glomerulosclerosis is indicative of irreversible nephron injury and loss of kidney function [48,75].

Positive staining for transforming growth factor β, a pro-fibrotic cytokine, has been observed in proximal tubular epithelial cells in biopsies from patients with FD, suggesting a role for fibrosis in the pathogenesis of FD nephropathy [56]. Active caspase 3 staining was also observed in the tubules from all FD biopsies and in the mesangial glomerular cells in 80% of biopsies, indicating that pathogenesis also involves the induction of apoptosis [56].

3.2. Cardiac manifestations and vascular damage in Fabry disease

Cardiac involvement is a leading cause of premature death in FD and one of the main determinants for prognosis in patients [76]. A predominantly cardiac variant of FD, in which patients present with later-onset isolated cardiac manifestations, has also been identified [77]. In a 1995 study of 230 male patients with FD with left ventricular hypertrophy, a 3% prevalence rate of the atypical FD variant was reported [78]. Electrophysiological abnormalities are seen very frequently in patients with FD, typically starting prior to the occurrence of structural and functional cardiac changes; atherosclerotic coronary artery disease is, however, rare [76,79]. The attribution of GL3 in conduction defects, endothelial dysfunction, and subsequently cardiac damage has been examined in multiple studies.

3.2.1. Preclinical

In a *GLA* knock-down hybrid endothelial cell line, GL3 accumulation was associated with a >60% reduction in endothelial nitric oxide synthase (eNOS) activity [59]. In addition, eNOS uncoupling was confirmed by the observed increase in free plasma and protein-bound aortic 3-nitrotyrosine (3NT; a specific marker for reactive nitrogen species) levels in *GLA* knockout mice [59]. The role of GL3 in endothelial dysfunction is further supported by evidence in human microvascular cardiac endothelial cells. The presence of GL3 inhibited total eNOS expression in both unstimulated and stimulated cells (by tumor necrosis factor-alpha), increased inducible nitric oxide synthase (iNOS) expression (induced by inflammation), and

increased expression of inflammatory cyclooxygenase-2 (COX-2), in unstimulated cells [58].

3.2.2. Clinical

GL3 accumulates in cardiomyocytes, conduction system cells, fibroblasts, endothelial cells and vascular smooth muscle cells within the heart [76,80]. At autopsy, extensive glycolipid deposits have been observed in the cardiac muscle fibers, vascular smooth muscle, endothelium and connective tissue cells of the mitral valve in a patient with FD [57]. Cardiac damage is typically characterized by myocardial hypertrophy; the development of left ventricular hypertrophy is likely triggered by the central pathophysiologic process of GL3 deposition, and has been associated with risk of adverse cardiovascular outcomes in patients with FD [79,81]. However, the increase in mass is not only caused by the accumulation of GL3 (which represents approximately 2% of the cardiac mass); instead, storage of GL3 and lyso-GL3 is thought to activate signaling pathways leading to hypertrophy, apoptosis, and fibrosis [76,79].

3.3. Neurological dysfunction in Fabry disease

Stroke is relatively common in patients with FD and is associated with high morbidity and mortality [82,83]. In a high proportion of patients with FD who experience stroke, this occurs prior to their FD diagnosis and in the absence of any renal or cardiac symptoms (reflecting a later-onset, rather than classic, Fabry phenotype) [83]. In addition to cerebrovascular manifestations, patients with FD can present with neurological symptoms directly affecting the central and peripheral nervous systems. Early neurological manifestations include neuropathic pain involving the peripheral nerves [12].

3.3.1. Preclinical

In a rat model of FD, there was evidence of glycolipid accumulation in the dorsal root ganglia (DRG) sensory neurons, likely impacting ion channel function within these cells [84]. In a mouse model of FD, it has been shown that ERT in combination with substrate reduction therapy reduced the number of vacuolated neurons in the DRG [85].

3.3.2. Clinical

The deposition of GL3 in the endothelium of intracranial blood vessels may play a direct role in the risk of ischemic stroke in patients with FD [82]. Additionally, GL3 accumulation induces oxidative stress and an increased release of reactive oxygen species, causing dilation of cerebrovascular vessels [86–88]. Accumulation of GL3 has been routinely observed in Schwann cells and DRG, with the latter thought to be partly responsible for the neuropathic pain experienced by most patients with FD [60,87]. For example, in an autopsy of a 37-year-old man with FD, swollen neurons containing glycolipids were observed in DRG [60]. This observation was confirmed in a case series of three autopsies that highlighted storage of glycolipids in pigmented cells of the substantia nigra and in anterior horn cells, as well as degeneration of nerve fibers in the dorsal root entry zone and substantia gelatinosa of the spinal cord [61]. It is hypothesized that deposition of GL3 interferes with membrane protein function, for example disrupting ion channel function, causing hyperexcitability and cytotoxicity, thereby resulting in neurological dysfunction, including neuropathic pain [89,90].

FD also affects auditory systems with hearing loss evident from childhood. In 113 audiograms of 47 children with FD, six children had hearing loss, of which five had sensorineural hearing loss, most likely caused by FD [91]. Compared with healthy children (zero decibel hearing loss), children with FD showed increased hearing loss at all frequencies ($p < 0.01$), which was most prominent at high frequencies (>8 kHz) [91]. In an observational study of 22 patients with FD, 54.5% were found to have abnormal auditive tests ranging from progressive hearing loss to sudden deafness [62]. Furthermore, hearing loss on high-tone frequencies was found in seven out of the 10 remaining patients without clinical impairment, despite their young age at the time of examination [62]. This loss of audition may be caused by the accumulation of GL3 within the endothelial and smooth muscle cells of vessels leading to the cochlea [62].

3.4. Skin damage in Fabry disease

Dermatologic disturbances, such as angiokeratoma and hypohidrosis, are among the most common early symptoms of FD [1,92,93]. GL3 accumulation has been observed in multiple dermal types, including vascular endothelial cells, vascular smooth muscle cells and the perineurium [94]. Angiokeratomas are caused by cumulative damage of the vascular endothelial and smooth muscle cells of the skin with vessel dilatation in the dermis [1,94]. Based on data from over 700 patients from the Fabry Outcome Survey, 78% of males and 50% of females had at least one dermatological abnormality, including angiokeratoma (66% and 36%, respectively), hypohidrosis (53% and 28%), telangiectasia (23% and 9%) and lymphoedema (16% and 6%) [93].

3.4.1. Preclinical

In a rat model of FD, animals developed skin manifestations, which included rougher coats, alopecia and xeroderma [95]. On histological examination, there was evidence of lipoatrophy, dilated sebaceous ducts, and macrophage and lymphocyte infiltration of the dermal layer. However, notably, there was no evidence of FD-associated vasculopathy, such as angiokeratomas [95].

3.4.2. Clinical

Compared with healthy volunteers, male patients with FD had a higher GL3 load in distal skin; furthermore, GL3 load was greater in those with more advanced disease (as reflected by impaired renal function) or small-fiber neuropathy than in patients without these complications [64]. In a study in pediatric patients, GL3 accumulation was documented in superficial skin capillary endothelial cells and/or deep vessel endothelial cells in most patients [47]. It has been suggested that topical proximity of dermal GL3 to skin nerve fibers may have an impact on skin innervation and/or skin nerve fiber sensitization [64]. Skin biopsies have been used to monitor GL3 clearance during treatment with ERT in a clinical trial [94]. Skin biopsies are a practical tool to assess disease progression as they are minimally invasive compared to kidney and heart biopsies, the skin is easily accessible, and dermatologic changes are often an early manifestation of FD [94,96].

3.5. Gastrointestinal manifestations in Fabry disease

Gastrointestinal symptoms such as abdominal pain, bloating, diarrhea, nausea and/or vomiting occur in up to 70% of male and almost half of female patients with FD, and are one of the earliest signs of the disease, frequently appearing in childhood and causing significant distress to affected individuals [1,68,97]. However, these symptoms are often under-reported and misdiagnosed [68]. They are thought to develop as a result of GL3 accumulation in myenteric and submucosal autonomic ganglia; as well as myenteric vessel occlusion and intestinal ischemia [68].

3.5.1. Preclinical

GL3 inclusions are present in DRG neurons of rodent preclinical models [98]. These inclusions lead to cell swelling, which is postulated to be the cause of pain. However, it is unknown whether these animal models exhibit specifically gastrointestinal pain [98].

3.5.2. Clinical

Overall, a wide range of histologic and radiologic abnormalities have been described in the gastrointestinal tracts of patients with FD. These abnormalities include autonomic neuropathy with vacuolated autonomic ganglia in the Meissner plexus, delayed gastric emptying,

loss of the gastrocolic reflex, thickening of the intestinal walls, dilation, diverticula (i.e., "outpouching" of the intestine), reduced peristaltic activity, constipation, and smoothing or loss of colonic haustration (i.e., contractions) [99,100]. The underlying pathophysiology of gastrointestinal manifestations is thought to be related to neuropathy, vasculopathy, and myopathy resulting from GL3 accumulation in enteric neurons, DRG, and spinal cord, smooth muscle cells, and in the vascular endothelium [101]. In addition to DRG neuron cell bodies, lipid inclusions are also prominent in the axons of peripheral nerves and correlate with axon morphological abnormalities including irregular-shaped axons, enlarged axons, and nonuniform myelin [98,102,103]. Blockage of the small arteries that supply peripheral nerves may disrupt the vasodilation/vasoconstriction balance, and combined with thrombosis, this imbalance may cause ischemic nerve damage [101]. The blood vessels of the gut may also be affected as a result of changes in signal transduction caused by lyso-GL3, resulting in migration of inflammatory mediators, localized inflammation, and smooth muscle cell hypertrophy. The following are also thought to be involved in gastrointestinal-related vasculopathy: increased cyclooxygenase activity resulting from high levels of GL3 in the vasculature; immunologic mechanisms involving natural killer T lymphocytes; thrombosis related to endothelial dysfunction, increased production of reactive oxygen species, and increased angiotensin II production; and ischemia resulting from GL3 accumulation in the vascular endothelium [101].

3.6. Other pathophysiological changes in Fabry disease

The mechanisms underlying premature organ damage in FD are multifactorial, but often occur as a result of GL3 accumulation in vascular endothelial cells, hence the multiorgan manifestations of the disease [58]. This leads to luminal encroachment and occlusion, which can cause ischemic events and tissue infarctions [7,104]. GL3 accumulation also causes dysregulation of several key endothelial pathways, including eNOS and iNOS, which lowers nitric oxide bioavailability and increases the formation of reactive oxygen species. This can impair vascular function and blood flow to vital organs, increasing the risk of vasculopathy and providing a pathologic microenvironment for accelerated development of vascular complications [58,104]. GL3 loading has also been shown to increase adhesion molecules in the vascular endothelium, increase levels of the vasoconstrictor COX-2, and facilitate the internalization of calcium-activated potassium channels, reducing intracellular calcium levels and downregulating eNOS [58,86,104,105]. Collectively, these contribute to the vasoconstrictive, prothrombotic, and pro-inflammatory phenotype that characterizes FD [58,86,104]. Interestingly, GL3 accumulation seems to affect microvascular endothelial cells earlier and to a greater extent than those in the macrovasculature, which is consistent with clinical data suggesting early and often silent involvement of the microcirculation in patients with FD [58].

Alongside direct damage to various cell types within the body, GL3 has also been implicated in stimulating an inflammatory state within the body [67]. In cultured peripheral blood mononuclear cells (PBMCs) from patients with FD, increased pro-inflammatory cytokine expression and production was observed, which was further increased following an inflammatory stimulus [67]. In control monocyte-derived dendritic cells and macrophages, the same pro-inflammatory profile was induced when cultured with GL3 and an inhibitor of α galactosidase A [67]. A higher apoptotic rate in PBMCs has been observed in patients with FD and directly attributed to elevated GL3 levels [106]. Platelet aggregation and increased plasma β-thromboglobulin levels have also been reported in patients with FD [107]. Additionally, a placebo-controlled study of 25 patients with FD found that the concentration of soluble intercellular adhesion molecule-1, vascular cell adhesion molecule-1, P-selectin, and plasminogen activator inhibitor were significantly higher in patients than controls; although thrombomodulin was significantly lower in this population [108]. There was also considerable evidence of endothelium and leukocyte activation in patients with FD compared with controls, consistent with a prothrombotic state. In conjunction with vascular structural changes, this may contribute to the incidence of thrombosis in patients with FD [108].

4. The effects of agalsidase beta on GL3 in affected tissues

To date, licensed treatment options for patients with FD include ERT (agalsidase alfa or agalsidase beta) and a pharmacological chaperone (migalastat) for a subset of patients with amenable mutations. Agalsidase beta is the only approved ERT for patients with FD in the US, with approvals in many different countries [18,19]. Agalsidase beta is internalized and transported to the lysosome where it hydrolyzes accumulated GL3. Studies evaluating the effect of agalsidase beta on GL3 in Fabry target tissues and/or function are shown in Table 2 (additional information is included in Supplementary Table 3). The efficacy of agalsidase alfa and migalastat is not discussed in this review.

4.1. Clearance of tissue GL3 by agalsidase beta

The effect of agalsidase beta on GL3 clearance was evaluated in a Phase 1/2 dose-escalation study in 15 males with classic FD [50] and a Phase 3 placebo-controlled study with an open-label extension (OLE) in 58 patients with classic FD [36]. In the Phase 1/2 study, there were rapid and marked reductions in plasma and tissue GL3 during treatment with agalsidase beta. In patients with pre- and post-treatment biopsies, mean GL3 content was decreased by 84% in the liver ($n = 13$), was markedly reduced in the kidney in four of five patients, and was modestly lowered in the endomyocardium of four of seven patients, with clearance both dose- and tissue-dependent [50]. In addition, GL3 deposits were cleared to near normal, or were markedly reduced, in the vascular endothelium of liver, skin, heart, and kidney. Importantly, patients with FD treated with agalsidase beta reported less pain and an increased ability to sweat, both consistent with GL3 clearance from the microvascular endothelium, and improvements in their quality of life [50].

In the Phase 3 study, 1 mg/kg agalsidase beta was shown to clear GL3 deposits by Month 5 of treatment in the capillary endothelium of the kidney, heart, and skin, with long-term GL3 clearance achieved at 54 months during the OLE phase of the study, when all 58 patients were treated with agalsidase beta (Fig. 2) [36,37,74,80,94]. Along with complete clearance of GL3 from the superficial capillary endothelium, after 5 months of treatment there was moderate clearance from vascular smooth muscle cells and the perineurium [94]. In addition, plasma GL3 levels were rapidly normalized with agalsidase beta treatment and maintained throughout the 54-month follow-up [74]. In the same cohort of 58 patients with FD, pre- and post-treatment kidney biopsies showed that after 11 months of agalsidase beta treatment there was complete clearance of glycolipid from the endothelium of all vasculature, mesangial cells of the glomerulus, and interstitial cells of the cortex [37]. Results from the 54-month OLE of this study revealed that long-term treatment with agalsidase beta may halt the accumulation of microvascular GL3, thereby preventing atherosclerotic disease [80]. Moderate clearance was also observed in smooth muscle cells of arterioles and small arteries, and evidence of clearance was observed in podocytes and distal tubular epithelium, which are typically more challenging to treat [74]. Furthermore, increased podocyte foot process width (a sign of injury) was significantly inversely correlated with the magnitude of the decrease in podocyte GL3 content [49]. The results suggest that short-term treatment with agalsidase beta maintains GL3 clearance in short-lived cells, and continued treatment reduces stores of GL3 in those cells typically more resistant to clearance [74].

Additionally, long-term treatment (5 years) with agalsidase beta was shown to result in complete GL3 clearance from mesangial and glomerular endothelial cells [45]. A dose-dependent clearance of GL3 in podocytes was also reported, with a significant correlation between podocyte GL3 inclusion clearance and reduction in albumin-to-

Table 2
Studies evaluating the effect of agalsidase beta on GL3 in Fabry target tissues and/or target tissue function.

Study	Study design and population	Key results
Eng et al. 2001A [50]	Phase 1/2 multidose, open label, single center, dose-escalation study* of 15 classically affected males with FD	• Reduction in plasma GL3 concentrations was dose-dependent for all infusion groups. • In patients with pre- and post-treatment biopsies, mean GL3 concentrations decreased 84% in liver (13/15 patients, was markedly reduced in kidney (4/5 patients), and after five doses was modestly lowered in the endomyocardium (4/7 patients). • GL3 was cleared or markedly reduced in the vascular endothelium of liver, skin, heart, and kidney. • Patients reported less pain and fatigue, increased ability to sweat, and improved quality-of-life measures.
Eng et al. 2001B [36]	Phase 3 multicenter, randomized, placebo-controlled, double-blind study† and OLE of 58 patients with FD	• 69% ($n = 20/29$) of patients who received agalsidase beta had no microvascular endothelial GL3 deposits in the kidney after 20 weeks (compared with 0/29 in the placebo group; $p < 0.001$). • Patients in the agalsidase beta group also had decreased microvascular endothelial deposits of GL3 in the skin ($p < 0.001$) and heart ($p < 0.001$). • Plasma levels of lyso-GL3 were directly correlated with clearance of the microvascular deposits. • After six months OLE, all patients in the former placebo group and 98% of patients in the former agalsidase beta group with biopsies had clearance of microvascular endothelial deposits of GL3.
Thurberg et al. 2002 [37]	11-month follow-up from the Phase 3 OLE† in 58 patients with FD who completed 20-week double-blind phase of the Phase 3 study	• At baseline, GL3 accumulated in nearly all renal cell types (including vascular endothelial cells, vascular smooth muscle cells, mesangial cells and interstitial cells, podocytes, and distal tubular epithelial cells). • Agalsidase beta completely cleared GL3 from the endothelium of all vasculature, as well as mesangial and interstitial cells. • Moderate clearance was observed in the smooth muscle cells of arterioles and small arteries. • Podocytes and distal tubular epithelium also demonstrated evidence for decreased GL3.
Wilcox et al. 2004 [109]	30-month follow up from the Phase 3 OLE† in 58 patients with FD who completed 20-week double-blind phase of the Phase 3 study	• Plasma GL3 levels remained normal during the 30-month extension period. • GL3 clearance in the capillary endothelial in 98% (39/40) of patients who had a skin biopsy taken after treatment for 30 months (original placebo group) or 36 months (original agalsidase beta group). • Mean serum creatinine level and eGFR also remained stable after 30–36 months of treatment.
Banikazemi et al. 2007 [110]	Multicenter, randomized double-blind placebo-controlled study† of 82 adults with advanced FD and mild to moderate kidney disease	• 13/31 (42%) patients in the placebo group and 14/1 (27%) in the agalsidase beta group experienced a clinical event. • Time to first clinical event (adjusted for baseline proteinuria) was delayed in the agalsidase beta group compared with placebo (HR 0.47 [95% CI, 0.21; 1.03]; $p = 0.06$).
Germain et al. 2007 [74]	54-month follow-up from the Phase 3 OLE† in 58 patients with FD who completed 20-week double-blind phase of the Phase 3 study	• All patients with available kidney biopsies ($n = 8$) maintained complete GL3 clearance in renal capillary endothelial cells and multiple cell types, after 54 months. • Complete clearance of skin (31/36) and heart (6/8) capillary endothelium was also observed. • Plasma GL3 levels remained within the normal range; serum creatinine/eGFR remained normal in patients with available data at month 54 ($n = 41$). • Six patients had renal disease progression; 4/6 were older than 40 years and had significant baseline proteinuria and > 50% glomerular sclerosis.
Wraith et al. 2008 [111]	Multicenter, open-label, 48-week study† of 16 pediatric patients with FD	• GL3 clearance from superficial dermal capillary endothelial cells was seen in all male patients ($n = 12$) at Week 24, and all available biopsies at Week 48. • Patient reports of post-prandial pain, nausea, and vomiting declined with treatment (statistically significant improvements by Week 24). • Renal and cardiac manifestations of FD were not present in most children in this study.
Lubanda et al. 2009 [41]	Open-label, single-arm, 96-week, study‡ of 21 ERT-naïve adult male patients	• GL3 clearance of the endothelial cells of the interstitial capillaries was achieved in all patients with 1.0 mg/kg and maintained in 90% with 0.3 mg/kg. • GL3 reduction or clearance was maintained with 0.3 mg/kg in approximately 70% of patients in other renal cell types (glomerular endothelial, mesangial, non-capillary endothelial, interstitial, distal tubule/collecting duct, smooth muscle cells, podocytes) and superficial dermal capillary endothelium.

Table 2 (*continued*)

Study	Study design and population	Key results
Germain et al. 2015 [112]	10-year outcome analysis[†] of 52 patients from the Phase 3 OLE and Fabry registry	• 81% of patients (42/52) did not experience any severe clinical event, and 94% (49/52) were alive at the end of the study period. • Mean eGFR decreased over time in patients with high and low renal involvement at baseline; however, decline was greater in the former (−6.82 versus −1.89 mL/min/1.73 m^2/year). • Mean left ventricular posterior wall thickness and interventricular septum thickness remained unchanged and normal. • Mean plasma GL3 normalized within 6 months.
Skrunes et al. 2017B [52]	Observational, single-center cohort study[§] of 20 patients (12 male, eight female) with classic FD starting ERT	• No statistical differences were found between group for baseline or final GFR or albuminuria. • Kidney biopsies showed significant reduction of podocyte GL3 in both the lower fixed dose group (−1.39 [SD = 1.04]; $p = 0.004$) and the higher dose group (−3.16 [SD = 2.39]; $p = 0.002$). • Arterial/arteriolar intima GL3 cleared significantly in the higher dose group, all seven patients with baseline intimal GL3 cleared the intima, one patient gained intimal GL3 inclusions ($p = 0.03$), and medial GL3 did not change statistically in either group.
Ramaswami et al. 2019 [65]	Randomized, open-label, parallel-group, 5-year, Phase 3b study[‖] of 31 ERT-naïve male pediatric patients with classic FD (without clinically evident kidney, heart, or brain involvement)	• There was complete, sustained GL3 clearance from the superficial skin capillary endothelium in most patients in both dosing regimens. • In the overall cohort, change from non-0 to 0-scores for superficial skin capillary endothelium GL3 were significant at years 1, 3, and 5. • Clearance was variable in patients with anti-agalsidase IgG antibody peak titers >3200. • GL3 accumulation and cellular/vascular injury were present in baseline kidney biopsies (n = 7).

eGFR, estimated glomerular filtration rate; ERT, enzyme replacement therapy; FD, Fabry disease; GL3, globotriaosylceramide; OLE, open-label extension.
*Agalsidase beta: Group A, 0.3 mg/kg every 2 weeks; Group B, 1.0 mg/kg every 2 weeks; Group C, 3.0 mg/kg every 2 weeks; Group D, 1.0 mg/kg every 48 h; Group E, 3.0 mg/kg every 48 h. [†]Agalsidase beta 1.0 mg/kg every 2 weeks. [‡]Agalsidase beta at 1.0 mg/kg every 2 weeks for 6 months followed by 18 months at 0.3 mg/kg every 2 weeks. [§]Lower fixed-dose group: agalsidase alpha or beta 0.2 mg/kg every 2 weeks; higher dose group: 0.2–1.0 mg/kg every 2 weeks. [‖]One of two low-dose agalsidase beta regimens: 0.5 mg/kg every 2 weeks or 1.0 mg/kg every 4 weeks for 5 years.

creatinine ratio ($p = 0.001$) [45]. There is also evidence to indicate that lower doses of agalsidase beta may be sufficient to maintain cellular GL3 clearance in some patients after transitioning from a standard therapeutic dose [41]. In an observational cohort study of 20 patients with classic FD, patients were treated with either agalsidase alfa or beta in two dose groups: the low fixed-dose group received 0.2 mg/kg every 2 weeks, and the higher-dose group received a range of doses from 0.2 to 1.0 mg/kg every 2 weeks, for up to 14 years [52]. There was a significant reduction in podocyte GL3 levels in both dose groups, although significantly greater GL3 clearance was reported in the higher-dose group in podocytes and arterial/arteriolar intima [52]. The relationship between the administered dose of agalsidase beta and the magnitude of GL3 clearance was also investigated in a low-dose study (0.5 mg/kg every 2 weeks or 1.0 mg/kg every 4 weeks) in 31 children with classic FD

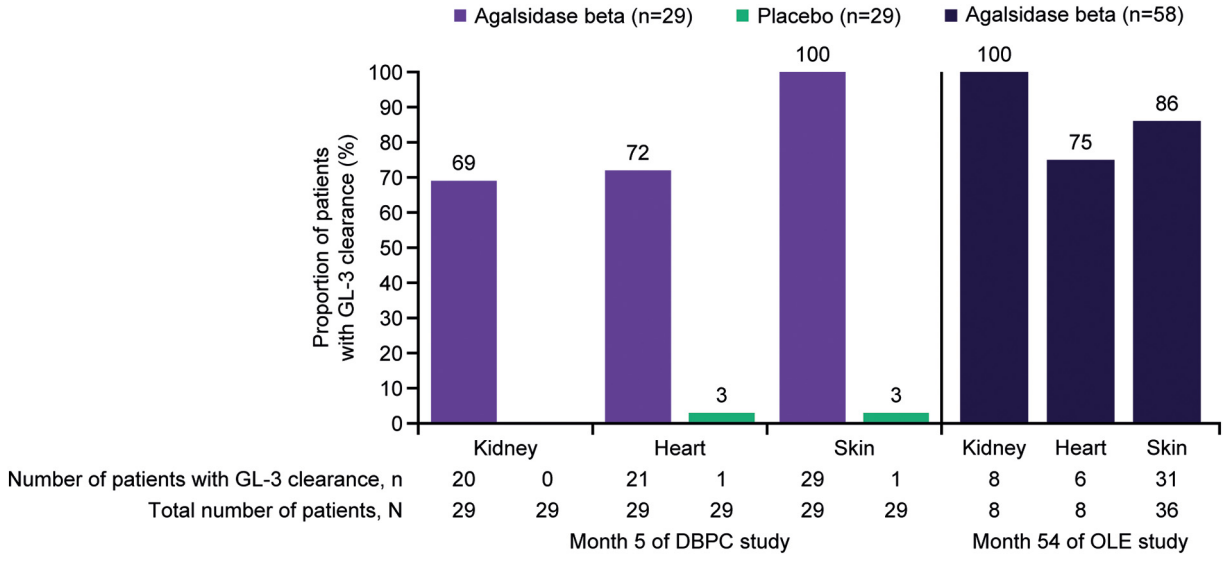

Fig. 2. Clearance of GL3 from kidney, heart, and skin capillary endothelium after treatment with agalsidase beta.
GL3 clearance was defined as specimens with no microvascular endothelial deposits of GL3 or only trace amounts (normal or nearly normal). Month 5 data are from the Phase 3 randomized, DBPC study in which 58 patients were randomized 1:1 to receive either agalsidase beta (1 mg/kg every 2 weeks) or placebo for 20 weeks [36,37,80,94]. All 58 patients entered the OLE in which they received biweekly 1.0 mg/kg agalsidase beta infusions for up to an additional 54 months. At Month 54 of the OLE, results were reported where biopsies were available [74,80].
DBPC, double-blind placebo controlled; GL3, globotriaosylceramide; OLE, open-label extension.

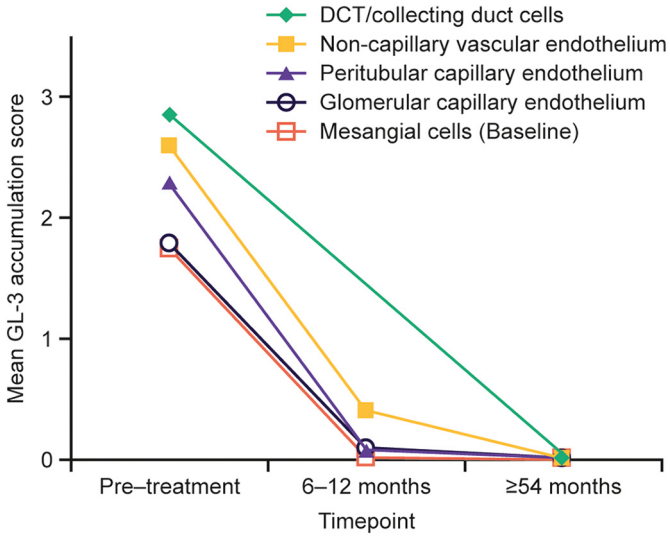

Fig. 3. Normalization of kidney cellular GL3 over time.
Figure used with permission from Germain DP, et al. Sustained, long-term renal stabilization after 54 months of agalsidase beta therapy in patients with Fabry disease. *J Am Soc Nephrol.* 2007;18:1547–1557; permission conveyed through Copyright Clearance Center, Inc. [74]. Agalsidase beta therapy reduced GL3 accumulation to zero at 54 months in various types of kidney cells. For DCT/collecting duct cells, baseline scores were obtained at the beginning of the double-blind trial and at month 54 on a 0 to 3 scale. For all other cell types, pretreatment scores were obtained before agalsidase beta treatment. Mesangial cells were scored on a scale of 0 to 2, and other cell types were scored on a scale of 0 to 3.
DCT, distal convoluted tubule; GL3, globotriaosylceramide.

with elevated GL3 in plasma or urine but no clinical evidence of organ damage [65]. Although both doses caused complete and sustained GL3 clearance from superficial skin capillary endothelium in the majority of patients [65], in a subset of patients (all with high antibody titers) the clearance was less robust than reported in studies of the approved dose of agalsidase beta in adults and children [36,111]. The use of GL3 as a marker for treatment response may also be dependent on when treatment has been initiated; in an autopsy of a patient with classic FD, histological examination reported that significant accumulation of GL3 was still present despite 6 years of agalsidase beta treatment (initiated at age 61 years) [113]. It was suggested that this was because treatment was started when the patient had already experienced symptomatic organ involvement, including left ventricular hypertrophy and kidney dysfunction, and because the patient had a high antibody titer against agalsidase beta [113]. Although such reports are rare, this evidence does underscore the importance of early treatment.

4.2. Treatment-related tissue GL3 clearance and clinical relevance of long-term structure, function, or outcomes

While it is hypothesized that the reduction in GL3 by agalsidase beta may restore the normalization of structure and function in disease-related tissues, a direct association between tissue GL3 clearance and long-term structure, function, or clinical progression has not been established in preclinical or clinical studies. This is attributable to three primary reasons: (1) Fabry sub-populations (later-onset, females, pediatric age group) show variable levels in tissues and plasma of the substrate GL3, (2) tissue biopsy is invasive in nature and is not routinely performed in the clinical management of FD (since there is no impact on clinical decision-making), and (3) despite GL3 accumulation in disease-related tissues, the *GLA* knockout mouse does not display an appreciable clinical disease phenotype. In the OLE of the Phase 3 agalsidase beta study, by Month 54, all patients with optional kidney biopsies ($n = 8$) maintained complete GL3 clearance in renal capillary endothelial cells and multiple cell types (Fig. 3). At Month 54 ($n = 41$), renal function was also measured; however, despite the clearance of GL3 from renal tissue based on light microscopy, the decline in renal function was variable. The best histological predictor of the decline of the eGFR was the absence/presence of 50% sclerotic glomeruli at baseline (Fig. 4), suggesting that early treatment of FD leads to the best renal protective effect. These data comprise the best correlate of tissue GL3 with clinical changes of the organ of interest.

Nonetheless, a link between reduction in GL3 and clinical benefit is biologically plausible and is supported indirectly by evidence demonstrating that treatment with agalsidase beta lowers the risk or slows the rate of clinical events, such as chronic dialysis, kidney transplant, myocardial infarction, and stroke [74,110,112]. This includes the OLE of the Phase 3 study of 58 patients with FD, as discussed above [36]. After 30–36 months follow-up in the OLE study, mean serum creatinine level and eGFR remained stable [109]. Similar results were observed

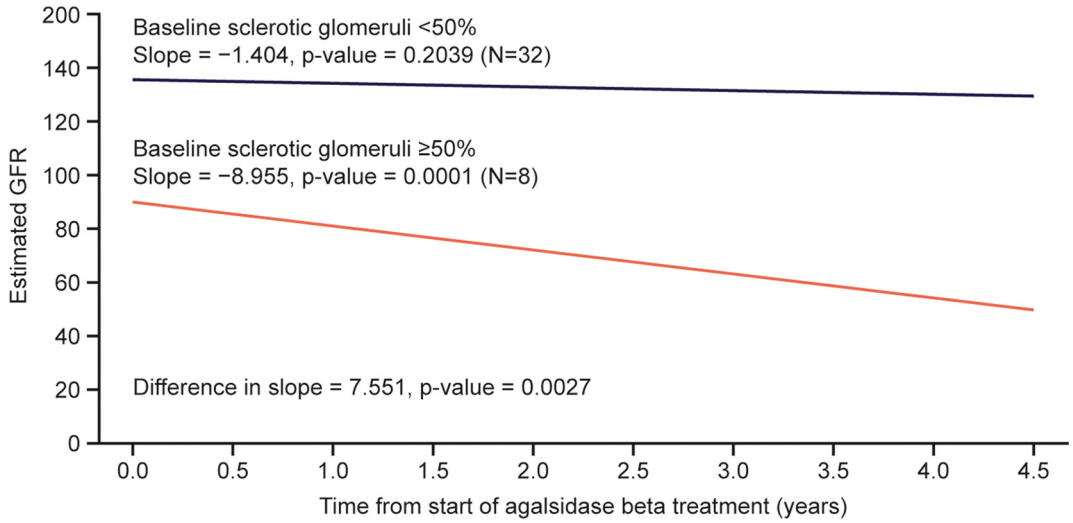

Fig. 4. eGFR decline by renal involvement (sclerotic glomeruli).
Figure used with permission from Germain DP, et al. Sustained, long-term renal stabilization after 54 months of agalsidase beta therapy in patients with Fabry disease. *J Am Soc Nephrol.* 2007;18:1547–1557; permission conveyed through Copyright Clearance Center, Inc. [74]. Subgroup analyses of patients in the "as treated" population show that those with pre-treatment glomerulosclerosis (≥50% sclerotic glomeruli) had a higher rate of decline in eGFR compared with patients with <50% sclerotic glomeruli at pre-treatment.
eGFR, estimated glomerular filtration rate.

after 54 months, although six patients had renal disease progression despite effective GL3 clearance with agalsidase beta [74]. However, four of these six patients were older than 40 years and had proteinuria and sclerotic glomeruli before treatment.

Among 52 patients with FD who participated in both the OLE study and continued to be followed observationally in the Fabry Registry, 10-year outcomes were assessed for agalsidase beta treatment at the FDA-approved dose of 1 mg/kg every 2 weeks [112]. Renal and cardiac function, as well as the occurrence of severe clinical events (defined as a composite of chronic dialysis, kidney transplant, myocardial infarction, congestive heart failure, major cardiac procedures, stroke and death) were assessed. eGFR decreased over time in patients with high and low renal involvement at baseline; however, decline was greater in the former (−6.82 versus −1.89 mL/min/1.73 m^2/year). Additionally, the mean left ventricular posterior wall thickness and interventricular septum thickness remained unchanged and normal in the overall population; however, it increased in the subgroup who initiated treatment after the age of 40 years. Overall, 42 patients (81%) did not experience any severe clinical events [112].

The incidence of clinical events in a randomized, double-blind, placebo-controlled study of agalsidase beta-treated patients with advanced FD was also evaluated [110]. Patients were treated for up to 35 months (median, 18.5 months) and the primary endpoint was time to first clinical event (renal, cardiac, or cerebrovascular; or death). Overall, 14/51 patients (27%) in the agalsidase beta group had an event, compared with 13/31 (42%) in the placebo group, suggesting treatment with agalsidase beta delayed time to first clinical event (hazard ratio, 0.47 [95% CI, 0.21 to 1.03]; $p = 0.06$) [110].

Finally, meta-analysis of clinical trials and long-term observational studies demonstrate that agalsidase beta stabilizes renal function in treated compared with untreated patients (as evidenced by slowing the decline of eGFR slopes by approximately 2.0 mL/min/1.73 m^2/year) and may delay progression to severe clinical events, if initiated before severe organ damage [114,115].

5. Conclusion

FD is a progressive and debilitating disease that causes severe organ damage. GL3 accumulation in the lysosomes of a range of cell types in FD and its metabolite lyso-GL3 are tissue toxic and progressively cause structural damage and functional loss. The intracellular accumulation of GL3 is correlated with the degree of damage in affected organs. Agalsidase beta is effective in substantially clearing GL3 from target tissues in FD and stabilizes disease progression. A direct association to confirm the clinical relevance between treatment-related tissue GL3 clearance and long-term structure, function or clinical progression has not been established due to lack of available data for such analyses. An indirect link between reduction in GL3 and clinical benefit is biologically plausible and is supported by evidence demonstrating that treatment with agalsidase beta slows eGFR decline and, in general, lowers the overall risk of clinical progression. Therefore, it is important to diagnose and treat patients with FD early, before the occurrence of irreversible damage secondary to glycosphingolipid accumulation.

Funding

This study was funded by Sanofi.

Disclosures of interest

CT: has on behalf of Haukeland University Hospital served as consultant for Sanofi, Amicus, Chiesi, Freeline Therapeutics and Avrobio, participates as investigator in clinical Fabry studies initiated by Sanofi, Protalix, Idorsia and Freeline Therapeutics and has received speaker honoraria from Sanofi, Amicus, Takeda and Chiesi. All honoraria received goes to Haukeland University Hospital.

BT: was an employee and shareholder of Sanofi at the time of study completion and is currently an independent consultant for Sanofi, Avrobio and Freeline Therapeutics.

PD: is an employee and shareholder of Sanofi.

NL: is an employee and shareholder of Sanofi.

MM: is an employee and shareholder of Sanofi.

JB: is an employee and shareholder of Sanofi.

KG: is an employee and shareholder of Sanofi.

HP: is an employee and shareholder of Sanofi.

AH: was an employee and shareholder of Sanofi at the time of study completion and is currently an employee of Eloxx Pharmaceuticals.

Data availability

No data was used for the research described in the article.

Acknowledgements

The authors thank Amy Watkins, PhD of Ashfield MedComms, an Inizio company, for medical writing assistance, which was funded by Sanofi in accordance with Good Publication Practice guidelines.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgme.2022.10.005.

References

[1] D.P. Germain, Fabry disease, Orphanet. J. Rare. Dis. 5 (2010) 30.
[2] M. Spada, S. Pagliardini, M. Yasuda, T. Tukel, G. Thiagarajan, H. Sakuraba, A. Ponzone, R.J. Desnick, High incidence of later-onset fabry disease revealed by newborn screening, Am. J. Hum. Genet. 79 (2006) 31–40.
[3] T.P. Mechtler, S. Stary, T.F. Metz, V.R. De Jesús, S. Greber-Platzer, A. Pollak, K.R. Herkner, B. Streubel, D.C. Kasper, Neonatal screening for lysosomal storage disorders: feasibility and incidence from a nationwide study in Austria, Lancet. 379 (2012) 335–341.
[4] C.R. Scott, S. Elliott, N. Buroker, L.I. Thomas, J. Keutzer, M. Glass, M.H. Gelb, F. Turecek, Identification of infants at risk for developing Fabry, Pompe, or mucopolysaccharidosis-I from newborn blood spots by tandem mass spectrometry, J. Pediatr. 163 (2013) 498–503.
[5] T. Inoue, K. Hattori, K. Ihara, A. Ishii, K. Nakamura, S. Hirose, Newborn screening for Fabry disease in Japan: prevalence and genotypes of Fabry disease in a pilot study, J. Hum. Genet. 58 (2013) 548–552.
[6] W.L. Hwu, Y.H. Chien, N.C. Lee, S.C. Chiang, R. Dobrovolny, A.C. Huang, H.Y. Yeh, M.C. Chao, S.J. Lin, T. Kitagawa, R.J. Desnick, L.W. Hsu, Newborn screening for Fabry disease in Taiwan reveals a high incidence of the later-onset GLA mutation c.936+919G>a (IVS4+919G>a), Hum. Mutat. 30 (2009) 1397–1405.
[7] C.M. Eng, D.P. Germain, M. Banikazemi, D.G. Warnock, C. Wanner, R.J. Hopkin, J. Bultas, P. Lee, K. Sims, S.E. Brodie, G.M. Pastores, J.M. Strotmann, W.R. Wilcox, Fabry disease: guidelines for the evaluation and management of multi-organ system involvement, Genet. Med. 8 (2006) 539–548.
[8] A. Ortiz, D.P. Germain, R.J. Desnick, J. Politei, M. Mauer, A. Burlina, C. Eng, R.J. Hopkin, D. Laney, A. Linhart, S. Waldek, E. Wallace, F. Weidemann, W.R. Wilcox, Fabry disease revisited: management and treatment recommendations for adult patients, Mol. Genet. Metab. 123 (2018) 416–427.
[9] A. Tuttolomondo, R. Pecoraro, I. Simonetta, S. Miceli, A. Pinto, G. Licata, Anderson-Fabry disease: a multiorgan disease, Curr. Pharm. Des. 19 (2013) 5974–5996.
[10] C. Ellaway, Paediatric Fabry disease, Transl Pediatr 5 (2016) 37–42.
[11] R.J. Hopkin, J. Bissler, M. Banikazemi, L. Clarke, C.M. Eng, D.P. Germain, R. Lemay, A. Tylki-Szymanska, W.R. Wilcox, Characterization of Fabry disease in 352 pediatric patients in the Fabry registry, Pediatr. Res. 64 (2008) 550–555.
[12] A. Tuttolomondo, R. Pecoraro, I. Simonetta, S. Miceli, V. Arnao, G. Licata, A. Pinto, Neurological complications of Anderson-Fabry disease, Curr. Pharm. Des. 19 (2013) 6014–6030.
[13] B. Hoffmann, M. Beck, G. Sunder-Plassmann, W. Borsini, R. Ricci, A. Mehta, Nature and prevalence of pain in Fabry disease and its response to enzyme replacement therapy–a retrospective analysis from the Fabry Outcome Survey, Clin. J. Pain 23 (2007) 535–542.
[14] M. Arends, C. Wanner, D. Hughes, A. Mehta, D. Oder, O.T. Watkinson, P.M. Elliott, G.E. Linthorst, F.A. Wijburg, M. Biegstraaten, C.E. Hollak, Characterization of classical and nonclassical Fabry disease: a multicenter study, J. Am. Soc. Nephrol. 28 (2017) 1631–1641.
[15] T. Alegra, F. Vairo, M.V. de Souza, B.C. Krug, I.V. Schwartz, Enzyme replacement therapy for Fabry disease: a systematic review and meta-analysis, Genet. Mol. Biol. 35 (2012) 947–954.
[16] EMA, Summary of Product Characteristics (Replagal), 2008.

[17] EMA, Summary of product characteristics (Fabrazyme), 2001.
[18] R.J. Desnick, Enzyme replacement therapy for Fabry disease: lessons from two α-galactosidase a orphan products and one FDA approval, Expert. Opin. Biol. Ther. 4 (2004) 1167–1176.
[19] Sanofi, Highlights of prescribing Information (Fabrazyme), 2001. https://products.sanofi.us/fabrazyme/fabrazyme.pdf.
[20] E.H. McCafferty, L.J. Scott, Migalastat: a review in Fabry disease, Drugs. 79 (2019) 543–554.
[21] I. Beirão, A. Cabrita, M. Torres, F. Silva, P. Aguiar, F. Laranjeira, A.M. Gomes, Biomarkers and imaging findings of Anderson-Fabry disease-what we know now, Diseases. 5 (2017) 15.
[22] C. Auray-Blais, D. Cyr, A. Ntwari, M.L. West, J. Cox-Brinkman, D.G. Bichet, D.P. Germain, R. Laframboise, S.B. Melançon, T. Stockley, J.T. Clarke, R. Drouin, Urinary globotriaosylceramide excretion correlates with the genotype in children and adults with Fabry disease, Mol. Genet. Metab. 93 (2008) 331–340.
[23] R. Schiffmann, S. Waldek, A. Benigni, C. Auray-Blais, Biomarkers of Fabry disease nephropathy, Clin. J. Am. Soc. Nephrol. 5 (2010) 360–364.
[24] A. Nowak, T.P. Mechtler, R.J. Desnick, D.C. Kasper, Plasma LysoGb3: a useful biomarker for the diagnosis and treatment of Fabry disease heterozygotes, Mol. Genet. Metab. 120 (2017) 57–61.
[25] H. Sakuraba, T. Togawa, T. Tsukimura, H. Kato, Plasma lyso-Gb3: a biomarker for monitoring fabry patients during enzyme replacement therapy, Clin. Exp. Nephrol. 22 (2018) 843–849.
[26] T. Togawa, T. Kodama, T. Suzuki, K. Sugawara, T. Tsukimura, T. Ohashi, N. Ishige, K. Suzuki, T. Kitagawa, H. Sakuraba, Plasma globotriaosylsphingosine as a biomarker of Fabry disease, Mol. Genet. Metab. 100 (2010) 257–261.
[27] E. Young, K. Mills, P. Morris, A. Vellodi, P. Lee, S. Waldek, B. Winchester, Is globotriaosylceramide a useful biomarker in Fabry disease? Acta Paediatr. Suppl. 94 (2005) 51–54, https://doi.org/10.1080/08035320510028111.
[28] J.M. Aerts, J.E. Groener, S. Kuiper, W.E. Donker-Koopman, A. Strijland, R. Ottenhoff, C. van Roomen, M. Mirzaian, F.A. Wijburg, G.E. Linthorst, A.C. Vedder, S.M. Rombach, J. Cox-Brinkman, P. Somerharju, R.G. Boot, C.E. Hollak, R.O. Brady, B.J. Poorthuis, Elevated globotriaosylsphingosine is a hallmark of Fabry disease, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 2812–2817.
[29] I. Simonetta, A. Tuttolomondo, M. Daidone, A. Pinto, Biomarkers in Anderson-Fabry disease, Int. J. Mol. Sci. 21 (2020) 8080.
[30] H. Maruyama, K. Miyata, M. Mikame, A. Taguchi, C. Guili, M. Shimura, K. Murayama, T. Inoue, S. Yamamoto, K. Sugimura, K. Tamita, T. Kawasaki, J. Kajihara, A. Onishi, H. Sugiyama, T. Sakai, I. Murata, T. Oda, S. Toyoda, K. Hanawa, T. Fujimura, S. Ura, M. Matsumura, H. Takano, S. Yamashita, G. Matsukura, R. Tazawa, T. Shiga, M. Ebato, H. Satoh, S. Ishii, Effectiveness of plasma lyso-Gb3 as a biomarker for selecting high-risk patients with Fabry disease from multispecialty clinics for genetic analysis, Genet. Med. 21 (2019) 44–52.
[31] C. Auray-Blais, P. Lavoie, M. Boutin, A. Ntwari, T.R. Hsu, C.K. Huang, D.M. Niu, Biomarkers associated with clinical manifestations in Fabry disease patients with a late-onset cardiac variant mutation, Clin. Chim. Acta. 466 (2017) 185–193.
[32] J. Kramer, F. Weidemann, Biomarkers for diagnosing and staging of Fabry disease, Curr. Med. Chem. 25 (2018) 1530–1537.
[33] A. Nowak, T.P. Mechtler, T. Hornemann, J. Gawinecka, E. Theswet, M.J. Hilz, D.C. Kasper, Genotype, phenotype and disease severity reflected by serum LysoGb3 levels in patients with Fabry disease, Mol. Genet. Metab. 123 (2018) 148–153.
[34] M. Niemann, A. Rolfs, S. Störk, B. Bijnens, F. Breunig, M. Beer, G. Ertl, C. Wanner, F. Weidemann, Gene mutations versus clinically relevant phenotypes: lyso-Gb3 defines Fabry disease, Circ. Cardiovasc. Genet. 7 (2014) 8–16.
[35] B.E. Smid, L. van der Tol, M. Biegstraaten, G.E. Linthorst, C.E. Hollak, B.J. Poorthuis, Plasma globotriaosylsphingosine in relation to phenotypes of Fabry disease, J. Med. Genet. 52 (2015) 262–268.
[36] C.M. Eng, N. Guffon, W.R. Wilcox, D.P. Germain, P. Lee, S. Waldek, L. Caplan, G.E. Linthorst, R.J. Desnick, Safety and efficacy of recombinant human alpha-galactosidase a replacement therapy in Fabry's disease, N. Engl. J. Med. 345 (2001) 9–16.
[37] B.L. Thurberg, H. Rennke, R.B. Colvin, S. Dikman, R.E. Gordon, A.B. Collins, R.J. Desnick, M. O'Callaghan, Globotriaosylceramide accumulation in the Fabry kidney is cleared from multiple cell types after enzyme replacement therapy, Kidney. Int. 62 (2002) 1933–1946.
[38] US Food & Drug Administration, Table of Surrogate Endpoints that Were the Basis of Drug Approval or Licensure, 2021.
[39] M.C. Gubler, G. Lenoir, J.P. Grünfeld, A. Ulmann, D. Droz, R. Habib, Early renal changes in hemizygous and heterozygous patients with Fabry's disease, Kidney. Int. 13 (1978) 223–235.
[40] C. Tøndel, L. Bostad, A. Hirth, E. Svarstad, Renal biopsy findings in children and adolescents with Fabry disease and minimal albuminuria, Am. J. Kidney. Dis. 51 (2008) 767–776.
[41] J.C. Lubanda, E. Anijalg, V. Bzdúch, B.L. Thurberg, B. Bénichou, A. Tylki-Szymanska, Evaluation of a low dose, after a standard therapeutic dose, of agalsidase beta during enzyme replacement therapy in patients with Fabry disease, Genet. Med. 11 (2009) 256–264.
[42] A.B. Fogo, L. Bostad, E. Svarstad, W.J. Cook, S. Moll, F. Barbey, L. Geldenhuys, M. West, D. Ferluga, B. Vujkovac, A.J. Howie, A. Burns, R. Reeve, S. Waldek, L.H. Noël, J.P. Grünfeld, C. Valbuena, J.P. Oliveira, J. Müller, F. Breunig, X. Zhang, D.G. Warnock, Scoring system for renal pathology in Fabry disease: report of the international study Group of Fabry Nephropathy (ISGFN), Nephrol. Dial. Transplant. 25 (2010) 2168–2177.
[43] B. Najafian, E. Svarstad, L. Bostad, M.C. Gubler, C. Tøndel, C. Whitley, M. Mauer, Progressive podocyte injury and globotriaosylceramide (GL-3) accumulation in young patients with Fabry disease, Kidney. Int. 79 (2011) 663–670.
[44] M.C. Liebau, F. Braun, K. Höpker, C. Weitbrecht, V. Bartels, R.U. Müller, S. Brodesser, M.A. Saleem, T. Benzing, B. Schermer, M. Cybulla, C.E. Kurschat, Dysregulated autophagy contributes to podocyte damage in Fabry's disease, PLoS. One. 8 (2013), e63506.
[45] C. Tøndel, L. Bostad, K.K. Larsen, A. Hirth, B.E. Vikse, G. Houge, E. Svarstad, Agalsidase benefits renal histology in young patients with Fabry disease, J. Am. Soc. Nephrol. 24 (2013) 137–148.
[46] C. Tøndel, T. Kanai, K.K. Larsen, S. Ito, J.M. Politei, D.G. Warnock, E. Svarstad, Foot process effacement is an early marker of nephropathy in young classic Fabry patients without albuminuria, Nephron. 129 (2015) 16–21.
[47] F.A. Wijburg, B. Bénichou, D.G. Bichet, L.A. Clarke, G. Dostalova, A. Fainboim, A. Fellgiebel, C. Forcelini, K. An Haack, R.J. Hopkin, M. Mauer, B. Najafian, C.R. Scott, S.P. Shankar, B.L. Thurberg, C. Tøndel, A. Tylki-Szymańska, U. Ramaswami, Characterization of early disease status in treatment-naive male paediatric patients with Fabry disease enrolled in a randomized clinical trial, PLoS. One. 10 (2015), e0124987.
[48] B. Fall, C.R. Scott, M. Mauer, S. Shankland, J. Pippin, J.A. Jefferson, E. Wallace, D. Warnock, B. Najafian, Urinary podocyte loss is increased in patients with Fabry disease and correlates with clinical severity of Fabry nephropathy, PLoS. One. 11 (2016), e0168346.
[49] B. Najafian, C. Tøndel, E. Svarstad, A. Sokolovkiy, K. Smith, M. Mauer, One year of enzyme replacement therapy reduces Globotriaosylceramide inclusions in podocytes in male adult patients with Fabry disease, PLoS. One. 11 (2016), e0152812.
[50] C.M. Eng, M. Banikazemi, R.E. Gordon, M. Goldman, R. Phelps, L. Kim, A. Gass, J. Winston, S. Dikman, J.T. Fallon, S. Brodie, C.B. Stacy, D. Mehta, R. Parsons, K. Norton, M. O'Callaghan, R.J. Desnick, A phase 1/2 clinical trial of enzyme replacement in fabry disease: pharmacokinetic, substrate clearance, and safety studies, Am. J. Hum. Genet. 68 (2001) 711–722.
[51] R. Skrunes, E. Svarstad, K. Kampevold Larsen, S. Leh, C. Tøndel, Reaccumulation of globotriaosylceramide in podocytes after agalsidase dose reduction in young Fabry patients, Nephrol. Dial. Transplant. 32 (2017) 807–813.
[52] R. Skrunes, C. Tøndel, S. Leh, K.K. Larsen, G. Houge, E.S. Davidsen, C. Hollak, A.B.P. van Kuilenburg, F.M. Vaz, E. Svarstad, Long-term dose-dependent Agalsidase effects on kidney histology in Fabry disease, Clin. J. Am. Soc. Nephrol. 12 (2017) 1470–1479.
[53] E. Svarstad, S. Leh, R. Skrunes, K. Kampevold Larsen, Ø. Eikrem, C. Tøndel, Bedside stereomicroscopy of Fabry kidney biopsies: An easily available method for diagnosis and assessment of sphingolipid deposits, Nephron. 138 (2018) 13–21.
[54] J. Politei, V. Alberton, O. Amoreo, N. Antongiovanni, M.N. Arán, M. Barán, G. Cabrera, S. Di Pietrantonio, C. Durand, A. Fainboim, J. Frabasil, F.G. Pizarro, R. Iotti, M. Liern, F. Perretta, D. Ripeau, F. Toniolo, H. Trimarchi, D.V. Rivas, E. Wallace, A.B. Schenone, Clinical parameters, LysoGb3, podocyturia, and kidney biopsy in children with Fabry disease: is a correlation possible? Pediatr. Nephrol. 33 (2018) 2095–2101.
[55] B. Najafian, C. Tøndel, E. Svarstad, M.C. Gubler, J.P. Oliveira, M. Mauer, Accumulation of Globotriaosylceramide in podocytes in Fabry nephropathy is associated with progressive podocyte loss, J. Am. Soc. Nephrol. 31 (2020) 865–875.
[56] P.A. Rozenfeld, M. de Los Angeles, P. Bolla, A. Quieto, S. Pisani, P. Feriozzi, C. Bondar Neuman, Pathogenesis of Fabry nephropathy: the pathways leading to fibrosis, Mol. Genet. Metab. 129 (2020) 132–141.
[57] V.J. Ferrans, R.G. Hibbs, C.D. Burda, The heart in Fabry's disease. A histochemical and electron microscopic study, Am. J. Cardiol. 24 (1969) 95–110.
[58] M. Namdar, C. Gebhard, R. Studiger, Y. Shi, P. Mocharla, C. Schmied, P. Brugada, T.F. Lüscher, G.G. Camici, Globotriaosylsphingosine accumulation and not alpha-galactosidase-a deficiency causes endothelial dysfunction in Fabry disease, PLoS. One. 7 (2012), e36373.
[59] L. Shu, A. Vivekanandan-Giri, S. Pennathur, B.E. Smid, J.M. Aerts, C.E. Hollak, J.A. Shayman, Establishing 3-nitrotyrosine as a biomarker for the vasculopathy of Fabry disease, Kidney Int. 86 (2014) 58–66.
[60] N. Gadoth, U. Sandbank, Involvement of dorsal root ganglia in Fabry's disease, J. Med. Genet. 20 (1983) 309–312.
[61] P. Kahn, Anderson-Fabry disease: a histopathological study of three cases with observations on the mechanism of production of pain, J. Neurol. Neurosurg. Psychiatry. 36 (1973) 1053–1062.
[62] D.P. Germain, P. Avan, A. Chassaing, P. Bonfils, Patients affected with Fabry disease have an increased incidence of progressive hearing loss and sudden deafness: an investigation of twenty-two hemizygous male patients, BMC Med. Genet. 3 (2002) 10.
[63] P.A. Schachern, D.A. Shea, M.M. Paparella, T.H. Yoon, Otologic histopathology of Fabry's disease, Ann. Otol. Rhinol. Laryngol. 98 (1989) 359–363.
[64] N. Üçeyler, N. Schröter, W. Kafke, D. Kramer, C. Wanner, F. Weidemann, C. Sommer, Skin Globotriaosylceramide 3 load is increased in men with advanced Fabry disease, PLoS. One. 11 (2016), e0166484.
[65] U. Ramaswami, D.G. Bichet, L.A. Clarke, G. Dostalova, A. Fainboim, A. Fellgiebel, C.M. Forcelini, K. An Haack, R.J. Hopkin, M. Mauer, B. Najafian, C.R. Scott, S.P. Shankar, B.L. Thurberg, C. Tøndel, A. Tylki-Szymanska, B. Bénichou, F.A. Wijburg, Low-dose agalsidase beta treatment in male pediatric patients with Fabry disease: a 5-year randomized controlled trial, Mol. Genet. Metab. 127 (2019) 86–94.
[66] J. Cox-Brinkman, A. Vedder, C. Hollak, L. Richfield, A. Mehta, K. Orteu, F. Wijburg, P. Hammond, Three-dimensional face shape in Fabry disease, Eur. J. Hum. Genet. 15 (2007) 535–542.
[67] P.N. De Francesco, J.M. Mucci, R. Ceci, C.A. Fossati, P.A. Rozenfeld, Fabry disease peripheral blood immune cells release inflammatory cytokines: role of globotriaosylceramide, Mol. Genet. Metab. 109 (2013) 93–99.

[68] J. Politei, C. Durand, A.B. Schenone, A. Torres, J. Mukdsi, B.L., Thurberg, chronic intestinal pseudo-obstruction. Did you search for lysosomal storage diseases? Mol. Genet. Metab. Rep. 11 (2017) 8–11.
[69] G. Quinn, A. Abedini, H. Liu, Z. Ma, A. Cucchiara, A. Havasi, J. Hill, M. Palmer, K. Susztak, Renal histologic analysis provides complementary information to kidney function measurement for patients with early diabetic or hypertensive disease, J. Am. Soc. Nephrol. 32 (2021) 2863–2876.
[70] W.R. Wilcox, J.P. Oliveira, R.J. Hopkin, A. Ortiz, M. Banikazemi, U. Feldt-Rasmussen, K. Sims, S. Waldek, G.M. Pastores, P. Lee, C.M. Eng, L. Marodi, K.E. Stanford, F. Breunig, C. Wanner, D.G. Warnock, R.M. Lemay, D.P. Germain, Females with Fabry disease frequently have major organ involvement: lessons from the Fabry registry, Mol. Genet. Metab. 93 (2008) 112–128.
[71] B. Najafian, A. Silvestroni, A. Sokolovskiy, C. Tøndel, E. Svarstad, B. Obrisca, G. Ismail, M.D. Holida, M. Mauer, A novel unbiased method reveals progressive podocyte globotriaosylceramide accumulation and loss with age in females with Fabry disease, Kidney. Int. 102 (2022) 173–182.
[72] C.E. Kurschat, Fabry disease-what cardiologists can learn from the nephrologist: a narrative review, Cardiovasc. Diagn. Ther. 11 (2021) 672–682.
[73] J. Kleinert, F. Dehout, A. Schwarting, A.G. de Lorenzo, R. Ricci, C. Kampmann, M. Beck, U. Ramaswami, A. Linhart, A. Gal, G. Houge, U. Widmer, A. Mehta, G. Sunder-Plassmann, Prevalence of uncontrolled hypertension in patients with Fabry disease, Am. J. Hypertens. 19 (2006) 782–787.
[74] D.P. Germain, S. Waldek, M. Banikazemi, D.A. Bushinsky, J. Charrow, R.J. Desnick, P. Lee, T. Loew, A.C. Vedder, R. Abichandani, W.R. Wilcox, N. Guffon, Sustained, long-term renal stabilization after 54 months of agalsidase beta therapy in patients with Fabry disease, J. Am. Soc. Nephrol. 18 (2007) 1547–1557.
[75] S.J. Shankland, J.W. Pippin, J.S. Duffield, Progenitor cells and podocyte regeneration, Semin. Nephrol. 34 (2014) 418–428.
[76] A. Linhart, P.M. Elliott, The heart in Anderson-Fabry disease and other lysosomal storage disorders, Heart. 93 (2007) 528–535.
[77] T. Kubo, Fabry disease and its cardiac involvement, J. Gen. Fam. Med. 18 (2017) 225–229.
[78] S. Nakao, T. Takenaka, M. Maeda, C. Kodama, A. Tanaka, M. Tahara, A. Yoshida, M. Kuriyama, H. Hayashibe, H. Sakuraba, et al., An atypical variant of Fabry's disease in men with left ventricular hypertrophy, N. Engl. J. Med. 333 (1995) 288–293.
[79] S. Baig, R. Vijapurapu, F. Alharbi, S. Nordin, R. Kozor, J. Moon, B. Bembi, T. Geberhiwot, R.P. Steeds, Diagnosis and treatment of the cardiovascular consequences of Fabry disease, QJM. 112 (2019) 3–9.
[80] B.L. Thurberg, J.T. Fallon, R. Mitchell, T. Aretz, R.E. Gordon, M.W. O'Callaghan, Cardiac microvascular pathology in Fabry disease: evaluation of endomyocardial biopsies before and after enzyme replacement therapy, Circulation. 119 (2009) 2561–2567.
[81] S. Feriozzi, A. Linhart, U. Ramaswami, V. Kalampoki, A. Gurevich, D. Hughes, Effects of baseline left ventricular hypertrophy and decreased renal function on cardiovascular and renal outcomes in patients with Fabry disease treated with Agalsidase alfa: a Fabry outcome survey study, Clin. Ther. 42 (2020) 2321–2330.e2320.
[82] A. Burlina, J. Politei, The Central Nervous System Involvement in Fabry Disease:A Review, J. Inborn. Errors. Metab. Screen. 4 (2016).
[83] K. Sims, J. Politei, M. Banikazemi, P. Lee, Stroke in Fabry disease frequently occurs before diagnosis and in the absence of other clinical events: natural history data from the Fabry registry, Stroke. 40 (2009) 788–794.
[84] J.J. Miller, K. Aoki, F. Moehring, C.A. Murphy, C.L. O'Hara, M. Tiemeyer, C.L. Stucky, N.M. Dahms, Neuropathic pain in a Fabry disease rat model, JCI Insight. 3 (2018), e99171.
[85] J. Marshall, K.M. Ashe, D. Bangari, K. McEachern, W.L. Chuang, J. Pacheco, D.P. Copeland, R.J. Desnick, J.A. Shayman, R.K. Scheule, S.H. Cheng, Substrate reduction augments the efficacy of enzyme therapy in a mouse model of Fabry disease, PLoS. One. 5 (2010), e15033.
[86] E. Kolodny, A. Fellgiebel, M.J. Hilz, K. Sims, P. Caruso, T.G. Phan, J. Politei, R. Manara, A. Burlina, Cerebrovascular involvement in Fabry disease: current status of knowledge, Stroke. 46 (2015) 302–313.
[87] R. Schiffmann, D.F. Moore, Neurological Manifestations of Fabry Disease, Oxford PharmaGenesis, Oxford, 2006.
[88] J.S. Shen, X.L. Meng, D.F. Moore, J.M. Quirk, J.A. Shayman, R. Schiffmann, C.R. Kaneski, Globotriaosylceramide induces oxidative stress and up-regulates cell adhesion molecule expression in Fabry disease endothelial cells, Mol. Genet. Metab. 95 (2008) 163–168.
[89] A.P. Burlina, K.B. Sims, J.M. Politei, G.J. Bennett, R. Baron, C. Sommer, A.T. Møller, M.J. Hilz, Early diagnosis of peripheral nervous system involvement in Fabry disease and treatment of neuropathic pain: the report of an expert panel, BMC Neurol. 11 (2011) 61.
[90] J.M. Politei, C. Durand, A.B. Schenone, Small Fiber neuropathy in Fabry disease: a review of pathophysiology and treatment, J. Inborn. Errors. Metab. Screen. 4 (2016).
[91] E. Suntjens, W.A. Dreschler, J. Hess-Erga, R. Skrunes, F.A. Wijburg, G.E. Linthorst, C. Tøndel, M. Biegstraaten, Hearing loss in children with Fabry disease, J. Inherit. Metab. Dis. 40 (2017) 725–731.
[92] O. Lidove, R. Jaussaud, S. Aractingi, Dermatological and soft-tissue manifestations of Fabry disease: Characteristics and response to enzyme replacement therapy, in: A. Mehta, M. Beck, G. Sunder-Plassmann (Eds.), Fabry Disease: Perspectives from 5 Years of FOS, Oxford PharmaGenesis. Copyright © 2006, Oxford PharmaGenesisTM, Oxford, 2006.
[93] C.H. Orteu, T. Jansen, O. Lidove, R. Jaussaud, D.A. Hughes, G. Pintos-Morell, U. Ramaswami, R. Parini, G. Sunder-Plassman, M. Beck, A.B. Mehta, Fabry disease and the skin: data from FOS, the Fabry outcome survey, Br. J. Dermatol. 157 (2007) 331–337.
[94] B.L. Thurberg, H. Randolph Byers, S.R. Granter, R.G. Phelps, R.E. Gordon, M. O'Callaghan, Monitoring the 3-year efficacy of enzyme replacement therapy in fabry disease by repeated skin biopsies, J. Invest. Dermatol. 122 (2004) 900–908.
[95] J.J. Miller, K. Aoki, C.A. Mascari, A.K. Beltrame, O. Sokumbi, P.E. North, M. Tiemeyer, A.J. Kriegel, N.M. Dahms, α-Galactosidase A-deficient rats accumulate glycosphingolipids and develop cardiorenal phenotypes of Fabry disease, FASEB J. 33 (2019) 418–429.
[96] P.C. Luna, P. Boggio, M. Larralde, Dermatologic Aspects of Fabry Disease, 4, 2016 2326409816661353.
[97] W.R. Wilcox, U. Feldt-Rasmussen, A.M. Martins, A. Ortiz, R.M. Lemay, A. Jovanovic, D.P. Germain, C. Varas, K. Nicholls, F. Weidemann, R.J. Hopkin, Improvement of Fabry disease-related gastrointestinal symptoms in a significant proportion of female patients treated with Agalsidase Beta: data from the Fabry registry, JIMD Rep. 38 (2018) 45–51.
[98] A.J. Burand Jr., C.L. Stucky, Fabry disease pain: patient and preclinical parallels, Pain. 162 (2021) 1305–1321.
[99] C.E. Argoff, N.W. Barton, R.O. Brady, H.A. Ziessman, Gastrointestinal symptoms and delayed gastric emptying in Fabry's disease: response to metoclopramide, Nucl. Med. Commun. 19 (1998) 887–891.
[100] B. Hoffmann, S. Keshav, Gastrointestinal symptoms in Fabry disease: everything is possible, including treatment, Acta. Paediatr. 96 (2007) 84–86.
[101] P. Buda, J. Książyk, A. Tylki-Szymanska, Gastroenterological complications of Anderson-Fabry disease, Curr. Pharm. Des. 19 (2013) 6009–6013.
[102] A. Onishi, P.J. Dyck, Loss of small peripheral sensory neurons in Fabry disease. Histologic and morphometric evaluation of cutaneous nerves, spinal ganglia, and posterior columns, Arch. Neurol. 31 (1974) 120–127.
[103] T. Tabira, I. Goto, Y. Kuroiwa, M. Kikuchi, Neuropathological and biochemical studies in Fabry's disease, Acta. Neuropathol. 30 (1974) 345–354.
[104] C.A. Stamerra, R. Del Pinto, P. di Giosia, C. Ferri, A. Sahebkar, Anderson-Fabry disease: from endothelial dysfunction to emerging therapies, Adv. Pharmacol. Pharm. Sci. 2021 (2021) 5548445.
[105] S. Choi, J.A. Kim, H.-Y. Na, S.-E. Cho, S. Park, S.-C. Jung, S.H. Suh, Globotriaosylceramide induces lysosomal degradation of endothelial KCa3.1 in Fabry disease, Arterioscler. Thromb. Vasc. Biol. 34 (2014) 81–89.
[106] P.N. De Francesco, J.M. Mucci, R. Ceci, C.A. Fossati, P.A. Rozenfeld, Higher apoptotic state in Fabry disease peripheral blood mononuclear cells.: effect of globotriaosylceramide, Mol. Genet. Metab. 104 (2011) 319–324.
[107] H. Sakuraba, T. Igarashi, T. Shibata, Y. Suzuki, Effect of vitamin E and ticlopidine on platelet aggregation in Fabry's disease, Clin. Genet. 31 (1987) 349–354.
[108] T. DeGraba, S. Azhar, F. Dignat-George, E. Brown, B. Boutière, G. Altarescu, R. McCarron, R. Schiffmann, Profile of endothelial and leukocyte activation in Fabry patients, Ann. Neurol. 47 (2000) 229–233.
[109] W.R. Wilcox, M. Banikazemi, N. Guffon, S. Waldek, P. Lee, G.E. Linthorst, R.J. Desnick, D.P. Germain, Long-term safety and efficacy of enzyme replacement therapy for Fabry disease, Am. J. Hum. Genet. 75 (2004) 65–74.
[110] M. Banikazemi, J. Bultas, S. Waldek, W.R. Wilcox, C.B. Whitley, M. McDonald, R. Finkel, S. Packman, D.G. Bichet, D.G. Warnock, R.J. Desnick, Agalsidase-beta therapy for advanced Fabry disease: a randomized trial, Ann. Intern. Med. 146 (2007) 77–86.
[111] J.E. Wraith, A. Tylki-Szymanska, N. Guffon, Y.H. Lien, M. Tsimaratos, A. Vellodi, D.P. Germain, Safety and efficacy of enzyme replacement therapy with agalsidase beta: an international, open-label study in pediatric patients with Fabry disease, J. Pediatr. 152 (2008) 563-570, 570.e561.
[112] D.P. Germain, J. Charrow, R.J. Desnick, N. Guffon, J. Kempf, R.H. Lachmann, R. Lemay, G.E. Linthorst, S. Packman, C.R. Scott, S. Waldek, D.G. Warnock, N.J. Weinreb, W.R. Wilcox, Ten-year outcome of enzyme replacement therapy with agalsidase beta in patients with Fabry disease, J. Med. Genet. 52 (2015) 353–358.
[113] K. Hongo, T. Harada, E. Fukuro, M. Kobayashi, T. Ohashi, Y. Eto, Massive accumulation of globotriaosylceramide in various tissues from a Fabry patient with a high antibody titer against alpha-galactosidase a after 6 years of enzyme replacement therapy, Mol. Genet. Metab. Rep. 24 (2020), 100623.
[114] A. Ortiz, S. Kanters, A. Hamed, P. DasMahapatra, E. Poggio, M. Maski, M. Aguiar, E. Ponce, J.P. Jansen, D. Ayers, R. Goldgrub, R.J. Desnick, Agalsidase beta treatment slows estimated glomerular filtration rate loss in classic Fabry disease patients: results from an individual patient data meta-analysis, Clin. Kidney. J. 14 (2021) 1136–1146.
[115] J.L. Batista, M. Maski, S. Richards, B. Gudivada, E. Ponce, R.J. Desnick, Overall slowing of decline in kidney function and reduction in severe clinical events in Fabry disease patients treated with agalsidase beta: a matched analysis, Presented at 18th Annual WORLDSymposium, February 7–11, 2022; Virtual congress, 2022 , LB-07.