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Fabry Disease Nephropathy: Histological Changes With Non-Classical Mutations and Genetic Variants of Unknown Significance

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ABSTRACT

Rationale & Objective: Fabry disease (FD) is an X-linked genetic disorder that causes lysosomal storage of glycosphingolipids, primarily globotryaosilceramide (Gb3) and its derivative globotryaosilsphingosine (Lyso-Gb3), with multiorgan dysfunction including chronic kidney disease. Afflicted individuals may be carriers of gene variants that are of uncertain significance (GVUS). We described kidney pathology at early-stages of FD-related kidney disease to gain insights into their association with GVUS and sex.

Study design: Single-center, case series.

Setting & Participants: Thirty-five consecutively biopsied patients (aged 48.1±15.4 years, 22 females) from among 64 patients, with genetically diagnosed FD. Patients were retrospectively screened using the International Study Group of Fabry Nephropathy Scoring System.

Observations: Genetic mutation type, p.N215S and D313Y, sex, age, eGFR (estimated glomerular filtration rate), plasma Lyso-Gb3 (pLyso-Gb3) levels, and histological parameters, including deposition of Gb3 were recorded. Genetic analyses showed mostly missense mutations, with the p.N215S variant in 15 and the benign polymorphism D313Y in 4 of the biopsied patients. Morphological lesions were similar for men and women except for interstitial fibrosis and arteriolar hyalinosis being more common in men. Even early in their clinical course, patients with normal/mild albuminuria, podocyte, tubular, peritubular capillary vacuoles/inclusions, and chronicity parameters, i.e., glomerulosclerosis, interstitial fibrosis, tubular atrophy, were present and were associatated with pLyso-Gb3, eGFR and age.

Limitations: Retrospective design and inclusion of outpatients partially based on family pedigree.

Conclusions: In early stages of kidney disease in the setting of FD, numerous histological abnormalities are apparent. These observations suggest that early kidney biopsies may reveal activity of kidney involvement and may inform clinical management.

Keywords: Fabry disease; Fabry nephropathy; α-GAL A; Lysosomal storage disease; Chronic kidney disease; Kidney biopsy; Podocyte vacuoles; Glomerulosclerosis; Interstitial fibrosis; Proteinuria; Enzymatic replacement therapy.

INTRODUCTION

Fabry disease (FD) is an X-linked inborn error of metabolism, due to mutations in the *GLA* gene encoding α-galactosidase A (α-GLA A). Defective α-GLA A activity leads to progressive lysosomal storage of glycosphingolipids, primarily globotriaosylceramide (Gb3) and its deacylated form globotriaosylsphingosine (Lyso-Gb3), in various cell types and body fluids [1]. Although Gb3 storage starts during fetal development [2], affected children are commonly asymptomatic while evolving to target organ failure as Gb3 deposits accumulate [3]

Kidney involvement advancing to severe kidney failure (CKD G5) [4] is associated with morbidity [5] and is a common cause of premature death. About 40% of adult women with proteinuria have a risk of premature death at the same median age as in men. [6] Thus the increased risk of mortality may be present even before kidney failure. The reported prevalence of chronic hemodialysis is 0.33% in men and 0.1% in women. [7, 8]

Early-stage FD nephropathy is often clinically silent. Depending on age, sex and genetics, clinical and laboratory markers may be elusive, making early diagnosis difficult, especially in heterozygous women. [9, 10]

Conversely, in later stages, chronic histological lesions, mainly glomerulosclerosis, interstitial fibrosis and arteriosclerosis are found in both sexes. [8]

In 2010, the International Study Group of Fabry Nephropathy (ISGFN) introduced a standardized scoring system for FD nephropathy and established that both men and women have early specific morphological findings, even in the absence of clinical signs and symptoms, [11]. Fogo and Tøndel's findings suggest that baseline biopsies could contribute to FD management optimization. [11, 12]

Although current guidelines (https://garrod.ca/wp-content/uploads/2020/02/Canadian-Fabry-Treatment-Guidelines-2019-final.pdf) strongly recommend biopsy of clinically involved organs, kidney biopsy typically occurs only when the disease is advanced, at about 11 years from clinical

onset [13]. This diagnostic delay considerably reduces therapeutic effectiveness and leads to worse outcomes. [14]

The present single-center retrospective case series included patients with genetically confirmed FD. The aim was to describe the kidney pathology at early-stages of FD related CKD to gain insights into their association with gene variants and sex.

METHODS

Study design and patients

From 2012 to 2020, 64 outpatients or proband patients (12%) were identified through family pedigree analysis (drawn from 21 families) performed at the Nephrological Rare Disease Clinic of the University Hospital of Bologna. These cases consisted of 26 men (40.6%) aged 45.4 ± 19.0 Table-S1 describes their main demographic and clinical characteristics by biopsy status and collected at the time of admission.

Kidney biopsy was performed in 35 patients who met the following criteria: (1) carriers of classic causal *GLA* gene mutations, gene variants of uncertain significance (GVUS), or *de novo* mutations; (2) CKD stage 1 or 2 (n=30) or 3 or above (n=5); (3) atypical FD nephropathy clinical course for suspected overlapping disease, e.g., IgA nephropathy, crescentic glomerulonephritis, multiple myeloma.

Kidney biopsies were performed, with patient consent in accordance with current clinical practice (real-time ultrasound-guided percutaneous biopsies using an automated spring-loaded biopsy device). The procedures were straightforward with few complications (post-biopsy bleeding, hematuria and minor perirenal hematomas). [15]

Cardiac biopsy was recommended [16] when cardiac involvement is suspected on the basis of clinical symptoms, ECG or echocardiography abnormalities (e.g. dysrhythmias or left ventricular hypertrophy, respectively), or specific MRI alterations (late gadolinium enhancement and abnormal myocardial T1 relaxation time) [16]

Cardiac biopsy was performed on four patients. Via right heart catheterization, a flexible endomyocardial bioptome was inserted into the right ventricle and 2-3 specimens were obtained

from the interventricular septum and submitted to histological examination. [17] The samples were observed under light microscopy (LM) and transmission electron microscopy (TEM). Immunohistochemistry with Gb3 antibodies was used to visualize deposits site and diffusion. No post-procedure complications were reported.

The study was approved by the institutional Ethics Committee (ref. 918/2021/Oss/AOUBo, 20/10/2021) and conducted in accordance with the declaration of Helsinki.

Genetic, clinical and laboratory data

Genetic analysis was carried out on dried blood spot filter paper by means of next generation sequencing (NGS) or multiplex ligation-dependent probe amplification (MLPA), as previously described. [18, 19]

The eGFR was computed using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula, [20] validated in patients with FD. [21] CKD was categorized into stages according to the National Kidney Foundation criteria. [22] [23]. We also recorded measured 24-hour creatinine clearance (mCrClearance), calculated in all patients using the standard equation (U_{Cr}*U_{vol}/P_{Cr}*t). Microalbuminuria and 24-hour urine protein excretion were determined through a turbidimetric method. Urinary albumin-to-creatinine ratio (UACR) was assessed using nephelometry. Microalbuminuria and UACR were measured in overnight urine samples. Plasma Lyso-Gb3 (pLysoGb3) levels were determined by tandem mass spectrometry.

Histological and ultrastructural analysis (in Item S1)

Routine histological and ultrastructural processing pipelines for kidney biopsies were utilized for specimen preparation. Histological scoring was done according to ISGFN guidelines [11]. LM of Formalin-Fixed Paraffin-Embedded sections stained with Periodic Acid-Schiff (Figure 1A) and Acid Fuchsin Orange G (Figure 1B) showed empty vacuoles in podocytes, quantitatively expressed by the mean podocyte score (MPS). Toluidine blue-stained semithin sections of resin embedded kidney tissue showed inclusions in podocytes (Figure 2A), tubular epithelial cells,

vascular smooth muscle cells (Figure 2B), and endothelial cells of peritubular capillaries (Figure 2C). TEM detection of Zebra bodies provided a detailed map of the involvement of the different renal cortex compartments (Figure 3A-E). Chronicity changes were scored on LM using the semiquantitative scale and methodology reported in the Supplementary Material (S1).

Statistical analysis (in Item S2)

Data were retrieved from the clinical records closest in time to patients' first consultations. The relationships between histological parameters and clinical characteristics were tested with bivariate tests or Spearman correlation, and confirmed using nonparametric kernel regression with 500 bootstrap replications (for not normally distributed histological parameters) or logistic regression (for histological parameters expressed as absence/presence). In these regressions, sex was included as the exposure and age as potential confounder. The association between CKD and LM scores was evaluated by a nonparametric trend analysis, and tested in an age-adjusted linear regression of eGFR.

RESULTS

Patient characteristics

Genetic analysis identified missense mutations in 43/64 patients (67.2%), making these the most frequent *GLA* mutation type (Table S2). In two patients from one family (mother and son), a *de novo* mutation, c.-265G>A, was identified by mRNA *GLA* gene analysis on fibroblast cultures from skin biopsy and by MLPA on blood samples.

The biopsied (n=35) and non-biopsied (n=29) patients had similar demographic and clinical features (Table S1). Among biopsied patients, women had significantly lower serum creatinine, pLysoGb3 and microalbuminuria values, higher mCrClearance (Table 1). The majority of cases had mild nephropathy. Specifically, 20 were in CKD stage 1(57.1%; 6 men, 14 women; age 41.6 ± 13.6 years), 10 in CKD stage 2 (28.6%; 5 men, 5 women; age 56.8 ± 14.8 years), and five in CKD stages 3-5 (14.3%; 4 men, 1 woman; age 60.8 ± 3.8 years).

Relationships of histological findings with sex, clinical parameters and CKD stage

Kidney biopsy revealed FD nephropathy in all 35 patients. No significant sex differences were observed in MPS scoring (Table 2). Men showed a significantly higher frequency of interstitial fibrosis (p=0.02) and arteriolar hyalinosis (p=0.02) (Table 2).

The MPS was significantly associated with inclusions at all sites, except proximal tubules (Table 3). Patients with inclusions tended to be younger and have higher eGFR, but the age difference only reached statistical difference for proximal tubular inclusions (p=0.01).

Table 4 shows chronicity markers in biopsied FD patients stratified by CKD stage. LM revealed significantly lower percentages of global sclerosis, interstitial fibrosis, arterial sclerosis, and arteriolar hyalinosis in patients with milder kidney impairment (stages 1 and 2 versus stage 3 or above).

Baseline eGFR was associated with histological parameters of chronicity after adjusting for age (Table 5). For example, eGFR was found to decrease by 0.27 mL/min/1.73 m^2 for each 1% increase in global glomerulosclerosis, by 1.03 mL/min/1.73 m^2 for each 1% increase in interstitial fibrosis, and by 10.70 mL/min/1.73 m^2 for each 1-point increase in the average arterial sclerosis score.

Histological chronicity parameters were correlated with each other and with several clinical variables in both sexes (Table 6A-C). In this case series, global sclerosis and tubulointerstitial fibrosis correlated positively with age at biopsy and with serum creatinine levels, and negatively with eGFR.

Finally, regressions of histological parameters by sex adjusted for age (Table 7) revealed that male sex was associated with an increased risk of peritubular capillary inclusions (OR=9.28; 95%CI 1.24-69.18, p=0.03), arteriolar hyalinosis (OR=9.78; 95%CI 1.26-79.95, p=0.03), and interstitial fibrosis (OR=9.50; 95% CI 2.53-18.27, p=0.02). However, these estimates should be interpreted with caution given their wide confidence intervals.

A novel aspect of the present study is the inclusion of GVUS carriers among biopsied patients.

Notably, 20/43 (46.5%) carried the p.N215S mutation, classified as later-onset cardiac variant [24]. Kidney biopsy was performed in 15 p.N215S carriers (M5/F10), revealing FD nephropathy. Under LM, all 15 biopsied p.N215S carriers (Table 8) had varying degrees of specific morphological lesions, namely vacuolation in podocytes (14/15, mild/moderate and severe in 2 patients), vascular compartments and peri-glomerular Bowman capsule. Podocyte hypertrophy, foot process effacement and zebra bodies (15/15) were demonstrated by TEM (Figure 4A-4B). Semithin sections confirmed FD nephropathy, highlighting inclusions in the parietal epithelium (6/15) and distal tubule epithelium (3/15). (Table 8) Applying ISGFN scoring system, we estimated in both sexes MPS (F 0.91/M 1%), TI (tubule-interstitial fibrosis) (F 7.5/M 11.2%), GS (global glomerulosclerosis) moderate-severe in F5/M5. In conclusion, histological FD nephropathy was present in all our patients with p.N215S.

Four asymptomatic patients (2 M, 74/72 years, 2 F 40/43 years) underwent cardiac biopsy due to MRI signs of cardiomyopathy. The two women's cardiac biopsy confirmed FD heart involvement with histological samples highlighting sarcoplasmatic vacuoles, mild focal subendocardic fibrosis, lipofuscin deposits in sarcolemma, arterio-arteriolar sclerosis/hyalinosis, and zebra bodies in myocardiocytes. Myocardiocyte vacuoles showed focal reactivity on immunohistochemistry with anti-Gb3 antibodies. In men, cardiac biopsy revealed many larger vacuoles and zebra bodies in myocardiocytes, mild-severe interstitial fibrosis, and strong positivity to Gb3 antibodies.

As in the classical form, ocular pathology was manifested in 16/20 patients, 6 of whom had cornea verticillata. Cornea verticillata, acroparesthesia and gastrointestinal symptoms were common elements reminiscent of a classic form. In non-classic FD form, cornea verticillata could suggest mutation pathogenicity in non-classical FD. [25]

Four patients (2M/2F) showed D313Y variant c.937G>T. Although D313Y is classified as a benign polymorphism, clinical and histological findings in these patients showed kidney FD

involvement (Table 9). All were carriers of variant c.937G>T and had low levels of α-GAL A (normal range >= 15.3 μmol/l/h)

Among these cases with D313Y polymorphism, two had FD nephropathy, one refused biopsy, and one presented superimposed monoclonal immune-deposition disease (MIDD) and histological features confounded by coexisting kidney disease [26]. As documented in Figures 5A-5B, biopsied D313Y patients presented pyknotic mitochondria (including the MIDD patient), endothelial activation, small and large vacuoles especially in podocytes and tubules, microvacuolization containing multi-lipidic droplets and lipofuscins, arteriosclerosis, and arteriolar hyalinosis. Pyknotic mitochondria was also observed with p.N215S carriers, and deserves to be further explored (Table 8).

DISCUSSION

Despite the availability and improvement of genetic testing and tailored therapies, kidney biopsy remains controversial in FD management, even though this procedure is recommended by authoritative sources [27, 28]. Biopsies of the kidney and other involved organs are rarely routinely performed in FD, especially in early stages, in women, and in GVUS carriers.

Our study is likely amongst the largest systematic kidney biopsy descriptions in FD, with a high percentage of heterozygous women (62.9%).

Only Fogo's multicenter (11 centers) study [11] had a larger cohort (35 males and 24 females) including five men carrying p.N215S mutation. Clinical data from their biopsied patients were very similar to those recorded in our case series, highlighting mostly mild disease: average Cr 1.3 mg/dl, CKD stage 1-2 in 78% and nearly half of women, UACR in the normal range or mildly increased in both sexes.

Furthermore, their scoring system revealed podocytes and tubular vacuoles and inclusion bodies. Chronicity lesions preceded clinical signs even in patients without albuminuria and proteinuria.

Our studies share histopathological similarities, such as atherosclerotic lesions of peritubular capillaries and vasculature mostly in men, and arteriolar hyalinosis in women. These corroborated biopsy findings expand previous reported histological features [29]

Important novelties in our study are the mutation pathogenicity and FD nephropathy progression in later-onset GVUS . New *GLA* mutations are increasing, with about 1000 to date recorded in the Human Gene Database (www.hgmd.org). The KDIGO 2017 Controversies Conference [28] highlighted the need to "elucidate the role of GVUS and investigate potential genotype-phenotype relations" for clinical assessment and histopathology disclosure. Similarly, Houge et al. [30] remarked that a "new general model for an improvement of genotype-phenotype correlations focusing on GVUS has recently been proposed and needs further validation".

GVUS induced FD was found in all of our biopsied p.N215S patients (15/15) and putatively in 3/4 D313Y carriers. Morphological features deserve to be further explored, and the specific association of some of them with genetic and clinical aspects support a genotype-phenotype correlation. [31]

The p.N215S mutation has been associated with a later-onset cardiac phenotype with cardiomyopathy as severe as in classic FD. Three studies reported clinical findings in cohorts of patients with this mutation, but lacked detailed nephrological data. [32,33,34].

A further study [35] analyzed phenotype data from the largest sample of p.N215S patients (66 women, 59 men) enrolled in the Fabry Registry and confirmed cardiac mutation pathogenicity. Kidney involvement occurred in 17% of p.N215S men (mostly aged 65-74 years), and rarely in women (3%). p.N215S patients' mean eGFR values remained >60 ml/min/1.73 m^2 in both men and women, for all age groups except men aged 65-74 years [mean eGFR (*SD*) 54.1 (21.5) ml/min/1.73 m^2]; five patients (3 women) required dialysis treatment.

No cardiac or kidney biopsy data from p.N215S patients were available in the Fabry Registry.

The four cases of p.N215S carriers who underwent kidney and heart biopsies confirmed involvement of both organs. LM observations and semithin sections revealed asymmetrical distribution within the glomerulus of the inclusions, were always present in podocytes and not

observed in mesangial cells. In other reports, mesangial cells are described as one of the sites of accumulation of specific inclusions, but we were not able to confirm this finding. Thus, our observation supports previous evidence that later-onset FD gene variants lack endothelial and mesangial inclusions. [36] Hsu et al. [37] made a similar observation when examining endomyocardial biopsies in a novel later-onset Fabry variant (IVS4 + 919G > A), suggesting that this mutation is a true pathogenic mutation. We can only speculate about the mechanism of the asymmetrical distribution in mesangial cells, as a different cell turnover rate, different capacity to break down Gb3 deposits, or other as-yet unknown mechanism could be implicated.

Cardiac biopsy of classical FD patients revealed significant Gb3 accumulation in endothelium and cardiomyocytes. The degree of endothelial Gb3 deposition has been used as a scoring system for monitoring long-term cardiac therapeutic outcomes of enzyme replacement therpayin classic FD. [27]. Cardiac biopsy of later-onset patients revealed that Gb3 accumulation was confined to the myocardiocytes and did not involve endothelial cells [38], as described in other reports [39–40] and confirmed in the IVS4 + 919G > A variant. [37] Myocardiocyte vacuoles showed focal reactivity on immunohistochemistry with anti-Gb3 antibodies. In men, cardiac biopsy revealed many larger vacuoles and zebra bodies in myocardiocytes, mild-severe interstitial fibrosis, and strong positivity to Gb3 antibodies. Among p.N215S carriers, cardiac involvement may become as severe as in classic Fabry patients, especially in adult men.

Recently, later-onset FD phenotypes were 10 times more frequent in most populations. [41] Given their characteristics, the term “later-onset” FD might indeed be more appropriate than “variant” FD. Kidney or cardiac biopsy can identify pathogenicity of novel mutation. Cardiac manifestations are the main cause of death, carrying the highest prognostic impact. It would be appropriate in these patients with early biopsy-proven double organ involvement to have an early therapeutic approach.

Table 9 shows clinical, laboratory and histological data of our D313Y cases carried C.937G<T mutation, the same variant reported in 17/62 patients in a large polycentric study in Greece [42]

in which D313Y pathogenicity was confirmed by kidney biopsy. In that study, the mutation was detected in 7M/10F patients, but in no healthy patient. Five patients had CKD-G5 treated with dialysis, one not specified CKD, and one adaptive focal and segmental glomerulosclerosis due to FD nephropathy.

The D313Y mutation seems to be related to a later-onset, milder phenotype with normal/high α-GLA A levels and classical clinical symptoms, such as acroparesthesia, cornea verticillata, gastro-intestinal involvement, and hearing loss.

Four new D313Y variants recently identified [42,43], namely c.835C>T, c.280T>A, c.924A>C and c.511G>A, are recognized as novel *GLA* mutations causing FD, with all affected patients (4/4) showing CKD associated with biopsy-proven FD nephropathy, or CKD-G5 on dialysis, cardiomyopathy with LVH and major neurological manifestations. However, the cells of the vascular endothelium are targeted, and the damage takes the form of vasculopathy and microvascular obstruction. [43,44]

Kidney biopsies showed focal microvacuoles and enlargement of podocytes, as well as lysosomal deposits in many cell types.

Additional findings were mitochondrial pyknosis and non-specific lipidic inclusions, i.e., vacuoles containing uni- or multi-lipidic-droplets, lipofuscins, as well as zebra bodies. Giant mitochondria were also observed, given that Gb3 inclusions have been proven to elicit mitochondrial alterations. Mitochondrial pyknosis has previously been described as a type of ultrastructural injury due to metabolic, ischemic, and toxic drivers [45,46]. Its presence can affect critical cellular pathways (apoptosis, lipid metabolism, energy balance). This finding suggests the potential involvement of other pathogenic players through activation of inflammation and oxidative stress. NGS techniques have identified causal nuclear and mitochondrial genes in hereditary podocytopathies, including Alport disease and FD [47].

Changing the landscape of D313Y variants, the limited literature data gathered without the aid of TEM offer strong evidence that the D313Y mutation could actually be pathogenic. It seems to be related to a later-onset phenotype associated with normal plasma LysoGb3 levels. This mutation

could be classified as a “pseudodeficient allele”, implying that it is a sequence variant encoding an enzyme that is transported to the lysosomes, where it shows about 75% of normal enzymatic activity. [42]

These findings support the theory that later-onset FD should be considered a unique entity that is different from classical FD, owing to the primary involvement of cardiomyocytes instead of endothelial cells.

Classical FD is characterized by frequent central nervous system involvement, which may be caused by the deposition of glycosphingolipids in cerebrovascular endothelial cells, a consequence of cardiogenic embolism from cardiomyopathy, valvular heart disease, ischemic heart disease, and/or arrhythmias. [48,49]

In this comparison between patients with later-onset and classical FD, the prevalence of infarction was similar in each group (32.0% for later-onset patients and 33.3% for classical Fabry patients). The incidence of stroke found in our patients is higher than in the general population, but similar to other studies. [48]

Our data suggest that the genotype-phenotype relationship is not always predictable, and in such cases, biopsy could be a decisive diagnostic tool. *GLA* non-classic variants and GVUS could expand the phenotypic heterogeneity even within the same family, highlighting the need for patient-specific clinical evaluation and a multi-biopsy policy [8]. In CKD patients with *GLA* GVUS, kidney biopsy with TEM analysis has been proposed as the only valid procedure to confirm or exclude FD nephropathy. Other criteria are currently not considered suitable surrogates for biopsy. [31,50]

The main limitations of this study are its single-center retrospective cross-sectional design and the lack of a control group of age-matched patients without FD. Its main strengths are the large number of cases, including disregarded subgroups (women and GVUS carriers), the

identification of mild FD nephropathy by biopsy procedures, the application of the ISGFN scoring system, the systematic pedigree analysis for proband identification.

We argue that knowledge of targets cells and structures involved in FD pathways could lead to the identification of single organ pathomechanisms and, therefore, novel targeted therapies or preventive strategies. [51]

In conclusion, this series suggests that there are at least two FD forms, classical and later-onset, the latter secondary to as yet unknown genotype-phenotype correlations, which we know to involve several separate drivers and pathways of many cells leading to a distinct phenotype. [52,53,54]

We aimed to describe histological findings in early-stage FD patients with CKD and to highlight genotype-phenotype correlations. Just like Fogo, “for now, we conclude that important information is provided by kidney biopsy in FD that is not available from routine assessment of kidney function and proteinuria. Our results support the role of kidney biopsy in the baseline evaluation of all Fabry patients, even with mild disease”. Identifying cells and structures involved in FD pathways may clarify pathological mechanisms and, thereby, inform future novel therapies or preventive strategies.

Article Information

Authors’ Contributions: Research area: MS, IC, VD, RL; Study design: MS, IC, ET; Data acquisition: BF, DM, ET, VP, BE, GC; Data analysis: MC, MD, AG, VD; Data interpretation: MC, MD, GZ; Statistical analysis: DG, RL; Supervision: MS, GLM, GP; Mentorship: GLM, GP. Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual’s own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work, even one in which the author was not directly involved, are appropriately investigated and resolved, including with documentation in the literature if appropriate.

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FIGURES CAPTIONS

Figure 1 (A-B). LM podocyte vacuolization in periodic acid-schiff (PAS) and acid fuchsin orange G (AFOG) staining**.** A) Glomerulus with podocytes showing foamy, pale, lacy cytoplasm due to lipid accumulation of Gb3. Lipids have been dissolved in the routine processing (PAS, 400x magnification); B) The same feature (arrows) is better represented in the AFOG stain (AFOG, 400x magnification).

Figure 2 (A-B-C). Toluidine blue-stained semithin sections of podocytes, vascular smooth muscle cells, and endothelial cells of peritubular capillaries. A) Inclusion (*) of different sizes in podocyte cytoplasm (400x magnification); B) vascular medial inclusion in the cytoplasm of smooth muscle cells of an arteriole (400x magnification); C) Cytoplasmic inclusions in endothelial cells (red°) of peritubular capillaries (400x magnification).

Figure 3 (A-B-C-D-E). TEM analysis of zebra bodies in different renal cortex compartments Detailed map of the involvement of the different renal cortex compartments by TEM, identifying specific inclusions and zebra bodies, of different sizes and morphology. A) Small and large zebra bodies engulfing the cytoplasm of epithelial parietal cells. B) Distal tubule epithelial cells showing specific inclusions. C) Arteriolar endothelial cells containing numerous zebra bodies and showing degenerative findings (chromatin changes, cytoplasmic vacuoles); on the left, a few degenerating endothelial cells are splitting off the vascular wall. D) Peritubular capillary endothelial cells show small zebra body inclusions and multilayering of the basal membrane as a feature of chronic injury. E) Peritubular capillary mural-interstitial cells with small specific inclusions. Scale bar = 2 µm. (3400x magnification, High Voltage 80 kV).

Figure 4 (A-B). TEM observation in FD in carriers of p.N215S mutation

Electron microscopy findings of renal biopsy from two female patients with FDcarrying the N215S mutation in *GLA*. A) Low magnification showing hypertrophic podocytes with specific lipidic inclusions (arrows) and close association (circle) between multidroplet lipidic inclusions (L) and zebra bodies. Scale bar = 2 um; B) Podocytes showing multidroplet lipidic inclusions (L) and pyknotic mitochondria (arrows). Scale bar = 2 um. (3400x magnification, High Voltage 80 kV).

Figure 5 (A-B). TEM observation in carriers of D313Y mutation. Electron microscopy findings of renal biopsy from a male patient with FD carrying the D313Y polymorphism in *GLA*. A) Hypertrophic podocytes with a large multidroplet lipidic inclusions (L) and pyknotic mitochondria (arrows). Scale bar = 2 um. B) Podocyte showing many pyknotic mitochondria (arrows). Scale bar = 1 um. (3400x magnification, High Voltage 80 kV).

Table 1. Patients with biopsy: comparison by sex. Comparisons were made by the Mann-Whitney test with a significance level set at $p<0.05$. Significance is indicated in bold.

	Males (n=13)			**Females (n=22)**			**p-value**
	median	**range**	**IQR**	**median**	**range**	**IQR**	
Age (years)	48.0	18-72	37-62	46.5	21-81	42.6-57.6	0.9
sCreat (μmol/L)	87.5	57-202	80-120	61.9	36-583	53-71	**0.001**
eGFR (mL/min/1.73 m^2)	89.00	29-136	73-96	102	8-120	81-111	0.2
mCrClearance (mL/min) *	96	64-140	69.5-119	123	7.8-248	90-159	0.1
pLysoGb3 (ng/mL)	18.1	1.1-96.9	4.3-30.5	3.2	0.9-12.7	1.5-4.8	**0.001**
UACR (mg/g)	18.3	2.0-554	13.3-128	9.3	2.8-2650	5.8-22.8	0.2
24-hour urine protein excretion (mg/24 h)	234	60-4900	130-515	137.5	21-2296	78-203.5	0.1
Microalbuminuria (mg/L)	27.4	7-943	16.5-169	13.5	3-1301	5.8-25.4	**0.05**

* $U_{Cr}*U_{vol}/P_{Cr}*t$

eGFR, estimated glomerular filtration rate; mCrClearance, measured creatinine clearance; sCreat, serum creatinine; UACR, urinary albumin-to-creatinine ratio; pLysoGb3, plasmatic Lyso-Gb3

Table 2. Histological renal findings in FD patients, overall and by sex. Data are given as mean ± standard deviation (SD) for continuous variables or number with percentage in brackets for categorical variables. Comparisons were carried out using Mann-Whitney test, or Fisher's exact test (*), or chi-square test (§), as appropriate with a significance level set at $p<0.05$. Significance is indicated in bold.

	All (n=35)	**Men (n=13)**	**Women (n=22)**	**Test; p-value**
Semithin sections				
MPS; Podocyte vacuoles (0-3)	1.23 ± 0.83	1.24 ± 0.99	1.23 ± 0.77	0.20; 0.9
Podocyte inclusions (0-4)	1.87 ± 0.90	1.88 ± 0.90	1.87 ± 0.93	0.09; 0.9
Parietal epithelial inclusions	13/26 (50.0%)	7/12 (58.3%)	6/14 (42.9%)	0.62; 0.4§
Distal tubule inclusions	20/35 (57.1%)	8/13 (61.5%)	12/22 (54.5%)	0.8*
Proximal tubule inclusions	19/35 (54.3%)	8/13 (61.5%)	11/22 (50.0%)	0.7*
Peritubular capillary inclusions	13/35 (39.2%)	6/13 (46.2%)	7/22 (31.9%)	0.6*
Vascular intimal inclusions	12/35 (34.3%)	6/13 (46.2%)	6/22 (27.3%)	0.4*
Vascular medial inclusions	16/35 (45.7%)	6/13 (46.2%)	10/22 (45.5%)	0.9*
Light microscopy				
Segmental sclerosis (mild + severe) (%)	1.5 ± 4.1	1.0 ± 3.3	1.7 ± 4.4	0.45; 0.7
Global sclerosis (%)	11.9 ± 27.7	23.2 ± 44.2	6.0 ± 10.3	-1.51; 0.1
Glomeruli without sclerosis (%)	79.8 ± 30.2	73.4 ± 32.4	83.4 ± 29.1	1.03; 0.3
Interstitial fibrosis (%)	10.3 ± 10.7	16.7 ± 13.2	6.7 ± 7.1	**-2.32; 0.02**
Average arterial sclerosis (0-3)	0.72 ± 0.89	0.92 ± 1.00	0.60 ± 0.82	-0.94; 0.3
Most severe arterial sclerosis (0-3)	0.97 ± 1.00	1.08 ± 1.08	0.90 ± 0.97	-0.46; 0.6
Arteriolar hyalinosis	16/33 (48.5%)	9/12 (75.0%)	7/21 (33.3%)	**5.31; 0.02**§
Arterial hyalinosis	4/33 (12.1%)	2/12 (16.7%)	2/21 (9.5%)	0.6*

MPS, mean podocyte score

Table 3. Association between Fabry disease nephropathy histological findings and clinical parameters. Data are given as mean ± SD, and comparisons were carried out using Mann-Whitney test. Significance is indicated in bold.

	Age (years)	**eGFR (mL/min/1.73 m²)**	**pLysoGb3 (ng/mL)**	**MPS**
Parietal epithelial inclusions				
Absent	49.9 ± 17.1	77.5 ± 34.7	4.8 ± 8.1	0.75 ± 0.75
Present	46.5 ± 17.7	100.1 ± 21.5	20.3 ± 26.2	1.59 ± 0.85
test; p-value	0.10; 0.918	-1.49; 0.137	**-2.75; 0.006**	**-2.29; 0.022**
Proximal tubular inclusions				
Absent	52.2 ± 14.3	85.1 ± 27.8	6.1 ± 8.0	1.12 ± 0.82
Present	32.7 ± 13.7	100.6 ± 43.0	34.1 ± 33.3	1.61 ± 0.87
test; p-value	**2.46; 0.014**	-1.74; 0.082	**-2.26; 0.024**	-0.81; 0.418
Distal tubular inclusions				
Absent	51.1 ± 17.2	82.1 ± 33.3	4.8 ± 7.3	0.78 ± 0.68
Present	45.9 ± 14.8	92.9 ± 27.6	19.9 ± 25.2	1.77 ± 0.77
test; p-value	0.70; 0.485	-0.85; 0.396	**-3.21; 0.001**	**-3.17; 0.002**
Peritubular capillary inclusions				
Absent	51.0 ± 15.4	87.6 ± 29.8	4.8 ± 6.1	1.04 ± 0.78
Present	40.5 ± 16.2	91.7 ± 36.5	34.7 ± 29.3	1.88 ± 0.83
test; p-value	1.44; 0.151	-0.28; 0.777	**-3.65; <0.001**	**-2.05; 0.040**
Vascular intimal inclusions				
Absent	51.5 ± 16.1	86.6 ± 32.6	4.5 ± 6.7	0.81 ± 0.62
Present	43.2 ± 16.7	90.6 ± 32.3	25.7 ± 27.9	1.69 ± 0.72
test; p-value	1.10; 0.272	-0.17; 0.863	**-3.38; 0.001**	**-3.10; 0.002**
Vascular medial inclusions				
Absent	51.8 ± 16.5	84.7 ± 32.5	4.6 ± 6.9	0.81 ± 0.64
Present	40.9 ± 14.9	97.7 ± 16.0	29.9 ± 32.6	1.76 ± 0.78
test; p-value	1.39; 0.164	-0.64; 0.523	**-2.86; 0.004**	**-2.78; 0.006**

MPS, mean podocytes score; pLysoGb3, plasmatic LysoGb3

Table 4. Histological kidney findings in FD patients according to CKD stage. Data are given as mean ± SD for continuous variables or number with percentage in brackets for categorical variables. Comparisons were made using the nonparametric Cuzick's test for trend and chi-square test for trend, for continuous and categorical variables respectively, with a significance level set at $p<0.05$. Significance is indicated in bold.

	Stage 1 (n=20)	Stage 2 (n=10)	Stage 3-5 (n=5)	Test; p-value
Semithin sections				
MPS; Podocyte vacuoles (0-3)	1.22 ± 0.85	1.57 ± 0.74	0.55 ± 0.64	-0.43; 0.7
Podocyte inclusions (0-4)	1.98 ± 0.77	2.29 ± 0.61	0.62 ± 0.95	-1.52; 0.1
Distal tubule inclusions	10/17 (58.8%)	5/10 (50.0%)	1/5 (20.0%)	2.06; 0.2
Proximal tubule inclusions	5/17 (29.4%)	0/10 (0%)	1/5 (20.0%)	1.15; 0.3
Peritubular capillary inclusions	4/18 (22.2%)	3/9 (33.3%)	1/5 (20.0%)	0.02; 0.9
Vascular intimal inclusions	6/16 (37.5%)	4/9 (44.4%)	1/5 (20.0%)	0.24; 0.6
Vascular medial inclusions	5/14 (35.7%)	3/8 (37.5%)	0/4 (0%)	1.23; 0.3
Light microscopy				
Segmental sclerosis (mild + severe) (%)	1.2 ± 3.6	1.6 ± 4.8	2.8 ± 5.6	0.67; 0.5
Global sclerosis (%)	3.1 ± 6.2	25.5 ± 48.4	23.3 ± 15.7	**3.10; 0.002**
Glomeruli without sclerosis (%)	82.6 ± 30.5	72.6 ± 36.5	82.1 ±10.4	-1.39; 0.2
Interstitial fibrosis (%)	6.0 ± 9.1	13.3 ± 9.4	25.0 ± 5.8	**3.69; <0.001**
Average arterial sclerosis (0-3)	0.4 ± 0.8	1.1 ± 0.9	1.5 ± 0.6	**3.24; 0.001**
Most severe arterial sclerosis (0-3)	0.6 ± 0.9	1.3 ± 1.0	1.8 ± 1.0	**2.62; 0.009**
Arteriolar hyalinosis	6/20 (30.0%)	6/9 (66.7%)	4/4 (100.0%)	**8.17; 0.004**
Arterial hyalinosis	2/20 (10.0%)	1/9 (11.1%)	1/4 (25.0%)	0.51; 0.5

MPS, mean podocytes score

Table 5. Linear regressions of eGFR at baseline on histological parameters, adjusted for age. Significant p-values are highlighted in bold.

Predictors	Coeff. men; 95%CI; p-value
Global sclerosis (% glomeruli)	-0.267 (-0.546; 0.011); 0.06
Age	**-1.065 (-1.561; -0.569); <0.001**
Interstitial fibrosis (%)	**-1.031 (-1.729; -0.334); 0.005**
Age	**-0.838 (-1.323; -0.352); 0.001**
Average arterial sclerosis (0-3)	**-10.688 (-19.530; -1.864); 0.02**
Age	**-0.941 (-1.453; -0.429); 0.001**
Most severe arterial sclerosis (0-3)	**-8.306 (-16.235; -0.376); 0.04**
Age	**-0.988 (-1.504; -0.471); 0.001**
Arteriolar hyalinosis	-16.636 (-33.607; 0.335); 0.05
Age	**-0.838 (-1.396; -0.280); 0.005**

Table 6. Spearman correlations between clinical and histological parameters in biopsied patients: all (A: n=35), men (B: n=13) and women (C: n=22). In bold, the correlations >0.400 except those regarding variables correlated by construction. Significance is indicated in bold

A. All biopsied patients	Age at biopsy	Serum Creatinine	eGFR	pLysoGb3	UACR	24-hour urine protein excretion	MPS	Segmental sclerosis (LM)	Global sclerosis (LM)	Glomeruli without sclerosis	Tubulointerstitial fibrosis	Arteries most severe sclerosis	Arteries average sclerosis	Average podocyte inclusions
Age at biopsy	1.000													
Serum Creatinine	0.285	1.000												
eGFR	-0.728	-0.778	1.000											
pLysoGb3	0.133	0.367	-0.162	1.000										
UACR	0.355	0.342	-0.345	0.332	1.000									
24-hour urine protein excretion	-0.105	0.451	-0.212	0.073	0.557	1.000								
MPS	0.141	0.023	-0.108	0.352	-0.008	-0.276	1.000							
Segmental sclerosis (LM)	0.259	0.032	-0.115	0.228	0.274	0.007	-0.104	1.000						
Global sclerosis (LM)	**0.407**	**0.441**	**-0.529**	0.366	0.262	0.312	0.175	0.251	1.000					
Glomeruli without sclerosis	-0.099	-0.202	0.281	-0.018	**-0.493**	**-0.425**	-0.140	0.082	-0.145	1.000				
Tubulointerstitial fibrosis	**0.590**	**0.470**	**-0.619**	0.262	**0.456**	0.335	-0.005	-0.006	**0.522**	-0.296	1.000			
Arteries, most severe sclerosis	**0.456**	0.314	**-0.515**	0.172	**0.452**	0.291	-0.018	0.227	**0.402**	-0.399	**0.567**	1.000		
Arteries, average sclerosis	**0.454**	**0.486**	**-0.626**	0.235	**0.410**	0.397	-0.086	0.200	0.329	**-0.441**	**0.589**	0.865	1.000	
Average podocyte inclusions	-0.128	-0.190	0.134	0.138	-0.218	-0.289	**0.723**	-0.358	-0.020	-0.232	-0.105	-0.128	-0.110	1.000

B. Male biopsied patients	Age at biopsy	Serum Creatinine	eGFR	pLysoGb3	UACR	24-hour urine protein excretion	MPS	Segmental sclerosis (LM)	Global sclerosis (LM)	Glomeruli without sclerosis	Tubulointerstitial fibrosis	Arteries most severe sclerosis	Arteries average sclerosis	Average podocyte inclusions
Age at biopsy	1.000													
Serum Creatinine	0.361	1.000												
eGFR	-0.686	-0.843	1.000											
pLysoGb3	**-0.509**	-0.207	0.399	1.000										
UACR	**0.476**	0.238	-0.210	-0.045	10.000									
24-hour urine protein excretion	0.070	0.516	-0.182	-0.084	0.732	1.000								
MPS	-0.301	-0.306	**0.437**	**0.433**	-0.091	0.036	1.000							
Segmental sclerosis (LM)	0.301	**0.501**	**-0.500**	0.200	0.300	0.301	**-0.451**	1.000						
Global sclerosis (LM)	0.170	**0.846**	**-0.782**	-0.060	0.093	**0.469**	0.061	0.205	1.000					
Glomeruli without sclerosis	-0.025	-0.312	0.221	0.161	**-0.427**	**-0.563**	-0.224	0.000	**-0.511**	1.000				
Tubulointerstitial fibrosis	0.331	**0.673**	**-0.579**	-0.075	0.352	0.318	-0.056	0.204	**0.703**	**-0.464**	1.000			
Arteries, most severe sclerosis	0.234	**0.612**	**-0.511**	0.006	**0.578**	**0.562**	**-0.413**	**0.485**	**0.550**	**-0.523**	**0.711**	1.000		
Arteries, average sclerosis	**0.412**	**0.523**	**-0.522**	-0.006	**0.622**	**0.406**	-0.284	**0.430**	**0.441**	**-0.523**	**0.813**	0.923	1.000	
Average podocyte inclusions	**0.450**	0.058	-0.159	-0.029	0.232	0.174	0.399	0.205	0.059	-0.305	-0.022	-0.046	0.092	1.000

C. Female biopsied patients	Age at biopsy	Serum Creatinine	eGFR	pLysoGb3	UACR	24-hour urine protein excretion	MPS	Segmental sclerosis (LM)	Global sclerosis (LM)	Glomeruli without sclerosis	Tubulointerstitial fibrosis	Arteries most severe sclerosis	Arteries average sclerosis	Average podocyte inclusions
Age at biopsy	1.000													
Serum Creatinine	0.237	1.000												
eGFR	-0.712	-0.750	1.000											
pLysoGb3	**0.567**	0.158	**-0.463**	1.000										
UACR	0.328	-0.028	-0.199	0.198	1.000									
24-hour urine protein excretion	-0.238	0.166	0.097	-0.140	0.413	1.000								
MPS	**0.591**	0.284	**-0.595**	0.312	0.066	**-0.434**	1.000							
Segmental sclerosis (LM)	0.312	-0.027	-0.006	0.350	0.329	-0.092	-0.003	1.000						
Global sclerosis (LM)	**0.505**	0.079	-0.298	**0.561**	0.243	0.092	0.254	0.309	1.000					
Glomeruli without sclerosis	-0.110	-0.034	0.194	0.107	**-0.561**	-0.290	-0.194	0.179	0.033	1.000				
Tubulointerstitial fibrosis	**0.670**	-0.109	-0.327	0.266	**0.402**	0.151	0.144	-0.035	0.264	-0.324	1.000			
Arteries, most severe sclerosis	**0.495**	0.356	**-0.543**	0.362	0.301	0.263	0.170	0.122	0.162	-0.378	**0.511**	1.000		
Arteries, average sclerosis	**0.453**	0.121	-0.383	0.304	**0.414**	0.216	0.177	0.155	**0.418**	-0.338	**0.452**	**0.830**	1.000	
Average podocyte inclusions	0.237	-0.039	-0.210	0.115	-0.329	**-0.616**	**0.696**	-0.308	0.032	-0.161	0.179	0.161	0.014	1.000

Table 7. Multivariable regressions of histological parameters of men, adjusted for age. Significance is indicated in bold.

Dependent variable	Coeff. men; 95%CI; p-value	Coeff. age; 95%CI; p-value
Semithin sections		
Segmental sclerosis (mild + severe) (%)^	-6.11; (-24.69; 11.25); 0.5	0.32; (-0.41; 1.13); 0.4
Global sclerosis (%)^	1.81; (-3.15; 6.40); 0.4	0.03; (-0.06; 0.13); 0.5
Podocyte inclusions^	0.14; (-0.51; 0.82); 0.7	-0.01; (-0.04; 0.02); 0.6
Parietal epithelial inclusions§	1.27; (0.30; 5.47); 0.7	0.99; (0.95; 1.04); 0.7
Distal tubule inclusions§	0.76; (0.18; 3.18); 0.7	0.98; (0.94; 1.02); 0.3
Proximal tubule inclusions§	6.01; (0.57; 63.62); 0.1	**0.90; (0.82; 0.98); 0.02**
Peritubular capillary inclusions§	**9.28; (1.24; 69.18); 0.03**	0.95; (0.89; 1.01); 0.1
Vascular intimal inclusions§	1.77; (0.39; 8.06); 0.5	0.97; (0.92; 1.02); 0.2
Vascular medial inclusions§	2.64; (0.44; 15.92); 0.3	0.95; (0.89; 1.01); 0.1
Light microscopy		
MPS; Podocyte vacuoles (0-3)^	0.31; (-0.67; 0.89); 0.4	0.01; (-0.03; 0.04); 0.5
Segmental sclerosis (mild + severe) (%)^	-0.94; (-4.07; 1.65); 0.5	0.06; (-0.01; 0.17); 0.2
Global sclerosis (%)^	16.53; (-3.07; 50.37); 0.3	0.15; (-2.03; 0.86); 0.8
Glomeruli without sclerosis (%)^	-26.64; (-57.31; 44.92); 0.2	-1.41; (-3.95; 4.41); 0.5
Interstitial fibrosis (%)^	**9.50; (2.53; 18.27); 0.02**	0.25; (-0.26; 0.44); 0.1
Average arterial sclerosis (0-3)^	0.26; (-0.37; 0.97); 0.4	0.02; (-0.01; 0.04); 0.09
Most severe arterial sclerosis (0-3)^	0.12; (-0.62; 0.91); 0.8	0.02; (-0.02; 0.04); 0.2
Arteriolar hyalinosis§	**9.78; (1.26; 79.95); 0.03**	**1.10; (1.02; 1.18); 0.01**
Arterial hyalinosis§	1.93; (0.23; 15.95); 0.5	0.99; (0.93; 1.06); 0.8

MPS, mean podocytes score
^ Nonparametric kernel regression
§Logistic regression (coefficients are odds-ratios).

Table 8. Toluidine-blue stained semithin sections in p.N215S and D313Y patients

MUTATION	**pN215S**															Total	**D313Y**			Total
Empty vacuoles in podocytes	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	14	0	0	0	0
Histology	-	-	GM	GM	-	-	-	GM	-	-	-	-	-	GM	GM	-	-	MIDD	-	-
Mean podocyte score (MPS)	0.625	1.2	1.21	0.56	1.16	2.4	1	0.86	0.9	1	0.07	0.25	0.35	1.3	1	0.92	0	0	0	0
Podocytes hypertrophy (0= absent; 1=segmental; 2=global)	1	1	1	1	1	2	1	1	1	1	0	1	1	1	1	14	0	0	0	0
Foot process fusion (0=interdigitating prevalent, 1=<80%, 2=>80%)	0	1	1	1	1	2	1	1	1	1	0	1	1	1	1	50%	40	60	0	30%
Endothelial cells inclution (E), mesangium (M) (0=absent, 1= present)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zebra bodies podocytes (0=assenti, 1=presenti)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15	0	0	0	0
Fogo score	1.85	2	1	2	2	3	-	3	1	-	0.88	3	3	2.38	1.28	2.03	0	0	0	0
Parethial epithelial inclusions	1	0	1	1	0	0	0	0	1	0	0	0	0	1	1	6	0	0	0	0
Proximal tubular inclusions	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Distal tubular inclusions	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	3	0	0	0	0
Peritubular capillary inclusions	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vascular intimal inclusions	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vascular medial inclusions	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyknotic mitochondria	0	0	0	0	0	0	1	1	1	0	0	1	0	1	0	5	1	1	1	3

GM= Glomerulomegaly

Table 9. Clinical and histological findings in D313Y patients, all with c.937G>T variant and low α-GAL activity

Patient and genetic data	Clinical, biochemical and histological data (when performed)
M, 58 years **D313Y c.937G>T** No family history	Cataract, acroparesthesias, nerve VI paralysis (sudden double vision) CKD G3 (Cr 1.8 mg/dl, eVFG 40, Proteinuria 119 mg/24h, alpha1-micro 28U/l, UACR 2mg/g) Lyso-Gb3 1,1ng/ml, alpha-GAL da 9.1 a 11 micromol/l/h (>=15.3) Kidney biopsy 2018 Semithin sections: podocytes, tubules inclusions TEM: podocytes, tubules, endothelium zebra bodies and *mitochondrial pyknosis* Kidney biopsy 2019: small and larger vacuoles, podocytes and tubules, glomerular ischemia, arteriosclerosis, arteriolar hyalinosis Skin biopsy: no Gb3 deposits small fiber neuropathy
F, 36 years **D313Y c.937G>T** Family history: positive (mother with different mutation c.818T>C)	Acroparesthesias, ocular signs (microaneurysms of conjunctival vessels and tortuosities of retinal vessels) alpha-GAL A 10.3 micromol/L/h >15.3 (pathological) Kidney biopsy LM: hypertrophic podocytes, foamy cytoplasm Endothelial activation, focal segmental GS, arteriolar hyalinosis TEM: endothelium, tubules, podocytes with *mitochondrial pyknosis* and lipofuscins
M, 28 years **D313Y c.937G>T** Family history: positive (2 male siblings with “petit mal” epilepsy)	2015 headache, hemiparesis, aphasia of understanding and speech, EEG: aspecific frontal and temporal alterations 2016 headache preceded by sensory disorders and facialnerve deficit; brain CT/MRI: no alterations Lyso-Gb3 0.8 ng/ml (<1.8) Alpha-GAL A 9.1 micromol/L/h >15.3 Kidney biopsy refused
F, 64 years **D313Y c.937G>T**	Long course CKD G3 + LCDD + AL kappa Alpha-GAL A 11.5 micromol/L/h >15.3 (pathological) Kidney biopsy: MIDD, *pyknotic mitochondria*