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CLINICAL REPORT

Gene variants of unknown significance in Fabry disease: Clinical characteristics of *c.376A>G (p.Ser126Gly)*

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Abstract

Background: Anderson–Fabry disease (FD) is an X-linked lysosomal storage disorder with varying organ involvement and symptoms, depending on the underlying mutation in the alpha-galactosidase A gene (HGNC: *GLA*). With genetic testing becoming more readily available, it is crucial to precisely evaluate pathogenicity of each genetic variant, in order to determine whether there is or might be not a need for FD-specific therapy in affected patients and relatives at the time point of presentation or in the future.

Methods: This case series investigates the clinical impact of the specific *GLA* gene variant *c.376A>G (p.Ser126Gly)* in five (one heterozygous and one homozygous female, three males) individuals from different families, who visited our center between 2009 and 2021. Comprehensive neurological, nephrological and cardiac examinations were performed in all cases. One patient received a follow-up examination after 12 years.

Results: Index events leading to suspicion of FD were mainly unspecific neurological symptoms. However, FD-specific biomarkers, imaging examinations (i.e., brain MRI, heart MRI), and tissue-specific diagnostics, including kidney and skin biopsies, did not reveal evidence for FD-specific symptoms or organ involvement but showed normal results in all cases. This includes findings from 12-year follow-up in one patient with renal biopsy.

Conclusion: These findings suggest that *p.Ser126Gly* represents a benign *GLA* gene variant which per se does not cause FD. Precise clinical evaluation in individuals diagnosed with genetic variations of unknown significance should be performed to distinguish common symptoms broadly prevalent in the general population from those secondary to FD.

KEYWORDS

diagnosis in Fabry disease, Fabry disease, gene variant, genotype/phenotype correlation, lysosomal storage disease

1 | BACKGROUND

Anderson–Fabry disease (FD) (OMIM: *#301500*) is an X-linked lysosomal storage disorder (Germain, 2010). The incidence of FD varies in literature from 1:40,000 to 1:117,000 (Mehta et al., 2004; Meikle et al., 1999; van der Tol, Cassiman, et al., 2014). Due to the broad range of symptoms and organ manifestations, FD is a genetic disorder with particularly complex clinical presentation. Classic symptoms, which decrease life expectation, include renal failure and hypertrophic cardiomyopathy (Cairns et al., 2018). Additionally, FD can affect the central and peripheral nervous systems, the gastrointestinal tract, ears, eyes, and skin (Zarate & Hopkin, 2008). Because of the X-linked inheritance, men are affected more frequently and more seriously. More than 1000 distinct mutations in the alpha-galactosidase A gene (HGNC: *GLA*) (OMIM: *#30064*; HGNC: *4296*) have been described so far (McCafferty & Scott, 2019). Besides "classical" mutations, known to usually cause the full clinical picture of FD, many variants of unknown or attenuated clinical significance are known to date. Some mutations can lead to a "late-onset" or "organ-specific" form of FD with mild symptoms and/or later onset of apparent disease (Oder et al., 2017). In addition, due to increasing disease awareness and better availability of genetic testing, there is increasing evidence of genetic variants with no relevant degree of disease (Oder et al., 2018). One key aspect in the management of patients with a genetic variant in the *GLA* is to evaluate if there is a need for FD-specific therapy (Ortiz et al., 2018). This decision can be difficult, especially in women, who show particularly high variance in disease penetration, in young male patients without manifest disease, where a "preventive" initiation of treatment is considered and in patients of both genders carrying genetic variations of unknown clinical significance (Wanner et al., 2019) Early and unnecessary treatment of benign genetic variants should clearly be avoided to prevent stigma and/or potential side effects of related individuals and their relatives, but also for health-economic reasons (Wanner et al., 2018).

This case series highlights clinical characteristics of patients carrying the *c.376A>G (p.Ser126Gly)* (NCBI reference sequence: NM_000169.3:c.376A>G) mutation. Until now, scientific evidence of clinical presentation linked with this mutation has been controversial. Branton et al. described *p.Ser126Gly* in 2002 as a pathogenic variant of FD (Branton et al., 2002). A Belgian prevalence study from 2010 linked the mutation to appearance of late-onset FD (Brouns et al., 2010). In contrast, literature research at "*International Fabry Disease Genotype-Phenotype Database (dbFGP)*" describes *p.Ser126Gly* as a likely benign variant of FD causing mutations. However, due to inconclusive clinical data available, dbFGP also recommends further assessments to confirm that this mutation is a benign variant (dbFGP.org, 2021, April 7).

2 | METHODS

We extensively examined five individuals from different families carrying the *p.Ser126Gly* variant of the *GLA* gene. All five patients visited the Fabry Center for Interdisciplinary Therapy (FAZiT) Würzburg, Germany. Every patient at FAZiT obtains a standardized full clinical, laboratory, and imaging examination, focusing on all possible aspects of FD. This clinical work-up involved a complete cardiac examination with echocardiographic and cardiac magnetic resonance tomography imaging (MRI), paying particular attention to cardiac hypertrophy and signs of fibrosis of the left ventricle. A Holter- and exercise-ECG for detection of cardiac arrhythmias is also implemented. Renal function assessment includes glomerular filtration rate (GFR) calculated with creatinine as well as Cystatin C, proteinuria, and renal biopsy if indicated. Patients also underwent complete neurological examination, nerve conduction studies, quantitative sensory testing (QST), and skin punch biopsy at the lower leg and back to determine the intraepidermal nerve fiber density (IENFD). Additionally, cerebral and spinal MRI scans were performed. In one case, a 12-year follow-up was possible to conduct.

This multidisciplinary approach at FAZiT ensures a complete evaluation of patient's clinical condition. If indicated, experts from other disciplines for example, ophthalmology, otorhinolaryngology, or dermatology are consulted on site.

3 | CASE PRESENTATIONS

All patients carried the *p.Ser126Gly* variant and were evaluated for clinical signs or symptoms of potential FD. After evaluation, the patients were assessed for FD-specific therapy indication. Clinical characteristics of these patients are shown in Table 1.

Patient 1 was a man in his late 40's, who presented at FAZiT with symptoms of acral pain, hypohidrosis, and heat intolerance. Past medical history includes hyperthyroidism from a multinodular goiter treated by thyroidectomy with postoperative hypothyroidism. Other history includes depression and Scheuermann's disease.

Patient 2 was a man in his early 60's, who presented with unspecific cognitive impairment, namely loss of short-term memory. Besides that, he had arterial hypertension.

TABLE 1 Baseline characteristics of each patient

Parameters	Patient 1, m, age: Late 40's	Patient 2, m, age: Early 60's	Patient 3, m, age: Early 50's	Patient 4, f, age: Early 40's	Patient 5, f, age: Early 30's
Patient history	• Acral pain • Hypohidrosis • Heat intolerance • Vertigo (2–3 times a week)	• Short-term memory disturbance	• Lacunar cerebral stroke (age of 51 years)	• Positive tested family member for FD • Diarrhea	• Positive tested family member for FD
Medical history	• Thyroidectomy (nodular goiter) • Hypoparathyroidism • Depression • Small-fiber neuropathy • Tinnitus and loss of hearing (blast injury)	• Arterial hypertension • Lumbar spinal stenosis • Tinnitus	• Hyperlipidemia • Tension neck syndrome • Smoking history	• Depression • Migraine (since childhood)	• Tinnitus (in stress situations) • WPW syndrome
Fabry specific biomarkers	A-Gal-activity: 0.39 (0.4–1.0 nmol/min/mg protein) Lyso-GB3: 0.6 (<0.9 ng/ml)	A-Gal-activity: 0.42 (0.4–1.0 nmol/min/mg protein) Lyso-GB3: 0.8 (<0.9 ng/ml)	A-Gal-activity: 0.6 (0.4–1.0 nmol/min/mg protein) Lyso-GB3: 0.6 (<0.9 ng/ml)	A-Gal-activity: 0.5 (0.4–1.0 nmol/min/mg protein) Lyso-GB3: 0.6 (<0.9 ng/ml)	A-Gal-activity: 0.27 (0.4–1.0 nmol/min/mg protein) Lyso-GB3: 0.5 (<0.9 ng/ml)
Heart	ECG: normal TTE: normal[a] 24 h tape: HR: 57–114/min Ergometry: max. 200 W Cardiac-MRI: normal[a] NTproBNP & hs-Troponin: normal[a]	ECG: T-neg. V1-V2 TTE: normal[a] 24 h tape: HR: 52–94/min Ergometry: n.p. Cardiac-MRI: normal[a] NTproBNP & hs-Troponin: normal[a]	ECG: normal TTE: normal[a] 24 h tape: HR: 47–120/min Ergometry: max. 200 W Cardiac-MRI: normal[a] NTproBNP & hs-Troponin: normal[a]	ECG: normal TTE: normal[a] 24 h tape: n. p. Ergometry: max. 100 W Cardiac-MRI: normal[a] NT-proBNP & hs-Troponin: normal[a]	ECG: normal TTE: normal[a] 24 h tape: HR: 47–120/min Ergometry: max. 200 W Cardiac-MRI: normal[a] NT-proBNP & hs-Troponin: normal[a]
Neuro-logical examination	Small fiber neuropathy with no other FD-specific findings	No clear signs of a severe cognitive disorder	Old lacunar infarction No clear correlation between infarction & FD	Normal finding	Normal finding
Skin biopsy	Reduction of distal and proximal Intraepidermal nerve fibers IENFD: Leg: 4.1 fibers/mm Back: 13.9 fibers/mm	Normal IENFD: Leg: 6.4 fibers/mm Back: 19.5 fibers/mm	Reduction of distal and proximal Intraepidermal nerve fibers IENFD: Leg: 2.1 fibers/mm Back: 16.6 fibers/mm	Normal IENFD: Leg: 5.5 fibers/mm	n. p.

Abbreviations: A-Gal-activity, Alpha-galactosidase enzyme activity; ECG, electrocardiography; FD, Fabry disease; GFR, glomerular filtration rate; HR, heart rate; IENFD, intraepidermal nerve fiber density; MRI, magnetic resonance imaging; n. p., not performed; NTproBNP, pro B-type natriuretic peptide; TTE, transthoracic echocardiogram; WPW syndrome, Wolff–Parkinson–White syndrome.

[a]Exact results are shown in Table 2.

Patient 3 was a man in his early 50's, who suffered from lacunar cerebral stroke at the age of 51. His medical record showed hyperlipidemia, tension neck syndrome, and smoking history.

Patient 4 was a woman in her early 40's, who was referred to our center because of FD-specific positive genetic testing in a family member. The patient suffered from episodes of migraine since childhood and depression.

Patient 5 was a woman in her early 30's, who was also referred to our center because of FD-specific positive genetic testing in a family member. No FD-specific signs or symptoms could be found. Medical history showed Wolff–Parkinson–White Syndrome and Tinnitus. Genetic testing revealed a homozygous gene variant which indicates, that both, mother and father have the specific FD-variant. *p.Ser126Gly* has an assumed heterozygous allele frequency of 0.0007 (prevalence: 7: 10,000), which results in an assumed allele frequency of 0.00000049 for the homozygous variant. Therefore, the prevalence for homozygous *p.Ser126Gly* variant is 4.9: 10,000,000 (Phan et al., 2020; "Reference SNP (rs) Report: rs149391489," 2021). In a country like Germany with approximately 80,000,000 citizens homozygous allele frequency should be present in about 40 people. According to *Patient 5* only the mother received a genetic test (positive for *p.Ser126Gly*), but both parents—at the time point of investigation in their early 60's—are reported to be healthy and show no symptoms related to FD.

In four patients, alpha-galactosidase A enzyme activity was normal. The homozygous female (*Patient 5*) showed an intermediate level of enzyme activity (mean: 0.436 nmol/min/mg protein; range 0.27–0.6). Lyso-GB3-levels were normal in all five patients (mean: 0.62 ng/ml; range: 0.5–0.8). There were no other laboratory signs for FD. Kidney function at baseline was also normal (eGFR mean: 83.2 ml/min/1.73 m^2; range: 74–103) in all patients, with no signs of proteinuria.

Cardiac evaluation showed normal results in all five patients. ECG, Holter-ECG, and Exercise-ECG showed no abnormalities such as cardiac bradycardia or arrhythmia. In one patient, we found unspecific T-segment deviation. All MRI-examinations showed no pathological results, especially no cardiac hypertrophy or intramural late gadolinium enhancement (LGE). The cardiac marker NT-proBNP (mean: 57.8 pg/ml; range: 16–92) was normal and hs-troponin was low. Detailed results of our examinations are shown in Table 2.

Three out of five patients reported neurological symptoms, which was the reason why genetic testing for FD was initially performed. *Patient 1* had a small fiber neuropathy, with a reduction of distal and proximal intraepidermal nerve fibers density in skin biopsies. In this case, we could not find any other signs of FD. *Patient 2* primarily had signs of short-term memory disturbance leading to genetic testing, even though this does not represent a FD-specific symptom. In our clinical evaluation no other symptoms were found, that would determine FD. *Patient 3* had a lacunar cerebral stroke at an early age. No other FD-specific symptoms that would undermine FD could be evaluated.

3.1 | Follow-up

Patient 4 presented for follow-up examination 12 years after the initial visit. The results are shown in Figure 1. Alpha-galactosidase A enzyme activity (0.45 nmol/min/mg Protein) as well as Lyso-GB3 (<0.5 ng/ml) were still in normal range. Interestingly, we found a reduced glomerular filtration rate (eGFR CKD-EPI formula: 59 ml/min/1.73 m^2) calculated by blood creatinine. The patient had no relevant proteinuria (total protein in urine <40 mg/L). To confirm the estimated measurement we calculated the GFR from 24-h urine collection (73 ml/min) with a similar reduced result. Because of the unclear etiology of impaired renal function, a renal biopsy was performed. Histological evaluation did not reveal any FD-specific pathology, as shown in Figure 1a. Cardiac evaluation showed no abnormal results, all investigations including cardiac MRI examination did not reveal any evidence for FD-related organ involvement of the heart. In particular, no cardiac hypertrophy and cardiac fibrosis measured by gadolinium late enhancement (LGE) was found as shown in Figure 1b. The patients had a normal systolic and diastolic heart function in all imaging. Neurological examinations did not reveal FD-specific findings. Brain MRI scans were also normal (Figure 1c). Over the whole follow-up period, the patient never suffered from FD-associated pain or showed signs of autonomic dysregulation like hyper- or hypohidrosis. There were no clinical signs of small fiber neuropathy although skin biopsies taken from the lower leg and back showed progressive reduction of intraepidermal nerve fiber density (intraepidermal nerve fiber density: lower leg: 3.9 fibers/mm; back: 15.8 fibers/mm).

Because of an initial external report, which diagnosed *Cornea verticillata* in the patient's right eye, the patient received a repeat full ophthalmological assessment, which concluded that the patient has a mild corneal opacity but no signs of FD typical *Cornea verticillata*.

4 | CASE DISCUSSION

Prevalent literature discusses *p.Ser126Gly* as a controversial *GLA* gene variant. Because of many ongoing screening programs, an increased awareness of FD and a higher

TABLE 2 Results of patient's examinations (all patients)

Parameter	Norm values	Male ($n = 3$)	Female ($n = 2$)	All patients ($n = 5$)	All patients (range)
Age [years]		53 ± 7.2	34.5 ± 6.36	45.6 ± 11.8	30–61
Height [cm]		182.6 ± 6.4	168 ± 9.9	176.8 ± 10.5	161–190
Weight [kg]		83.6 ± 15	75 ± 7	80.2 ± 12.1	70–101
Laboratory parameters					
A-Gal-activity [nmol/min/mg protein]	0.4–1.0	0.47 ± 0.11	0.39 ± 0.16	0.436 ± 0.12	0.27–0.6
Lyso-GB3 [ng/ml]	<0.9	0.66 ± 0.11	0.55 ± 0.07	0.62 ± 0.11	0.5–0.8
NT-proBNP [pg/ml]		49 ± 30.4	71 ± 30	57.8 ± 28.8	16–92
hs-Troponin over norm [*n* (%)]—[pg/ml]	0–14	0 (0)	0 (0)	1 (20)	5–14.1
Creatinine [mg/dl]	0–0.95	1.02 ± 0.11	0.93 ± 0.35	0.99 ± 0.1	0.9–1.1
GFR (CKD-EPI) [ml/min/1.73 m^2]		85.3 ± 15.5	80 ± 8.5	83.2 ± 12.2	74–103
Cystatin C [mg/L]	0.61–0.95	0.89 ± 0.18	0.83 ± 0.6	0.87 ± 0.13	0.69–1.01
Proteinuria detected [*n*]		0 (0)	0 (0)	0 (0)	
Heart-specific features					
LVEF [%]	>55	59.6 ± 4.7	62.5 ± 3.5	60.8 ± 4.1	56–65
IVSd [mm]	<10	9.3 ± 1.2	6.5 ± 0.7	8.2 ± 1.79	6–10
LVPWd [mm]	<10	8 ± 1	6.5 ± 0.7	7.4 ± 1.14	6–9
LVMi [g/m^2]	<95	80 ± 14.4	50.25 ± 8.8	68.1 ± 19.7	44–96
Mitral valve—E/A	0.5–1.9	1.1 ± 0.26	1.65 ± 0.07	1.32 ± 0.36	0.8–1.7
E'septal [cm/s]	>7	8.0 ± 1.8	15.5 ± 5.0	11.04 ± 5	6–19.1
Cardiac-MRI myocardial mass (normalized)		61.6 ± 5.1	45 ± 1.41	55 ± 9.8	44–66
Cardiac-MRI stroke volume		45.3 ± 9	48 ± 11.3	46.4 ± 8.7	36–56
LGE in cardiac-MRI [*n* (%)]		0 (0)	0 (0)	0 (0)	

Abbreviations: A-Gal-activity, alpha-galactosidase enzyme activity; GFR, glomerular filtration rate; IVSd, interventricular septal thickness at diastole; LGE, late gadolinium enhancement; LVEF, left ventricular ejection fraction; LVMi, left ventricular mass index; LVPWd, left ventricular posterior wall thickness at diastole.

frequency in genetic testing, more patients with mutations of unclear significance such as *p.Ser126Gly* are found (Ortiz et al., 2018; van der Tol, Cassiman, et al., 2014). Ortiz et al. described, that pathogenic "classical" mutations are rarer and "non-classical" mutations are more common but difficult to evaluate. In these cases, symptoms can imitate FD but not arise from it (Ortiz et al., 2018). One of the main obstacles for physicians evaluating FD patients is to determine the individual need and ideal time point for specific therapy. Our current clinical report discusses findings from five individuals with *p.Ser126Gly*. Based on clinical evaluation, *p.Ser126Gly* represents a benign variant not causing FD.

Small fiber neuropathy as described in *Patient 1* can be a FD-related symptom, however with low specificity (Üçeyler & Sommer, 2012). In this case it is more likely, that small fiber neuropathy emerged because of a different reason like hypothyroidism (van der Tol, Smid, et al., 2014). *Patient 3* had a lacunar cerebral stroke. Similar to small fiber neuropathy in *Patient 1*, stroke or transient ischemic attack are among the typical spectrum of FD-related symptoms (Liu et al., 2018), but there are also many other potential underlying reasons, often questioning causality. In this case, no specific reason could be evaluated. Hyperlipidemia and smoking history are risk factors, which can lead to stroke. However, previous screening studies showed low prevalence of FD in young patients with cryptogenic stroke or transient ischemic attack (Dubuc et al., 2013; Reisin et al., 2018; Sarikaya et al., 2012).

After precise examination, it was not possible to confirm FD as cause of any of the patients' symptoms. Additionally, alpha-galactosidase A enzyme activity was only mildly reduced in one patient and lyso-GB3 Were in all cases normal. Slightly reduced enzyme activity in *Patient 5* was accompanied by homozygosity for *p.Ser126Gly*, representing a rarity. Despite slightly abnormal enzyme activity, no accumulation of Lyso-GB3 or FD-specific results were detected.

The follow-up of *Patient 4* demonstrates the importance of diagnosing FD patients with high accuracy. Because of its unspecific and wide-ranging symptoms,

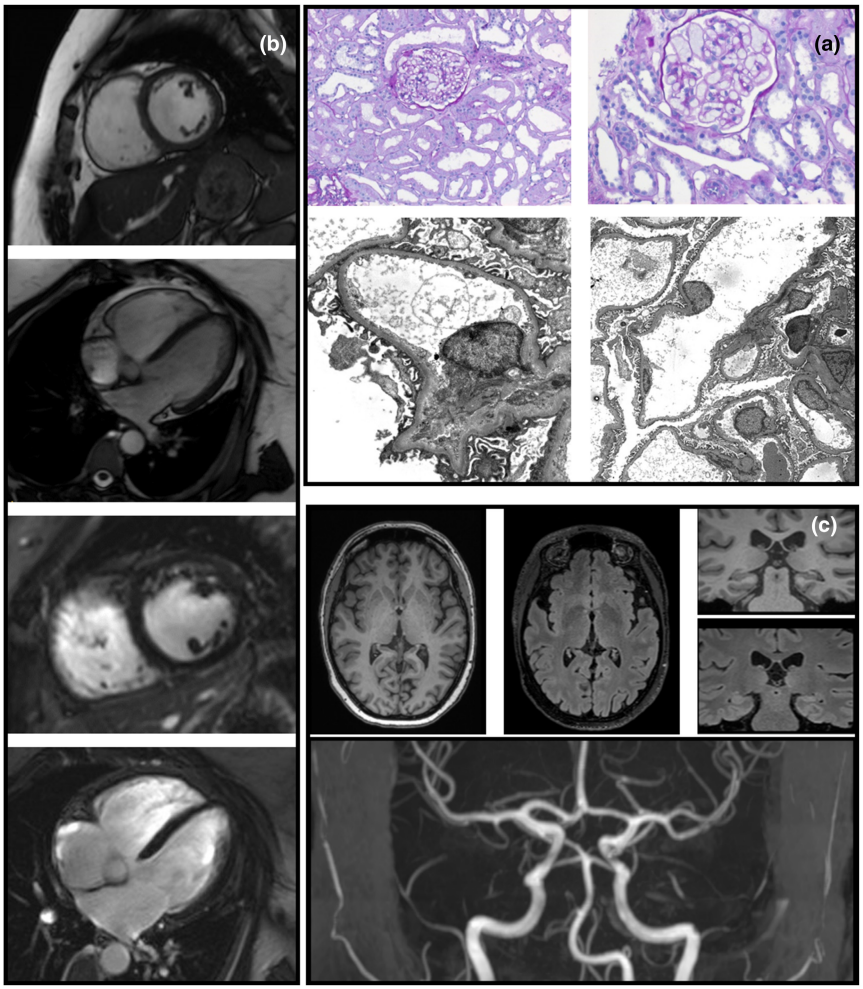

FIGURE 1 12-year follow-up visit of *patient 4*. (a) The upper picture shows PAS-stained glomeruli (20-fold and 40-fold microscopic magnification) with inconspicuous findings. Bottom: Electron microscope views (2000-fold and 5000-fold magnification) with no signs of Fabry-specific changes. (b) Cardiac MRI: Short-axis and long-axis view (upper two pictures: SSFP; lower two pictures: Late gadolinium enhanced T1-IR) reveal no signs of cardiac hypertrophy or fibrosis. (c) Brain MRI: The upper pictures show axial and coronal T1-weighted images and axial and coronal FLAIR images at the level of the basal ganglia, the pulvinar thalami appears isointense to the cortex. The bottom picture of an intracerebral angiography shows no abnormal findings

FD might be suggested in many different clinical findings. *Patient 4* was diagnosed for several potential FD symptoms such as corneal opacity and renal impairment, which in combination can be interpreted as FD-specific symptoms. Coupled with "positive" genetic testing, a diagnosis of FD can be assumed wrongly. In our case, we had to figure out if renal impairment arises from FD and if there is a need for therapy. After extensive investigations, including histology by renal biopsy, FD was not confirmed and FD-specific therapy was therefore not initiated.

Van der Tol et al. created an algorithm for patients with uncertain symptoms and a questionable FD (van der Tol, Smid, et al., 2014). All our examined patients with *p.Ser126Gly* showed insufficient evidence for FD when using this diagnostic tool.

With many new possibilities in genetic testing and rising awareness of FD, it is getting increasingly important to critically evaluate, if specific gene variants have a clinical impact, which might be seen as one key task for specialized FD centers. As shown in previous studies, especially some of the most prevalent genetic variants such as *D313Y* or *A143T* have comparable clinical presentations (Lenders et al., 2016; Oder et al., 2018). These mutations also demonstrate the importance of accurate diagnostics of *GLA* variants of unknown significance. In related cases, it is important to clearly identify and name "*healthy*" individuals and to release these patients from the stigma of being "*chronically and/or severely ill*". This approach is not only important for the patients', but also needed to protect health care systems from unnecessary therapy costs, which, especially in FD, are particularly substantial for each affected individual.

5 | CONCLUSION

Based on findings from extensive clinical characterization, *p.Ser126Gly* appears to be a benign *GLA* gene variant not related to development of FD. This prevents clinical necessity for FD-specific therapy or repetitive short-interval follow-up examinations. Especially in gene variants with unclear clinical significance, precise clinical evaluation and characterization of pathogenicity with highest possible accuracy should be aimed at.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Peter Nordbeck, Christoph Wanner, and Kolja Lau created the study conception and research design. Tereza Cairns, Lora Lorenz, Nurcan Üçeyler, Claudia Sommer, Magnus Schindehütte, and Kerstin Amann performed data collection and patients' examination. All authors performed analysis and interpretation of results. Kolja Lau and Peter Nordbeck wrote the manuscript. All authors reviewed the manuscript and approved the final version of the paper.

ETHICAL COMPLIANCE

Informed consent was obtained by the study participants. Permission to use patient- specific pictures in Figure 1 was obtained. All patients obtained consent to be part in HEAL-FABRY observational study (ClinicalTrials.gov Identifier: NCT03362164).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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