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# Prevalence of Fabry disease and *GLA* variants in young patients with acute stroke: The challenge to widen the screening. The Fabry-Stroke Italian Registry

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ABSTRACT

*Background:* Fabry disease (FD) is a treatable X-linked lysosomal storage disorder caused by *GLA* gene variants leading to alpha-galactosidase A deficiency. FD is a rare cause of stroke, and it is still controversial whether in stroke patients FD should be searched from the beginning or at the end of the diagnostic workup (in cryptogenic strokes).
*Methods:* Fabry-Stroke Italian Registry is a prospective, multicentric screening involving 33 stroke units. FD was sought by measuring α-galactosidase A activity (males) and by genetic tests (males with reduced enzyme activity and females) in patients aged 18–60 years hospitalized for TIA, ischemic stroke, or intracerebral hemorrhage. We diagnosed FD in patients with 1) already known pathogenic *GLA* variants; 2) novel *GLA* variants if additional clinical, laboratory, or family-derived criteria were present.
*Results:* Out of 1906 patients, we found a *GLA* variant in 15 (0.79%; 95%CI 0.44–1.29) with a certain FD diagnosis in 3 (0.16%; 95%CI 0.03–0.46) patients, none of whom had hemorrhage. We identified 1 novel pathogenic *GLA* variant. Ischemic stroke etiologies in carriers of *GLA* variants were: cardioaortic embolism (33%), small artery occlusion (27%), other causes (20%), and undetermined (20%). Mild severity, recurrence, previous TIA, acroparesthesias, hearing loss, and small artery occlusion were predictors of *GLA* variant.
*Conclusion:* In this large multicenter cohort the frequency of FD and *GLA* variants was consistent with previous reports. Limiting the screening for *GLA* variants to patients with cryptogenic stroke may miss up to 80% of diagnoses. Some easily recognizable clinical features could help select patients for FD screening.

## 1. Introduction

Fabry disease (FD) (OMIM 301500) is a rare hereditary disorder, and stroke is one of its neurological complications [1]. Classically reported birth prevalence in the general population, ranging from 1 in 476,000 to 1 in 117,000, largely underestimates the true frequency of the disease [2].

FD is an X-linked lysosomal storage disorder due to *GLA* gene variants that lead to deficiency of the alpha-galactosidase A enzyme (α-Gal) involved in the metabolism of glycosphingolipids [2]. Globotriaosylceramide (Gb3), globotriaosylsphingosine (LysoGb3), and other less well-known catabolites progressively accumulate in lysosomes and other subcellular components of different cells, including neurons, endothelial and smooth muscle cells, cardiomyocytes and specialized cells of the cardiac conduction system [3]. At a later stage further damage is mediated by oxidative stress [4], fibrosis [5] and inflammation [6].

FD has significant phenotypical heterogeneity. The degree of residual activity of α-Gal influences the onset of symptoms and the patient phenotype. In males, an absent or nearly absent activity leads to the classical form, while reduced levels (up to 20% of normal) of α-Gal activity are associated with the late-onset form [2]. In females, clinical manifestations are highly variable, ranging from asymptomatic to severe forms of disease, as a consequence of skewed X chromosome inactivation and other epigenetic mechanisms [7,8].

Since 1990, several mechanisms have been associated with ischemic stroke in Fabry patients (dolichoectasia of cerebral arteries, small vessels disease, cardiogenic embolism, impaired autonomic function, and changes in blood components) and intracerebral hemorrhage has also been comprised in the spectrum of cerebrovascular consequences of the disorder [9]. In 2009 K. Sims et al. reported that stroke occurrence in young people (men aged 35 to 45 years) with FD was 12 times greater than in the general population and emphasized that stroke was frequently the first manifestation of FD to attract clinical attention [10]. In Fabry females, stroke occurs only slightly less frequently and a few years later than in males [10,11].

The diagnosis of FD is differentially made between males and females [12,13]. In males, the detection of reduced or absent α-Gal activity in leukocytes is the diagnostic "gold-standard"; in females, it is always necessary to perform *GLA* gene analysis first because enzyme activity can be normal in a significative proportion of cases [14,15]. Based on the clinical suspicion, there has not been a significant reduction in diagnostic times over recent decades [16]. So, screening of newborns and "high-risk" adult populations (including stroke patients) is an increasingly used strategy to achieve more timely FD diagnoses. In this setting, Dried Blood Spot (DBS) assay is the most used assay for testing both males and females [17], particularly due to the small amount of blood required, the high stability of the enzyme in the spots, and the possibility of simultaneously investigate α-Gal activity and *GLA* gene.

To date, there is no agreement on how to investigate for FD patients admitted for an acute cerebrovascular event. Current stroke guidelines do not recommend routine screening for FD, and cerebrovascular screening programs identify a large number of benign or variants of unknown significance (VUS) in the *GLA* gene, that are clinically difficult to manage [18,19]. In the algorithm proposed by J.L. Saver for cryptogenic stroke, FD-specific investigations are recommended only after excluding more common causes of stroke [20], but this could lead to underdiagnosing those FD patients in whom stroke develops through

common pathophysiologic mechanisms.

Our Fabry-Stroke Italian Registry (FSIR), a nationwide FD screening initiative aims to provide further data about prevalence of FD and *GLA* variants in young adult patients presenting in neurologic stroke services due to acute TIAs or strokes, trying to catch potential "red flags", i.e. signs and symptoms that could trigger earlier the suspicion of FD, and prompt selective investigations.

## 2. Methods

FSIR is a prospective multicenter study involving 33 Italian neurological stroke units spread throughout Italy. The FSIR protocol was approved by the Ethical Committee of the Florence coordinating center (CEAVC: "Comitato Etico Area Vasta Centro" of Tuscany Region, Italy), and then confirmed by the committee of each participating hospital. Centers were selected for their interest in FD and expertise in stroke care and research, but they had very different catchment areas. They were activated in three timeframes (25 hospitals between March 2018 and February 2019; 6 hospitals in March 2019; 2 hospitals in March 2020). The patient enrollment was also partially delayed due to the COVID-19 pandemic and ended on March 31, 2021.

Detailed information about the study protocol is described elsewhere [21]. We report here a brief description of the study methods emphasizing inclusion criteria, screening and data collection procedures, clinical interpretation of the identified variants of *GLA* gene, and the correlated statistical analysis.

After informed consent was obtained, each patient aged 18–60 years and hospitalized for TIA, ischemic stroke or intracerebral hemorrhage was screened for FD and registered in an electronic database accessible at http://www.studiorifs.it with a confidential account. TIA was diagnosed by a stroke specialist in presence of a sudden onset of a focal neurological deficit in the brain or retina attributable to ischemia, lasting less than one hour.

We recorded socio-demographics, stroke type, severity of symptoms (NIHSS scale), recurrence, risk factors, localization at neuroimaging, pathogenesis, FD-suggestive symptoms, and results of FD screening for all cerebrovascular patients. TIA/ischemic stroke etiology was defined using the computerized CCS (Causative Classification of Stroke) algorithm [22]. Intracerebral hemorrhage pathogenesis was assessed by the SMASH-U algorhytm [23].

We used DBS both to estimate α-Gal deficiency and to perform genetic testing. In males, we first measured the activity of α-Gal using a fluorometric assay and then we performed the molecular analysis of the *GLA* gene (exon and intron-exon boundaries) in patients with reduced (<15.3 μmol/L/h) activity. In females, we directly performed molecular analysis to investigate the presence of *GLA* gene variants.

In positive patients, a lysoGb3 dosage by liquid chromatography-mass spectrometry was also performed on DBS (normal values ≤1.8 ng/mL).

Variants of the *GLA* gene sequence were classified as novel or known after consulting the HGMD (Human Gene Mutation Database) [24], revising the published scientific reports, and ascertaining their prevalence using the gnomAD (Genome Aggregation Database) v2.1.1 [25]. According to the American College of Medical Genetics and Genomics (ACMG) criteria each variant was classified as benign, probably benign, VUS, probably pathogenic, or pathogenic [26]. Patients with *GLA* variants underwent standardized evaluation to obtain detailed phenotypic characterization: history of neuropathic pain, hypohidrosis, hearing deficits and gastrointestinal complaints, skin examination, slit-lamp examination, urine examination, albuminuria, 24-h urine protein, glomerular filtration rate, ECG, echocardiography (including measurement of maximal wall thickness), carotid Doppler, brain MRI and MR angiography of intracranial vessels (if not contraindicated; alternatively, head CT scan and transcranial Doppler or CT angiography). Cardiac MRI and skin biopsy for the detection of small fiber neuropathy were not routinely performed, because they were not available in all the involved hospitals.

Due to the lack of α-Gal dosage in leukocytes, we were unable to use the criteria for a certain FD diagnosis proposed by Smid et al. in 2014 [27] and recommended from the European Fabry Working Group (EWFG) in 2015 [12]. Thus, we made a definite diagnosis of FD: 1) in patients carrying a probably pathogenic or pathogenic *GLA* variant (ACMG criteria); 2) in patients carrying a novel *GLA* variant and one of the following additional criteria: (i) ≥1 typical and otherwise unexplained sign of FD (acroparesthesias due to histologically documented small-fiber neuropathy, cornea verticillata, and cluster angiokeratoma); (ii) an increase (>1.8 ng/mL) in plasma lysoGb3; (iii) a family member with a definite FD diagnosis carrying the same *GLA* variant. The distinction between classic and late-onset phenotype was based on the presence of early FD symptoms in childhood-adolescence [2].

Statistical analysis was performed using SPSS software, version 28 (IBM, NY, USA). The prevalence of FD was estimated with a 95% confidence interval (binomial distribution). Demographic and clinical data were resumed with descriptive statistics. For the purposes of statistical analysis, each etiological category of CCS (cardioaortic embolism, other causes, small artery occlusion, large supra-aortic artery atherosclerosis) was considered merging the 3 subcategories "evident, probable, or possible" and all the undetermined subcategories (cryptogenic embolism, other cryptogenic, unclassifiable, and incomplete assessment) were considered together. Univariate analysis for identifying potential markers selectively associated with *GLA* variants was limited to patients presenting with TIA or ischemic stroke. Hemorrhagic patients were excluded because no *GLA* variant was detected in this subgroup. Due to the low number of positive patients identified by the screening process, we used the propensity score (Software R, package MatchIt ver4.3.2, method "nearest", ratio 1:6) to ascertain mechanisms potentially associated with *GLA* variants matching for age, sex, hypertension, hypercholesterolemia, diabetes, and smoking. *P* values <.05 were considered statistically significant.

## 3. Results

Out of the 1953 patients who gave informed consent, 32 were excluded for age ≥ 61 years, and 4 because the diagnosis of TIA, ischemic or hemorrhagic stroke was not confirmed. An additional 11 patients were excluded from the per-protocol population due to absent FD tests. Thus, in the statistical analysis a total of 1906 patients were included.

Median age was 51 (IQR 45–56) years, and 67% of the sample were males. Two hundred forty-six (13%) patients presented with TIA, 1487 (78%) with ischemic stroke, and 173 (9%) with intracerebral hemorrhage; in 207 (11%) cases the event was recurrent. Demographics, stroke severity, recurrence, risk factors, and results of FD tests according to the index event are reported in Table 1.

Fifteen (0.79%; 95% CI: 0.44–1.29) cases with variants in the *GLA* gene sequence were identified among the screened population. No variants were found in patients admitted for hemorrhagic stroke. The frequency of *GLA* variants was 0.63%, (95% CI: 0.27–1.24) in males and 1.10% (95% CI: 0.45–2.26) in females. Their prevalence among the ischemic subgroup of patients (1733) was 0.87% (95% CI: 0.49–1.42).

Table 2 reports the frequency of each *GLA* variant identified in the study compared with that expected in European (non-Finnish) individuals included in gnomAD and reports its pathogenicity in ClinVar according to ACMG criteria.

The novel variant c.1139C > T, p.(Pro380Leu) missense mutation in exon 7 was identified in a 50-year-old male. The patient presented with a cryptogenic ischemic stroke and a history of episodic acroparesthesias (a loss of epidermal nerve fibers with a poor sub epidermal neural plexus was documented by skin biopsy) and angiokeratoma. His α-Gal activity was 2.1 μmol/L/h in DBS (normal values ≥15.3 μmol/L/h) and plasmatic LysoGb3 value was at the upper limit of the normal range (1.8 ng/mL, normal values ≤1.8 ng/mL). The patient's clinical features and

**Table 1**
Main clinical characteristics of the cerebrovascular cohort screened for Fabry disease and results of diagnostic tests.

| | TIA | Ischemic Stroke | ICH | All | P |
|---|---|---|---|---|---|
| | *N* = 246 (13%) | *N* = 1487 (78%) | *N* = 173 (9%) | *N* = 1906 (100%) | |
| Age, years (median, IQR) | 49 (42–54) | 51 (45–56) | 52 (46–56) | 51 (45–56) | 0.002 |
| Males | 148 (60%) | 1005 (68%) | 119 (69%) | 1272 (67%) | 0.060 |
| NIHSS at onset (median, IQR) | 1 (0–3) | 4 (2–8) | 7 (4–12) | 4 (2–8) | <0.001 |
| NIHSS ≥4 at onset | 42 (17%) | 853 (57%) | 133 (77%) | 1028 (54%) | <0.001 |
| Recurrent event | 53 (22%) | 144 (10%) | 10 (6%) | 207 (11%) | <0.001 |
| Previous TIA | 26 (11%) | 49 (3%) | 2 (1%) | 77 (4%) | <0.001 |
| Previous ischemic stroke | 26 (11%) | 100 (7%) | 3 (2%) | 129 (7%) | 0.002 |
| Previous ICH | 0 (0.0%) | 9 (1%) | 4 (2%) | 13 (1%) | 0.014 |
| Hypertension | 103 (42%) | 822 (55%) | 143 (83%) | 1068 (56%) | <0.001 |
| Diabetes | 21 (9%) | 229 (15%) | 23 (14%) | 273 (14%) | 0.016 |
| Hypercholesterolemia | 100 (41%) | 692 (47%) | 61 (36%) | 853 (45%) | 0.008 |
| Atrial fibrillation | 8 (3%) | 93 (7%) | 3 (2%) | 104 (6%) | 0.010 |
| Smoking | 85 (37%) | 669 (47%) | 65 (41%) | 818 (45%) | 0.005 |
| Variants of *GLA* gene | 4 (1.6%) | 11 (0.7%) | 0 (0%) | 15 (0.8%) | 0.163 |
| Definite FD diagnosis | 1 (0.4%) | 2 (0.1%) | 0 (0%) | 3 (0.2%) | 0.528 |

TIA: transient ischemic attack; ICH: intracerebral hemorrhage; IQR: interquartile range; NIHSS: National Institutes of Health Stroke Scale.

genotype-phenotype correlation have been pre-published elsewhere, supporting the diagnosis of FD [28].

The known variants in the 14 remaining patients were: c.937G > T, p.(Asp313Tyr) x5, c.376 A < G, p.(Ser126Gly) x3, c.196G > C p.(Glu66Gln), c.427G > A, p.(Ala143Thr), c.337 T > C p.(Phe113Leu), c.801 + 1G > T splicing donor site, c.856C > G, p.(Leu286Val) missense mutation, and c.1181 T > C, p.(Leu394Pro) in compound heterozygosity with c.427G > A, p.(Ala143Thr). Among them, we formulated 2 additional diagnosis of FD: the 57-year-old patient with the pathogenic c.337 T > C p.(Phe113Leu) missense mutation was admitted for a cardioembolic ischemic stroke in presence of mechanical aortic valve prothesis. α-Gal activity was 2.8 μmol/L/h in DBS (normal values ≥15.3 μmol/L/h) and plasma lysoGb3 was 7.5 ng/mL (normal values ≤1.8 ng/mL). His history was remarkable for acroparesthesias, angiokeratoma, hearing loss, gastrointestinal disturbances, and cardiac involvement (moderate cardiac hypertrophy, pacemaker/implantable defibrillator). The 54-year-old woman with the splicing donor site mutation was admitted for a recurrent TIA and presented with a history of otherwise unexplained neuropathic pain, gastrointestinal disturbances, hearing loss, proteinuria, thickened leaflets of mitral and aortic valves, and cardiac arrhythmias. She refused ophthalmological and dermatological assessment. The plasma LysoGb3 (6.2 ng/mL) was abnormal.

A certain FD diagnosis was so obtained in 3 (0.16%; 95% CI: 0.03–0.46) patients (with the same frequency in males and females). Table 3 summarizes the results of the baseline multisystem staging process of all patients with *GLA* gene variants.

In comparison to the 1718 ischemic controls, patients with *GLA* gene variants presented more frequently with previous TIA, acroparesthesias, angiokeratoma, and hearing loss, and less frequently with hypercholesterolemia. In these patients we observed trends towards mild severity of symptoms, higher recurrence rates, and higher occurrence of gastrointestinal complaints. No statistically significant differences were found regarding stroke etiology (as classified based on CCS algorithm) between the 2 groups of patients but we observed a trend towards a higher frequency of small artery occlusion and the total absence of supra-aortic large artery atherosclerosis among patients carrying *GLA* gene variants. Table 4 reports the results of the univariate analysis between patients with *GLA* variants and ischemic controls.

After matching by the propensity score method, we documented significantly lower NIHSS, higher proportions of recurrent events, prior TIAs, small artery occlusion etiology on CCS, acroparesthesias, and hearing loss among patients with *GLA* gene variants. Trends towards higher frequency of lacunar stroke on neuroimaging and more common gastrointestinal disturbances were also observed. Details of this analysis are reported in Table 5.

## 4. Discussion

In our young-adults nationwide Italian TIA/stroke registry we have observed frequencies of FD and *GLA* gene variants substantially consistent with data hitherto reported from other countries screenings in similar cerebrovascular cohorts. To the best of our knowledge only the Stroke in Young Fabry Patients (SIFAP) study [29] has systematically investigated for FD a larger number of such patients. Differently from our study, stroke pathogenesis among patients with *GLA* variants was not reported. Between 2007 and 2010 SIFAP screened for FD 5023 patients aged 18 to 55 years with TIA, ischemic stroke, or intracerebral hemorrhage. Twenty-seven (0.53%) patients were reported to have a "definite" and additional 18 patients a "probable" diagnosis of FD. We think that the higher prevalence of FD estimated in the SIFAP study (0.53% versus 0.16% in FSIR) may be at least in part attributable to the different criteria used to diagnose FD. Some of the *GLA* variants believed to be causative of FD until 2010, were subsequently reclassified as VUS, likely benign, or benign variants [30], although the debate about their pathogenicity is still open, especially in the neurological setting. Our results completely overlap those reported from the 2018 D. Donhey's review of prevalence of FD and *GLA* variants in high-risk populations [31]. From the 16 studies conducted in the stroke setting in this analysis, 3904 males and 2074 females were tested for FD. The frequency of *GLA* variants was 0.67% in males and 1.11% in females. When only *GLA* variants associated with the classical or late-onset phenotypes were considered, prevalence decreased to 0.13% in males and 0.14% in females.

Identifying a sequence variant in the *GLA* gene of a stroke patient should not lead to the automatic conclusion that the patient has FD. If the *GLA* variant has already been described, a careful examination of the scientific literature with the support of geneticists experienced in FD is advisable [19]. Considering that the *GLA* gene has a great allelic heterogeneity (to date over 1000 *GLA* variants have been reported) [24], the possibility of detecting a novel variant during a screening program is not negligible. The pathogenic role of a new variant should be assessed on the basis of laboratory tests, such as dosage of α-Gal and lysoGb3, clinical phenotype, and, if possible, family studies [19]. In doubtful cases, testing for the glycosphingolipids storage in affected organs is the alternative strategy to confirm FD diagnosis, but, if the involved organ is the brain, this may be obviously tricky. Thus, there is still ongoing controversy in the literature as to whether some *GLA* variants (including the c.937G > T, c.376 A < G, and c.427G > A detected in our study) may be pathogenic or contrarily correspond to a polymorphism/neutral variant. For example, in a very recent meta-analysis, L. Palaiodimou et al. concluded that the c.937G > T variation seems to correlate with an atypical, mild, and late-onset FD phenotype with predominantly neurological manifestations [32]. The authors found an increased prevalence of this variant in neurological patients compared to the general population, and a higher frequency of neurological disturbances (compared to cardiac or renal complications) in its carriers [32]. Although no firm conclusions can be drawn in the absence of robust

**Table 2**
Frequency of *GLA* gene variants in the FSIR cohort compared to European (non-Finnish) individuals in the genome aggregation database (gnomAD) and interpretation of their pathogenicity in ClinVar.

| *GLA* variant | Amino acid change | Frequency in per-protocol FSIR cohort n. 1906 | Frequency in ischemic (TIA+ IS) FSIR cohort n. 1733 | Frequency in gnomAD European (non-Finnish) (male prevalence) | Frequency in gnomAD European (non-Finnish) (absolute prevalence) | Pathogenicity in ClinVar |
|---|---|---|---|---|---|---|
| c.801 + 1G > T | splicing defect | 0.05% | 0.06% | Absent | Absent | Not reported |
| c.1139C > T | p.(Pro380Leu) | 0.05% | 0.06% | Absent | Absent | Not reported |
| c.337 T > C | p.(Phe113Leu) | 0.05% | 0.06% | Absent | Absent | Pathogenic |
| c.856C > G | p.(Leu286Val) | 0.05% | 0.06% | Absent | Absent | Not reported |
| c.1181 T > C | p.(Leu394Pro) | 0.05% | 0.06% | 0.007% (1:14018) | 0.004% (1:26696) | Uncertain significance |
| c.196G > C | p.(Glu66Gln) | 0.05% | 0.06% | 0.00% (0:16230) | 0.00% (0: 46420)† | Conflicting interpretations of pathogenicity: Uncertain significance(5); Likely benign(5) |
| c.376 A > G | p.(Ser126Gly) | 0.16% | 0.17% | 0.07% (1:1338) | 0.07% (1:1426) | Conflicting interpretations of pathogenicity: Uncertain significance(2); Benign(2); Likely benign(11) |
| c.427G > A | p.(Ala143Thr) | 0.10% | 0.11% | 0.09% (1:1054) | 0.07% (1:1379) | Conflicting interpretations of pathogenicity: Pathogenic(1); Likely pathogenic(5); Uncertain significance(16); Likely benign(2) |
| c.937G > T | p.(Asp313Tyr) | 0.26% | 0.28% | 0.45% (1:220) | 0.44% (1:228) | Conflicting interpretations of pathogenicity: other Uncertain significance(2); Benign(7); Likely benign(13) |

FSIR: Fabry-Stroke Italian Registry; gnomAD: genome aggregation database; FD: Fabry disease; TIA: Transient Ischemic Attack, IS: ischemic stroke; ACMG: American College of Medical Genetics and Genomics. gnomAD was consulted on December 5, 2023 at https://gnomad.broadinstitute.org/gene/ENSG00000102393?dataset=gnomad_r4; ClinVar was consulted on December 5, 2023 at https://www.ncbi.nlm.nih.gov/clinvar/

† The p.(Glu66Gln) was identified in an East Asian male patient living in Italy. The frequency in the entire gnomAD East Asian population is 0.46% (1:215), while in the East Asian male population is 0.45% (1:220).

histopathological data, a role for this and other *GLA* gene variants in increasing susceptibility to cerebrovascular (such as stroke or white matter lesions of presumed vascular origin) or neurological manifestations cannot be ruled out. In addition, M. Živná et al. recently investigated an intriguing new mechanism of cell damage in FD, which might be most relevant in patients with *GLA* mutations causing the α-Gal misfolding [33]. In their paper, they documented in a family carrying the *GLA* variant c.1181 T > C (the same that we discovered in compound heterozygosity with the c.427G > A and we interpreted on the basis of our criteria as VUS), a defective proteostasis of mutated α-Gal, resulting in chronic endoplasmic reticulum stress and unfolded protein response, which they said represents a pathway of FD pathogenesis independent from glycosphingolipid accumulation. They called this mechanism AGALopathy and documented similar observations in the case of the *GLA* variant c.427G > A (another of the *GLA* variants we identified).

Further basic research studies may aid to better understand the role of these missense variants in patients with neurological disorders.

From our results the effectiveness of systematic FD screening in young adult patients presenting with acute TIA/stroke cannot be conclusively determined. However, the more detailed information gathered about clinical and pathogenic characteristics of screened cerebrovascular events allowed us to propose with more confidence which features should lead to suspect FD and *GLA* variant in the cerebrovascular setting.

It seems that, in the 18 to 60 years age groups we have analyzed, patients with ICH or ischemic stroke due to large artery atherosclerosis have a quite low probability of carrying a definite FD or a *GLA* variant. The frequency of FD in our ICH subgroup was null. ICH accounts for 11–22% of all incident strokes [34], and an even higher frequency may be expected in younger males [35]. We found a slightly lower prevalence of ICH (9%), so a selection bias cannot be excluded; unlike ischemic stroke patients, young ICH patients may be preferentially admitted to neurosurgery or intensive care. Nevertheless, in FD populations ICH appears to be less common than ischemic stroke. In the analysis of the international Fabry Registry by K. Sims et al., only 16 of 138 FD patients with stroke had ICH [10]. The median age of these patients was 48 years (26 to 57) in men and 58 years (33 to 65) in women, approximately 10 years more than for ischemic stroke. In a large FD screening study of 373 Chinese ICH patients of all ages, one case was identified as a definite carrier of FD [36], but no pathogenic mutation was identified within hemorrhagic patients in other screening studies conducted in both Asian and non-Asian patients [37–40].

Ischemic stroke due to small vessel occlusion seems to be the most rewarding category to screen for FD in young adulthood. Small arteries are known to be a target for glycosphingolipid accumulation in FD in terms of the underlying mechanism, and in the Fabry Registry this pathogenesis accounts for 70% of ischemic strokes [10]. The frequency of cardioaortic embolism was similar in patients with or without *GLA* variants probably because in the screened age range the frequency of atrial fibrillation related to FD is still low.

The recurrence of cerebrovascular events, particularly the relapse after a TIA, may be another potential marker leading to search for FD. A cerebrovascular relapse, despite secondary prevention and cardiovascular risk factors control, may suggest an unusual etiology. A high recurrence rate (43%) has been documented in patients diagnosed to be affected by FD after screening for cryptogenic strokes, or among patients with stroke presenting as the first symptom of FD [41]. Prompt testing for FD may be important in patients with recurrence to introduce appropriate etiological treatment. In fact, a recent meta-analysis has shown a beneficial effect on stroke prevention of enzyme replacement therapy [42].

The last “red flag” for *GLA* variants emerging from our young cerebrovascular population is the history of acroparesthesias or hearing loss. Conversely, no significant association with both kidney and heart disease was found. We detected a low frequency of *GLA* variants associated with classical and late-onset phenotypes, whereas signs of cardiac and

**Table 3**
Baseline assessment of patients carrying a variant of *GLA* gene.

| Sex | Age | *GLA* variant | Novel variant | α-Gal μmol/L/h | LysoGb3 ng/mL | Early symptoms | Index event | WMH | Renal involvement | Cardiac Involvement | Risk factors | Definite FD diagnosis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F | 54 | c.801 + 1G > T (splicing defect) | No | n.a. | 6.2 | AP HL GI | Recurrent TIA NIHSS 0 | n.a. | Proteinuria | Supraventricular tachycardia, thickened valve leaflets | No | Yes |
| M | 50 | c.1139C > T p.(Pro380Leu) | Yes | 2.1 | 1.8 | AP† AK | Ischemic stroke NIHSS 12 | Yes | No | No | Hypertension Smoking | Yes |
| M | 36 | c.856C > G; p.(Leu286Val) | No | 2.8 | 1.6 | No | Ischemic stroke NIHSS 2 | Yes | No | No | High lipoprotein (a) | No |
| F | 40 | c.427G > A p.(Ala143Thr) + c.1181 T > C p.(Leu394Pro) | No | n.a. | 1.3 | No | Ischemic stroke NIHSS 8 | Yes | No | No | Hypercholesterolemia Contraceptives Thrombophilia | No |
| M | 57 | c.337 T > C; p.(Phe113Leu) | No | 2.8 | 7.5 | AP AK GI HL | Ischemic stroke NIHSS 3 | No | No | Cardiac hypertrophy Arrhythmias + PM/ICD Mechanical aortic valve prothesis | Hypertension Diabetes | Yes |
| M | 46 | c.427G > A; p.(Ala143Thr) | No | 9.7 | 1.7 | No | Ischemic stroke NIHSS 7 | No | No | First degree AV block | No | No |
| F | 47 | c.376 A > G; p.(Ser126Gly) | No | n.a. | 1.1 | AP | Recurrent ischemic stroke NIHSS 2 | Yes | No | No | Hypertension Hypercholesterolemia Contraceptives Thrombophilia | No |
| F | 60 | c.376 A > G; p.(Ser126Gly) | No | n.a. | 0.7 | No | Ischemic stroke NIHSS 0 | Yes | No | No | Hypertension Smoking | No |
| F | 49 | c.376 A > G; p.(Ser126Gly) | No | n.a. | n.a. | AP | Recurrent TIA NIHSS 1 | Yes | Mild proteinuria | No | Hypertension | No |
| F | 49 | c.937G > T; p.(Asp313Tyr) | No | n.a. | 1.3 | No | Recurrent TIA NIHSS 1 | No | No | No | Hypercholesterolemia | No |
| M | 57 | c.937G > T; p.(Asp313Tyr) | No | 13.9 | 1.0 | No | Ischemic stroke NIHSS 2 | Yes | No | No | Smoking | No |
| F | 43 | c.937G > T; p.(Asp313Tyr) | No | n.a. | n.a. | AP GI | Ischemic stroke NIHSS 7 | n.a. | No | No | Hypertension Alcohol | No |
| M | 50 | c.937G > T; p.(Asp313Tyr) | No | 14.4 | 1.1 | No | Ischemic stroke NIHSS 0 | No | No | No | Hypertension Hypercholesterolemia | No |
| M | 59 | c.937G > T; p.(Asp313Tyr) | No | 11.9 | 0.6 | CV HL | TIA NIHSS 2 | Yes | No | Cardiac hypertrophy | No | No |
| M | 42 | c.196G > C; p.(Glu66Gln) | No | 18.1 | 1.0 | No | Ischemic Stroke NIHSS 2 | Yes | No | No | Hypertension Hypercholesterolemia | No |

† histological evidence of small fiber neuropathy. α-Gal: alpha galactosidase A (normal value: ≥ 15.3 μmol/L/h); LysoGb3: globotriaosylsphingosine (normal value: ≤ 1.8 ng/mL); WMH: white matter hyperintensities; AP: acroparesthesias; HL: hearing loss; GI: gastrointestinal disturbances; AK: angiokeratoma; CV: cornea verticillata; TIA: transient ischemic attack; NIHSS: National Institutes of Health Stroke Scale.

renal involvement should be expected only for this type of *GLA* variants. The relationship between *GLA* variants (of any type) and concomitant disorders of other parts of the nervous system would be an interesting area for future research.

### 4.1. Study limitations

The first potential concern relates to the criteria for FD diagnosis that we used in the FSIR. We classified only a few cases as affected by FD within the larger group of patients carrying a *GLA* variant. The understanding of the pathogenicity of a *GLA* variant is likely to evolve in parallel with the reports describing it. We cannot exclude the possibility that the prevalence of FD within our screened stroke population may be recalculated in the future. Moreover, the screening procedure we adopted may have underestimated in males the prevalence of some more benign *GLA* variants associated with normal enzyme activity on DBS.

Our prevalence data comes from a hospital setting, and this may have reduced the strength of the correlation between FD and TIA as index event, as many of the patients with TIA are not hospitalized in Italy.

Despite the relatively large cohort studied, the small number of cases identified with FD as potential cause of stroke precludes statistically affordable guidelines on features that should lead to investigation for FD after hospital admission. Definite recommendations can only come from larger studies or meta-analyses of studies using similar definition

**Table 4**
Univariate analysis comparing main cerebrovascular clinical features, risk factors, neuroimaging, potential clinical markers of Fabry disease, and stroke etiology between patients with *GLA* gene variants and ischemic controls of FSIR cohort.

| | Patients with *GLA* gene variant n. 15 | Ischemic controls n. 1718 | p |
|---|---|---|---|
| Main cerebrovascular clinical features | | | |
| Age (median, IQR) | 50 (43–57) | 51 (45–56) | 0.840 |
| Males (%) | 8 (53%) | 1145 (67%) | 0.277 |
| NIHSS (median, IQR) | 2 (1–7) | 4 (2–7) | 0.074 |
| Major stroke (NIHSS ≥4) | 4 (27%) | 892 (52%) | 0.051 |
| Index event | | | |
| TIA | 4 (27%) | 242 (14%) | 0.165 |
| Ischemic stroke | 11 (73%) | 1476 (86%) | |
| Recurrent event | 4 (27%) | 193 (11%) | 0.061 |
| Previous TIA | 3 (20%) | 72 (4%) | 0.003 |
| Previous ischemic stroke | 1 (7%) | 125 (7%) | 0.928 |
| Previous intracerebral hemorrhage | 0 (0.0%) | 9 (0.5%) | 0.779 |
| Lacunar syndrome | 6 (40%) | 494 (29%) | 0.261 |
| Risk factors | | | |
| Hypertension | 7 (47%) | 918 (54%) | 0.592 |
| Diabetes | 1 (7%) | 249 (15%) | 0.389 |
| Hypercholesterolemia | 2 (13%) | 789 (46%) | 0.011 |
| Smoking | 4 (27%) | 750 (46%) | 0.193 |
| Neuroimaging | | | |
| Lacunar infarct | 6 (40%) | 462 (27%) | 0.255 |
| Not lacunar infarct | 5 (33%) | 880 (51%) | 0.168 |
| Multiple infarcts | 5 (33%) | 499 (32%) | 0.769 |
| Supratentorial lesion | 11 (79%) | 1154 (74%) | |
| Infratentorial lesion | 2 (14%) | 274 (18%) | |
| Both | 1 (7%) | 129 (8%) | 0.930 |
| Potential clinical markers of Fabry disease | | | |
| Acroparesthesias | 4 (27%) | 136 (8%) | 0.009 |
| Angiokeratoma | 1 (7%) | 8 (1%) | 0.001 |
| Gastrointestinal disturbances | 3 (20%) | 128 (8%) | 0.071 |
| Hearing loss | 3 (20%) | 75 (4%) | 0.004 |
| High protein on urine spot analysis | 1 (7%) | 192 (12%) | 0.615 |
| Renal failure | 0 (0%) | 74 (4%) | 0.410 |
| Atrial fibrillation | 0 (0%) | 101 (6%) | 0.325 |
| Cardiac hypertrophy | 3 (20%) | 339 (21%) | 0.920 |
| Stroke etiology (CCS scale)* | | | |
| Cardioaortic embolism | 5 (33%) | 470 (27%) | 0.614 |
| Other causes | 3 (20%) | 307 (18%) | 0.837 |
| Small artery occlusion | 4 (27%) | 332 (19%) | 0.480 |
| Supra-aortic large artery atherosclerosis | 0 (0%) | 180 (11%) | 0.184 |
| Undetermined/incomplete evaluation | 3 (20%) | 421 (25%) | 0.679 |

* CCS: Causative Classification of Stroke; IQR: interquartile range; NIHSS: National Institutes of Health Stroke Scale; TIA: transient ischemic attack.

criteria.

## 5. Conclusion

In conclusion, in this multicenter Italian cohort of young adults presenting in the acute setting with TIA/stroke, the prevalence of *GLA* variants known to cause FD was confirmed to be low together with a higher prevalence of other more benign variants of the *GLA* gene. The detailed, although statistically not conclusive, data we have collected, may suggest that special attention should be paid to ischemic patients with mild severity, recurrence after a TIA, lacunar stroke, and complaints of acroparesthesias or hearing loss. In young stroke patients, ICH and large artery atherosclerosis do not seem to be related to FD. Limiting the search for FD to only patients with cryptogenic stroke can miss a great proportion of diagnoses.

**Table 5**
Comparison by propensity score method between 15 patients with *GLA* gene variant and 90 matched ischemic controls.

| | Patients with *GLA* gene variant n. 15 | Control group n. 90 | p |
|---|---|---|---|
| Main cerebrovascular clinical features | | | |
| Age (median, IQR) | 50 (43–57) | 50 (42–54) | 0.791 |
| NIHSS (median, IQR) | 2 (1–7) | 5 (2–10) | 0.038 |
| Major stroke (NIHSS ≥4) | 4 (27%) | 53 (59%) | 0.020 |
| Stroke as index event | 11 (73%) | 75 (83%) | 0.352 |
| Recurrent event | 4 (27%) | 8 (9%) | 0.045 |
| Previous TIA | 3 (20%) | 2 (2%) | 0.003 |
| Previous ischemic stroke | 1 (7%) | 6 (7%) | 1.000 |
| Previous intracerebral hemorrhage | 0 (0%) | 0 (0%) | – |
| Lacunar syndrome | 6 (40%) | 22 (26%) | 0.250 |
| Neuroimaging | | | |
| Lacunar infarct | 6 (40%) | 18 (20%) | 0.088 |
| Not lacunar infarct | 5 (33%) | 49 (54%) | 0.130 |
| Multiple infarcts | 5 (33%) | 25 (31%) | 0.873 |
| Supratentorial lesion | 11 (79%) | 63 (79%) | 0.821 |
| Infratentorial lesion | 2 (14%) | 8 (10%) | |
| Both | 1 (7%) | 9 (11%) | |
| Potential clinical markers of Fabry disease | | | |
| Acroparesthesias | 4 (27%) | 4 (5%) | 0.003 |
| Angiokeratoma | `1 (7%) | 2 (2%) | 0.350 |
| Gastrointestinal disturbances | 3 (20%) | 5 (6%) | 0.058 |
| Hearing loss | 3 (20%) | 4 (%%) | 0.027 |
| High protein on urine spot analysis | 1 (7%) | 9 (11%) | 0.596 |
| Renal failure | 0 (0%) | 1 (1%) | 0.682 |
| Atrial fibrillation | 0 (0%) | 10 (12%) | 0.164 |
| Cardiac hypertrophy | 3 (20%) | 15 (17%) | 0.811 |
| Stroke etiology (CCS scale) | | | |
| Cardioaortic embolism | 5 (33%) | 3 (39%) | 0.682 |
| Other causes | 3 (20%) | 18 (20%) | 1.000 |
| Small artery occlusion | 4 (27%) | 8 (9%) | 0.045 |
| Supra-aortic large artery atherosclerosis | 0 (0%) | 9 (10%) | 0.200 |
| Undetermined/incomplete evaluation | 3 (20%) | 20 (22%) | 0.847 |

We used the propensity score method with a ratio of 1:6 to obtain a control group homogeneous for age, sex, hypertension, hypercholesterolemia, diabetes, and smoking. CCS: Causative Classification of Stroke; IQR: interquartile range; NIHSS: National Institutes of Health Stroke Scale; TIA: transient ischemic attack.

## Contributorship

IR contributed to the conceptualization of the study, data curation, formal analysis, original drafting, revision, and editing of the manuscript; CS contributed to the data curation, formal analysis, original drafting, revision, and editing of the manuscript; PN contributed to the conceptualization of the study, formal analysis, and revision of the paper; GP contributed to the software supervision, formal analysis, and revision of the paper; MZ, VC, AN, JM, DO, DT, PP,CC, VP, LB, RB, US, MR, DMM, RT, GV, MD, GB, AMC, AC, SR, EC, GL, SS, MR, AG, BB, MM, LPC, RCD, IG, UA, MRDR, MM, ES, MV, RV, RFM, BP, VD, GP, AG, FDM, RS, MA, SN, FD, MLDA, MGC, AP, BC, FN, FDL, SF, MLD, SCB contributed to the data curation and revision of the manuscript, SB and DA contributed to the data curation, formal analysis, and revision of the manuscript, RM and AM contributed to the formal analysis and revised the paper, DI made a substantial contribution to the conceptualization, funding acquisition, investigation methodology, formal analysis, and revision of the manuscript; he is the guarantor of the study. All authors approved the manuscript.

## Declaration of competing interest

IR received travel grants and speaker's honoraria from Takeda, Sanofi, and Amicus; PN received speaker's honoraria from Takeda, Sanofi, and Amicus; MZ received fees as consultant and advisory board member from Takeda, Sanofi, and Amicus; SS received personal fees as speaker or advisor (Abbott, Allergan-Abbvie, AstraZeneca, Eli Lilly, Lundbeck, Novartis, NovoNordisk, Pfizer, Teva), research grants (Allergan, Novartis, Uriach), and fees for CME/education (Medscape, Neurodiem Ology Medical Education); UA received speaker's fees and honoraria from EISAI; AM received speaker's honoraria and travel grants from Takeda, Sanofi, and Amicus; DI received speaker's honoraria from Takeda. Other authors declared that they have no competing interests for FSIR study.

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