Prevalence of GLA gene mutations and polymorphisms in patients with multiple sclerosis: A cross-sectional study

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A R T I C L E   I N F O

A B S T R A C T

Purpose: Fabry Disease (FD) has been frequently proposed as possible underestimated differential diagnosis of Multiple Sclerosis (MS), but no study has been performed to test prevalence of GLA gene mutations in a population fulfilling diagnostic criteria of MS. Aim of this study is to determine the prevalence of GLA gene mutations in a large and representative population diagnosed with MS, simultaneously providing a critical revision of current literature reports of coexistence or misdiagnosis between these two conditions.

Methods: In this mono-centric cross-sectional study, 927 patients fulfilling McDonald diagnostic criteria and encompassing all MS phenotypes were enrolled. Patients underwent evaluation of α-GalA activity and genotyping. Both genetic variants annotated as pathogenic and GVUS were considered. Estimated alleles frequencies were then compared to the ones reported in the gnomAD database.

Results: GLA gene variants were found in seven individuals. Five patients carried variants previously described having controversial impact on FD phenotype, and the analysis of exome database revealed that they are not rare among healthy individuals. One patient showed a new variant never described before, and another one carried a late-onset FD cardiac variant.

Conclusions: The overall prevalence of GLA gene variants in MS patients is comparable to the one estimated in healthy population. This result is further supported by critical revision of current literature evidences of misdiagnosis between MS and FD, arguing in favour of independence between these disorders.

1. Introduction

Fabry Disease (FD) (OMIM#301500) is a rare X-linked lysosomal storage disorder caused by mutations in the GLA gene, resulting in a defect of enzyme α-galactosidase A (α-GalA), with progressive accumulation of undegraded glycosphingolipids (especially globotriaosylceramide – Gb3) in different tissues [1]. Estimated to range between 1:50.000 to 1:117.000 newborns [2], its prevalence has been recently reassessed, including genetic variants of unknown significance (GVUS) and milder forms, previously underdiagnosed [3–5]. Furthermore, recent studies suggested that FD incidence could be higher than reported especially when considering specific high-risk

populations, underlying the importance of including FD among differential diagnoses of a large variety of pathological conditions [6–8].

Being FD phenotype the result of a multi-domain disease model [9], its clinical manifestations can be extremely heterogeneous. Usually occurring during childhood or adolescence, classical FD include autonomic neuropathies, acroparesthesia, angiokeratomas, corneal and lenticular opacities, along with a systemic involvement affecting kidney, heart, gastrointestinal system and brain, sometimes leading to premature death [10]; these patients can benefit from a timely enzyme replacement therapy (ERT) in order to reduce symptom severity, slow down disease progression and improve overall prognosis [7,11]. Concerning central nervous system (CNS) involvement, the spectrum of possible signs and symptoms has been recently expanded to also include much rare findings [12–17].The most common manifestations are known to be represented by acute cerebrovascular events, probably the main causes of permanent impairment in FD patients [6], and high white matter lesion (WML) burden, that may mimic demyelinating disorders [1,18]. On this basis, FD has been recently proposed as a possible and underestimate differential diagnosis of Multiple Sclerosis (MS), one of the most common causes of neurological disability in young adults [19–21]. A number of cases of misdiagnosis or coexistence of MS and FD have been reported in the last years, despite the two conditions being generally distinguishable due to multi-organ involvement, different magnetic resonance imaging (MRI) findings, positive familiar history and/or type of neurological onset [22–27]. However, to date no study was performed to investigate number of GLA variants in a large and representative population fulfilling diagnostic criteria of MS. With this knowledge, we aimed to assess the prevalence of GLA gene variants in a setting of clinical definite MS and speculate on their possible clinical significance.

2. Materials and methods

2.1. Subjects

From September 2016 to September 2017, among all the patients referring to the MS centre of University of Naples "Federico II", we enrolled 927 consecutive unrelated MS patients (309 male [33.3%], 618 female [66.7%]). All subjects expressed written informed consent to participate in the study, and trained medical staff provided a comprehensive explanation of possible implications of the participation. The study was approved by the local institutional review board, in accordance to the Declaration of Helsinki.

Inclusion criteria were adult age (age $\geq$ 18 years), clinical diagnosis of MS according to the revised McDonald criteria [28] and availability to collect a blood sample. All MS phenotypes were included in the study, ranging from clinically isolated syndrome (CIS) to progressive courses, with the following proportion: 3 CIS (0.3%), 714 relapsing remitting (RR-MS) (77.0%), 172 secondary progressive (SP-MS) (18.6%) and 38 primary progressive (PP-MS) (4.1%).

At the date of the enrolment, 909 patients (98.0%) were under immuno-modulatory treatment with the following drugs: interferon β-1a (n = 238, 26.2%), fingolimod (n = 195, 21.5%), natalizumab (n = 111, 12.2%), dimethyl fumarate (n = 98, 10.9%), interferon β-1b (n = 83, 9.1%), glatiramer acetate (n = 69, 7.6%), teriflunomide (n = 69, 7.6%), alemtuzumab (n = 35, 3.8%), siponimod (n = 9, 0.9%), rituximab (n = 1, 0.1%), or ocrelizumab (n = 1, 0.1%).

Demographic and clinical data of all the subjects included in the analysis are available in Table 1.

All patients underwent a blood sampling collected during a scheduled routine clinical examination, and samples were sent to the designated study laboratory at the National Research Council of Italy, Institute for Research and Biomedical Innovation, Palermo (Italy) for FD screening. Positive screens included both genetic variants annotated as unknown, pathogenic or atypical, as well as GVUS. Participants with positive screens were referred to FD reference centre at University of

Naples "Federico II" for further investigation, diagnosis and clinical management according to the most recent guidelines [29,30].

2.2. α-GalA activity assay and genetic analysis

For all subjects the evaluation of α-GalA activity and the GLA gene test was performed using the Dried Blood Filter Paper test.

For male patients, a preliminary evaluation of α-GalA activity was performed, and subjects showing an enzymatic activity < 5 nmol/h/ml underwent genetic testing. On the other hand, all female subjects were directly tested for possible mutation of the GLA gene, and evaluation of α-GalA activity was performed in those showing a positive genetic test.

Genetic analysis was conducted as follows: DNA samples were isolated by column extraction (GenElute Blood Genomic DNA Kit, Miniprep, Sigma-Aldrich, USA), and concentrations were determined using a spectrophotometer. Eight target regions, containing the seven exons of the GLA gene (including the regulatory sequences flanking them as well as the cryptic exon) were investigated. Using an automated DNA sequencer at BMR Genomics, PCR products were purified and sequenced to detect the presence of mutations in GLA gene.

2.3. Alleles pathogenic annotation and frequency estimation

Pathogenicity of GLA variants was defined according to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), a NCBI free and open-access archive of human genetic variants providing information on their clinical relevance in determining phenotypical manifestations of the related disease [31]. FD alleles frequencies found in the screened MS population have been compared to the ones reported in gnomAD database (https://gnomad.broadinstitute.org) in November 2019, using the Fisher exact test with alpha = 0.05.

3. Results

In our group of 927MS patients, 7 subjects (5 females and 2 males) carried GLA genetic variants (gene location: NM_000169.2), corresponding to a prevalence of 0.75%. GLA gene variants were first mentioned in accordance to Human Genome Variation Society recommendations [32].

3.1. Patient #1

Patient #1 was a 35-year-old man who received a diagnosis of RR-MS eight years before, with a clinical onset characterized by vertigo and diplopia due to brainstem involvement; oligoclonal bands (OB) at diagnosis were not available. Genetic analysis showed the presence of a c.937G > T mutation in the exon 6 of the GLA gene, causing an amino acid replacement p.Asp313Tyr (D313Y) already reported in ClinVar database as a mutation with "conflicting interpretations of pathogenicity" for FD. When we analysed the exome database gnomAD, we found that this variant is present on 624/205260 normal alleles (minor allele frequency = 0.003). First-degree family assessment revealed no suspected FD. At the neurological examination he showed a motor deficit affecting inferior limbs, while the MR scan showed the presence of a low T2w lesion load, with presence of bilateral rounded hyperintense lesions of the corona radiata, along with some small punctuate foci in juxtacortical frontal WM (Fig. 1A); cervical spine posterior columns involvement was also visible at spine MRI examination.

3.2. Patient #2

Patient #2 was a 63-year-old woman with eleven years of RR-MS duration after an onset characterized by vertigo, visual disturbance and dizziness, with no significant gait abnormality; OB and visual evoked potentials resulted normal at diagnosis. At the genetic testing she presented a c.337 T > C mutation in the exon 2 of the GLA gene causing a

Table 1
Demographic and clinical data of all subjects included in the study.

	MS ($n = 927$)	CIS ($n = 3$)	RR-MS ($n = 714$)	SP-MS ($n = 172$)	PP-MS ($n = 38$)
Age (mean $\pm$ SD)	45.4 $\pm$ 12.1	29.7 $\pm$ 8.5	43.2 $\pm$ 11.4	53.4 $\pm$ 9.6	52.5 $\pm$ 12.3
Sex (M/F)	309/618	0/3	218/496	69/103	22/16
EDSS median (range)	3 (1–9)	1.5 (1–3.5)	3.2 (1–9)	6 (2–8)	6 (3.5–9)
DD (mean $\pm$ SD)	14.7 $\pm$ 9.4	1.7 $\pm$ 1.2	13.0 $\pm$ 8.6	21.9 $\pm$ 9.4	14.2 $\pm$ 8.0

Abbreviations -MS: Multiple Sclerosis; CIS: Clinically Isolated Syndrome; RR-MS: Relapsing Remitting Multiple Sclerosis; SP-MS: Secondary Progressive Multiple Sclerosis; PP-MS: Primary Progressive Multiple Sclerosis; SD: Standard Deviation; EDSS: Expanded Disability Status Scale; DD: disease duration.
Notes: Age and DD are expressed in years.

p.Phe113Leu (F113L) substitution, never reported before in ClinVar database, with normal enzyme levels. This variant has been previously associated to later-onset atypical FD, and was not present in gnomAD. Neurological examination showed the presence of nystagmus, dysmetria, lower limbs weakness, with relative preservation of the other exteroceptive or proprioceptive sensations. Brain MRI scan was characterized by diffuse and confluent WM hyperintensities affecting semioval centres and corona radiata bilaterally, periventricular WM and corpus callosum (Fig. 1B), while cervical spine was spared. First-degree family assessment revealed 2 suspected FD subjects, resulted positive for the same mutation at a subsequent genotyping.

3.3. Patient #3

Patient #3 was a 26-year-old female patient with eight years of RR-MS history, after her onset characterized by diplopia and dizziness with normal OB and visual evoked potentials. Genetic test provided a c.376A > G mutation in the exon 3 of the gene, causing a p.Ser126Gly (S126G) substitution, already reported in ClinVar database as a mutation with "conflicting interpretations of pathogenicity", with normal enzymatic activity. When we analysed the exome database gnomAD, we found that this variant is present on 74/205438 normal alleles (MAF = 0.0004). In addition, she showed the following polymorphisms of the GLA gene: IVS2-77_81del5, IVS4-16A > G and IVS6-22C > T in homozygosis, as well as −10C > T in heterozygosis. First-degree family assessment revealed no suspected FD. At neurological

examination, she presented with mild weakness and hypopallesthesia of lower limbs, hyperreflexia coupled to positive Babinski sign of the left inferior limb, and urinary retention. The MR scan showed the presence of low lesions burden, although with a typical pattern of distribution with T2 hyperintensities affecting juxtacortical and deep WM of both cerebral hemispheres, as well as the isthmus of the corpus callosum (Fig. 1C); short segment focal wedge-shaped involvement of the posterior column was also visible at MRI.

3.4. Patient #4

Patient #4 was a 47-year-old man with RR-MS duration of seventeen years with normal OB and altered visual evoked potentials. When screened, she showed the same genetic variant of patient #1 (single disease-neutral variant D313Y - p.Asp313Tyr - substitution caused by a c.937G > T mutation in the exon 6), with reduced enzyme levels. First-degree family assessment revealed no suspected FD. Neurological examination revealed decreased visual acuity coupled to hyperreflexia of both superior and inferior limbs. He experienced a typical MS onset, characterized by optic neuritis. Conversely, the evaluation of the MRI scan showed the presence of an atypical pattern of lesion distribution for MS with a mild lesion load characterized by sparse periventricular and basal ganglia T2 hyperintensities (Fig. 1D), along with a short segment posterior columns myelopathy.
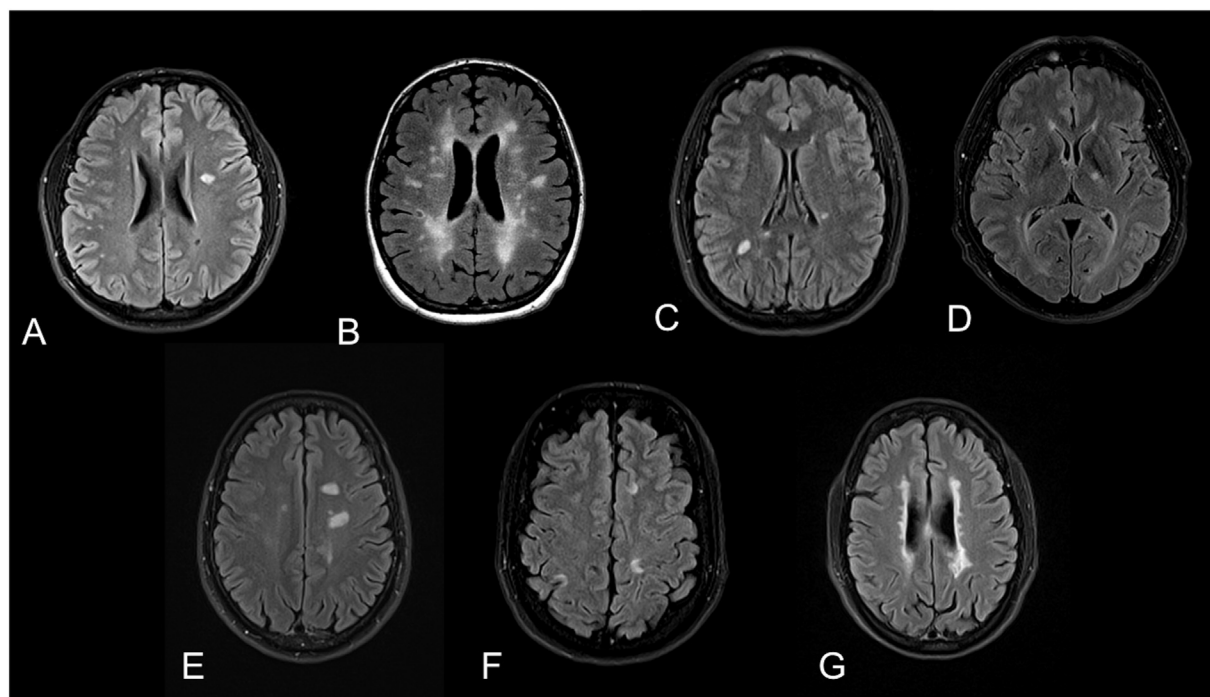
Fig. 1. Axial Fluid-Attenuated Inversion Recovery MRI sequences at the most representative level of MS patients with GLA gene mutations and/or polymorphisms.

Table 2
Demographic and MS related clinical data of subjects showing a mutation of the GLA gene.

Patient	Age	Sex	MS type	EDSS	DD	Total relapses number	ARR	Onset
1	35	M	RR-MS	1.5	7.4	2	0.27	Diplopia and vertigo
2	63	F	RR-MS	2.5	10.9	2	0.18	Vertigo, diplopia and dizziness
3	28	F	RR-MS	2	4.5	1	0.22	Diplopia and dizziness
4	48	M	RR-MS	3	16.0	6	0.37	Visual deficits
5	56	F	RR-MS	2	24.0	3	0.12	Lower limbs hypotonia and paraesthesia
6	34	F	RR-MS	4	16.8	12	0.72	Upper limbs dysesthesia and hypotonia
7	43	F	SP-MS	6	19.3	24	1.25	Visual and auditory deficits

Abbreviations. MS: Multiple Sclerosis; RR-MS: Relapsing Remitting Multiple Sclerosis; EDSS: Expanded Disability Status Scale; DD: disease duration; ARR: annualized relapse rate.
Notes: Age and DD are expressed in years.

3.5. Patient #5

Patient #5 was a 56-year-old female patient with twenty-four years of RR-MS history, after an onset characterized by hypotonia and hypopallesthesia of the superior limbs, with positive OB and altered visual evoked potentials. Genetic test provided a c.1238 T > C mutation in the exon 7 of the gene causing a p.Val41Ala (V413A) substitution (never described before and absent both in ClinVar and gnomAD), but normal enzymatic activity. In addition, she showed the following polymorphisms of the GLA gene: -12G > A; IVS4 + 68A > G; IVS6-22C > T. First-degree family assessment revealed 3 subjects in which the allele could have been inherited; at genetic screening two female subjects were found positive for the same mutation in heterozygosis, but no systemic manifestation referable to FD was recognized at clinical and instrumental examination. At neurological examination, she presented with hypotonia and hyporeflexia of both superior and inferior limbs. At cardiovascular examination, echocardiographic diagnosis of left ventricular hypertrophy with normal ejection fraction was performed. Brain MR scans showed the presence of a mild T2w lesion burden, with multiple bilateral rounded hyperintense lesions affecting semioval centres and corona radiata as well as the corpus callosum, with relative sparing of periventricular WM (Fig. 1E); cervical spine involvement was visible at spine MRI examination.

3.6. Patient #6

Patient #6 was a 34-year-old female with sixteen years of RR-MS duration, showing upper limbs dysesthesia and hypotonia as clinical onset, with positive OB and altered visual evoked potentials. Genetic test showed the same genetic variant of patient #1 (single disease-neutral variant D313Y - p.Asp313Tyr - substitution caused by a c.937G > T mutation in the exon 6), with moderately reduced enzymatic activity. First-degree family assessment revealed 3 subjects in which FD could have been suspected. At neurological examination, she presented with hypotonia and hyporeflexia of both superior and inferior limbs. At MRI scan a typical pattern of disseminated MS was observed, with multiple T2-weighted lesions affecting periventricular and juxtacortical WM as well as the midbrain (Fig. 1F) and the posterior columns of the cervical spine.

3.7. Patient #7

Patient #7 was a 43-year-old female with nineteen years of RR-MS history started with diplopia, visual and auditory deficits (positive OB and altered visual evoked potentials at diagnosis), with recent transition to a SP course. Genetic test showed also in this case the same genetic variant of patient #1 (single disease-neutral variant D313Y - p.Asp313Tyr - substitution caused by a c.937G > T mutation in the exon 6), with normal enzymatic activity. First-degree family assessment revealed a possible suspected FD in her mother, who died of hypertrophic left ventricular cardiomyopathy of unknown origin. At

neurological examination, she presented weakness and hypopallesthesia and hyperreflexia of inferior limbs along with visual deficits, in clinical worsening compared to previous controls. Subsequent brain MRI scan showed the presence of high lesion burden, with a typical distribution pattern involving bilateral juxtacortical and deep WM, not sparing the corpus callosum (Fig. 1F) and the cervical spine; after gadolinium administration, a large number of new U-shaped and ring-shaped contrast-enhancing lesions (N = 20) were visible within the cerebral hemispheres and the corpus callosum, consistently with the transition to SP course.

To summarize, four patients showed the D313Y variant, whose association with FD is still debated. Furthermore, the frequency that we found in MS population is comparable to the one reported in the general population (p = 1) at Fisher exact test. Also the pathogenicity of S126G variant, which we found in one patient, is still debated and considered as likely benign with only possible marginal effects on cerebrovascular phenotype. Also in this case we demonstrated that MS population does not differ from the general one (p = .43 at Fisher exact test). In one patient we found a new variant (V413A) never described before as associated with FD, and not present both in ClinVar and in gnomAD. The last patient showed the F113L variant previously described as associated to late-onset FD form invariably presenting with severe cardiac involvement [33–35], and at present not reported both in ClinVar and in gnomAD.

Demographic and MS related information of subjects showing GLA gene mutation are reported in Table 2, whereas complete list of clinical data concerning possible FD-related multi-organ involvement in these subjects is reported in Table 3.

4. Discussion

In the last few years FD has been increasingly proposed not only as an underestimated differential diagnosis of MS, but also as a possible comorbidity with MS [20–25,27,36–41]. The challenges in distinguishing these two disorders can be at least in part ascribed to the heterogeneous and sometimes insidious FD clinical presentation, especially in young adulthood when MS reaches its peak incidence and can therefore commonly be suspected. This is even more true in case of early MS manifestations suspicion, when the MS diagnostic criteria have not yet been fulfilled [20]. These evidences taken together raised the question whether demyelinating disorders could be evoked as confounders due to a potentially overlapping phenotype with FD. Given this knowledge, we decided to investigate the GLA gene variants in a representative population fulfilling diagnostic criteria for MS in order to elucidate the relation between GLA variants and MS development. In our population, we found one GLA gene variant responsible for late-onset FD phenotype (F113L) whose manifestations cannot be predicted exclusively by genotype, but no other variant clearly annotated as pathogenic for FD. In particular GLA variants D313Y and S126G, annotated as having uncertain clinical significance, showed a frequency comparable to the one observed in general population; these data are

Table 3
Clinical data of subjects with GLA gene mutation.

#	α-GalA activity	Mutation – AA substitution	Creatinine	Proteinuria	Cardiac involvement	Acroparaesthesia#	Hypohidrosis#	Angiokeratoma	Cardiovascular risk factors	Familial history
1	4.0	c.937G > T – p.Asp313Tyr (D313Y)	0.8	Absent	Absent	Present	Absent	Absent	Smoking	NTR
2	5.2	c.337 T > C – p.Phe113Leu (F113L)	0.7	Absent	Present*	Present	Present	Present	Mild essential hypertension	Mother: MI before 60y
3	6.0	c.376A > G – p.Ser126Gly (S126G)	0.8	Absent	Absent	Absent	Absent	Absent	NTR	NTR
4	1.5	c.937G > T–p.Asp313Tyr (D313Y)	0.7	Absent	Absent	Absent	Absent	Absent	Smoking; High LDL; Overweight (BMI = 26)	NTR
5	8.1	c.1238 T > C – p.Val413Ala (V413A)	0.7	Absent	Present*	Present	Absent	Absent	Mild essential hypertension	Mother: AD
6	4.0	c.937G > T – p.Asp313Tyr (D313Y)	0.7	Absent	Absent	Present	Absent	Present	NTR	Angiokeratomas + hearing disturbance in 3 family members
7	7.6	c.937G > T – p.Asp313Tyr (D131Y)	0.7	Absent	Absent	Present	Absent	Absent	NTR	Mother: LVH

Abbreviations: α-GalA: α-galactosidase A enzyme; AA: amino acid; NTR: nothing to report; NA: not available; MI: myocardial infarction; LDL: low density lipoprotein; BMI: body mass index; AD: Alzheimer's disease; LVH: Left ventricular hypertrophy.

Notes: Proteinuria is calculated over the 24 h; Creatinine levels are expressed in mg/dl, while α-GalA activity is expressed in nmol/h/ml (normal activity > 5 nmol/h/ml; reduced activity < 5 nmol/h/ml but > 1 nmol/h/ml; absent activity < 1 nmol/h/ml); *In both cases, cardiac involvement considered present due to the presence of left ventricular hypertrophy, in absence of other symptoms and signs of heart involvement.
#Anamnestic data.

further supported by the evidence that, in both the cases, pathological and biochemical findings have not been proved to be consistent with the diagnosis of FD manifest disease [42,43]. One variant (V413A) is a new GLA gene mutation, neither described in ClinVar database nor associated to FD; both in the index case and in the female siblings found positive at genetic testing, no clear evidence accounting for classical FD diagnosis was collected (although it should be noted that heterozygote female carriers are frequently asymptomatic or affected by an attenuated form of the disease). Moreover, there are two other minor alleles annotated for this codon in gnomAD, respectively c.1239 T > C (which is a silent mutation that does not alter the amino acid) and c.1237G > A (that results in p.Val313Ile). Neither of these variants is associated to FD and their clinical significance has never been defined. Finally, in our MS population only patient carrying F113L variant seems more likely to be a candidate to be a FD patient, although with a mild phenotype possibly due to heterozygosis and lyonization-related phenomena; indeed, F113L has been associated with a late-onset cardiac FD variant, with secondary cerebrovascular involvement and inconstant extra-cardiac manifestations [33–35]. However also in this case the frequency of FD-causative alleles in our population would be 1/1547 alleles, comparable to the one observed in the general population; therefore it is difficult to assess whether it should be considered a predisposing condition for MS, instead of a more likely occasional report of co-occurrence. Therefore, taken together, our results did not point in favour of a pathological interconnection between FD and MS.

In our series, clinical onset and neuroimaging findings evolution over time were consistent with the proposed diagnosis of MS. In particular, with the only exception of the T2-weighted low lesion load of patient #4, all subjects showed typical MRI features suggestive of MS with the presence of demyelinating lesions affecting juxtacortical and periventricular WM [44], posterior fossa and midline structures [45,46] including cervical spine [47], in absence of pathognomonic hallmarks related to FD [48].

Given the main result of this study, we questioned whether many evidences of challenging diagnosis between MS and FD were reported in literature, despite the presence in the diagnostic work-up of several "red flags" specific for one condition or another [18,28,49,50]. Indeed, it is known that one of the most critical points in the assessment of MS diagnosis relies in the reasonable exclusion of alternative disorders that could explain the presence of neurological signs and symptoms, according to the principle of "no better explanation" (NOBE) [51]. Despite the use of MS diagnostic criteria in daily clinical practice reduces the risk of misdiagnosis, this flow-chart is not completely free of misinterpretations especially when evaluating atypical patients.

When critically revising previous literature evidences of challenging diagnosis between MS and FD [20–25,27,36–41] in the light of the most recent revision of McDonald criteria, some elements worthy of attention emerged. First of all, it should be noted that in a large proportion of reported cases no diagnostic criteria was provided [21,22,36–40], although their use is necessary to discriminate possible mimickers (i.e. patients with juvenile stroke or transient ischemic attack [21]), or to demonstrate dissemination in space (DIS) and time (DIT) (i.e. patients with stable clinical [39] or MRI [22] findings over time). Moreover, some patients received a diagnosis of "possible MS" (no more contemplated in the recent MAGNIMS revision), deserving a revaluation according to updated MS diagnostic criteria; in these cases, the presence of systemic signs and symptoms should be included in the search for an alternative diagnosis that could better explain the global clinical presentation [28]. In about one third of the reported cases, patients received a diagnosis of "definite MS"; in these cases some discordant findings were also present, such as the sparing of midline structures [24,46] or the poor demonstration of dissemination in space and time [20,52], as well as the simultaneous presence of lacunar infarcts and sparse T2-weighted hyperintense lesions with concomitant hemosiderin deposition. These elements can be indicative of a vascular etiology [20,24,25] that should induce to reconsider MS diagnosis and search

for possible mimics.

Conversely in a minority of the literature reports [20–22,36,41] GVUS, hypomorphic alleles, newly described mutations and benign GLA polymorphisms were indicated as probably pathogenic for FD, suggesting a possible coexistence with MS and establishing a causal link that goes far beyond clinical evidences of pathogenicity. The prototype of this contradiction is the D313Y variant, frequently imputed for a possible role in neuronal damage [20,22], but at present not considered a disease-causing mutation (whose prevalence we have proven to be comparable to the general population) [53]. D313Y has been demonstrated to induce the so-called "pseudo-deficient" activity of α-GalA in plasma, simulating a clinically relevant reduction of tissue enzymatic levels that might lead to FD misdiagnosis [53]. In this light a more profound consideration should be dedicated to GVUS and other mutations with controversial genotypic-phenotypic correlation, being the presence of mutations in GLA gene necessary but not sufficient to achieve FD diagnosis. Indeed, the impact of GLA mutations on gene expression and α-GalA homeostasis depends on location, epigenetic factors and effects on the tridimensional structure of the enzyme; all these elements, taken together and coupled to other risk and environmental factors, determine the severity of the enzymatic deficiency [9]. Comprehensive FD manifestations can be thought as a multi-domain phenotype, where many determinants play a pivotal role in determining final clinical picture; robust evidences of impaired α-GalA activity and abnormal Gb3 deposition in different organs must therefore be considered the most reliable marker of disease severity, whereas the only genotyping can fail in identifying patients and distinguishing carriers.

Finally, only in a single literature report [23] MS and FD diagnostic criteria were both clearly satisfied at one time. Indeed, in this peculiar case the patient presented with a diagnosis of FD-classical variant (Q279K mutation), further confirmed by the evidence of reduced α-GalA activity and Gb3 deposition at renal biopsy; when clinical suspicion of MS was evoked, the patient was investigated with MRI that showed the presence of WML with evidence of dissemination in space and time. The diagnosis of MS was further supported by the presence of CSF OB and impaired visual evoked potentials; in this isolated case, the global clinical scenario is suggestive of an actual coexistence of the two disorders in the same subject (*see Supplementary Materials*). However, even recently, FD has not emerged as a robust alternative diagnosis even in a large scale NOBE analysis on first diagnosis demyelinating diseases cases [54].

For all the stated reasons, a proper knowledge of the mechanism of pathogenicity of GLA gene variants along with a correct interpretation of polymorphic clinical manifestations of FD is essential for healthcare professionals involved in FD patients' management. In a limited number of uncertain cases, when controversial clinical setting is present, genotyping supported by tissue biopsy might be necessary to confirm or exclude FD diagnosis.

Some limitations to this work should however be considered. The cross-sectional design of this study only allows for a limited number of evidences, leaving unresolved the potential role of GVUS and GLA gene polymorphisms in the definition of minor FD clinical manifestations. Furthermore, no standardized MR protocol including advanced imaging techniques was available in our sample, thus limiting the neuroradiological evaluation to the retrospective analysis of conventional imaging findings. In this light further prospective studies on larger populations are still required to better define more specific clinical and pathogenic details.

5. Conclusions

In conclusion, we found a prevalence of GLA gene variants in MS patients comparable to the one estimated in healthy population. This result, further supported by the critical revision of current literature evidences of misdiagnosis between MS and FD, argues in favour of the complete independence between these disorders. Therefore, possible cases of FD patients misdiagnosed with MS are more probably due to an insufficiently rigorous application of the current diagnostic criteria. Our results point to the importance of comprehensive anamnestic and clinical data collection along with a deep knowledge of possible pitfalls in the differential diagnosis between the two conditions, in order to provide patients with the most effective diagnostic assessment and subsequent prompt therapeutic management.

Ethical approval and informed consent

All procedures performed in in this study were in accordance with the standards of local ethical committee (Comitato Etico "C. Romano" – Università degli Studi di Napoli "Federico II", Napoli – n.62/10) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all participants included in the study.

Authors contribution

Each author contributed to all the four International Committee of Medical Journal Editors (ICMJE) criteria on authorship:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work;
- Drafting the work or revising it critically for important intellectual content;
- Final approval of the version to be published;
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declaration of Competing Interest

C.R. and G.P. received fees for speaking from Genzyme.

S.C. received fees for speaking from Genzyme and Shire, and fees for speaking, travel grant and honoraria for serving as consultant to Amicus.

E.R. received travel grant and honoraria for serving as consultant to Genzyme and Shire.

R.L. received fees for speaking, travel grant and honoraria for serving as consultant from Teva, Merck, Genzyme, Novartis, Roche and Biogen.

L.S., G.D. and M.I. received travel grant and honoraria as consultant to Genzyme and Shire.

E.T. received fees for speaking from Scientific Press, Ars Educandi and Shire Italy.

A.P. received travel grant and honoraria for serving as consultant to Genzyme, Shire and Amicus.

The other Authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2020.116782.

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