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SUPPLEMENTARY METHODS**Details on *GLA* variant 2-point scoring and validation**

The *GLA* variant 2-point scoring system derived from patients' Fabry Registry data was developed based on a modification of published criteria for classic Fabry disease in male patients.¹ Briefly, van der Tol et al.¹ used strict criteria to identify patients with a classic phenotype based on severely decreased or absent α -galactosidase activity in leukocytes of males and presence of at least one of the Fabry disease-specific clinical characteristics of acroparaesthesia, angiokeratomas and cornea verticillata. In the present initiative, acroparaesthesia was not used as a criterion because it was deemed to be too subjective and may result from carpal tunnel syndrome, which appears to have a higher prevalence in Fabry patients than in the general population.² α -galactosidase activity records were insufficient for the assessment. The *GLA* variant 2-point scores used in Stage 2 were calculated as follows: Total of positive Fabry Registry responses of diffuse angiokeratomas or cornea verticillata for the *GLA* variant divided by the total Fabry Registry responses ('yes' and 'no') of diffuse angiokeratomas and cornea verticillata status for that variant as a percentage. An example of calculation is provided below.

Male patients with the specific <i>GLA</i> variant	Fabry Registry responses			
	Available	Angiokeratomas 'Yes' (present) or 'No' (absent)	Available	Cornea verticillata 'Yes' (present) or 'No' (absent)
Patient #1	Yes	Yes	Yes	Yes
Patient #2	No	-	Yes	Yes
Patient #3	Yes	Yes	Yes	No
Patient #4	Yes	Yes	No	-

2-point score calculation:

- Total of positive responses of angiokeratomas or cornea verticillata = 5.
- Total of responses ('yes' and 'no') of angiokeratomas or cornea verticillata status = 6.
- 2-point score = 0.83 (i.e. 5/6) X 100% = 83%

SUPPLEMENTARY REFERENCES

1. van der Tol L, Smid BE, Poorthuis BJ, Biegstraaten M, Deprez RH, Linthorst GE, Hollak CE. A systematic review on screening for Fabry disease: prevalence of individuals with genetic variants of unknown significance. *J Med Genet* 2014;51:1–9.
2. Ghali J, Murugasu A, Day T, Nicholls K. Carpal tunnel syndrome in Fabry disease. *JIMD Rep* 2012;2:17–23.

Supplementary Table 1. The 55 *GLA* variants included in Stage 1. The variants had been reported to the Fabry Registry for ≥ 5 Fabry patients (males or females) and lacked fabry-database.org phenotype classification.

GLA variants (n=55)	Variant type	Classification in fabry-database.org^a	Preliminary workgroup classification in Stage 1 for variants subsequently included in Stage 2^b
p.Gln283*	Nonsense	Blank	Classic
p.Trp162*	Nonsense	Blank	Classic
p.Trp44Cys	Missense	Blank	Classic
p.Ser345Pro	Missense	Blank	Unclassified
p.Ala156Thr	Missense	Blank	Classic
p.Thr194Ile	Missense	Blank	Classic
p.Pro259Arg	Missense	Blank	Classic
p.Thr410Ile	Missense	Blank	Classic
p.Ser238Asn	Missense	Blank	Classic
p.Arg363His	Missense	Blank	Later-onset
p.Ala143Thr	Missense	Blank	Unclassified
p.Asp322Glu	Missense	Blank	Unclassified
c.1188_1189ins	Frameshift	Absent	Classic
p.Glu358del	Small deletion	Blank	Classic
p.Gln416*	Nonsense	Blank	Not included in Stage 2
p.Asp136Tyr	Missense	Blank	Not included in Stage 2
p.Ile242Phe	Missense	Blank	Not included in Stage 2
p.Leu16Pro	Missense	Blank	Not included in Stage 2
p.Cys63Tyr	Missense	Blank	Not included in Stage 2
p.Ile232Thr	Missense	Blank	Not included in Stage 2
p.Glu7*	Nonsense	Blank	Not included in Stage 2
p.Trp47*	Nonsense	Blank	Not included in Stage 2
p.Leu243*	Nonsense	Blank	Not included in Stage 2
p.Gln280*	Nonsense	Absent	Not included in Stage 2
p.Leu403*	Nonsense	Blank	Not included in Stage 2
p.Phe18Ser	Missense	Blank	Not included in Stage 2
p.Cys52Gly	Missense	Blank	Not included in Stage 2
p.Pro60Leu	Missense	Blank	Not included in Stage 2
p.Gly85Asn	Missense	Blank	Not included in Stage 2
p.Cys90Arg	Missense	Absent	Not included in Stage 2
p.Asp92Asn	Missense	Blank	Not included in Stage 2
p.Leu129Pro	Missense	Blank	Not included in Stage 2
p.Ile270Thr	Missense	Blank	Not included in Stage 2
p.Ala292Thr	Missense	Blank	Not included in Stage 2
p.Leu294Ser	Missense	Absent	Not included in Stage 2
p.Leu300Pro	Missense	Blank	Not included in Stage 2
p.Gly325Asp	Missense	Blank	Not included in Stage 2

GLA variants (n=55)	Variant type	Classification in fabry-database.org^a	Preliminary workgroup classification in Stage 1 for variants subsequently included in Stage 2^b
p.Gly325Ser	Missense	Absent	Not included in Stage 2
p.Ile354Lys	Missense	Blank	Not included in Stage 2
p.Arg363Pro	Missense	Absent	Not included in Stage 2
p.Thr412Asn	Missense	Absent	Not included in Stage 2
p.Leu415Pro	Missense	Blank	Not included in Stage 2
g.IVS2-2A>G	Splice site	Blank	Not included in Stage 2
g.IVS3-1G>C	Splice site	Blank	Not included in Stage 2
g.IVS4-2A>T	Splice site	Blank	Not included in Stage 2
g.IVS5-2_-3del ^c	Splice site	Absent	Not included in Stage 2
g.IVS5-3_-2del ^c	Splice site	Absent	Not included in Stage 2
g.IVS6-1G>A	Splice site	Blank	Not included in Stage 2
g.IVS6-10G>A	Splice site	Absent	Not included in Stage 2
c.265delC	Frameshift	Absent	Not included in Stage 2
c.734_792del	Frameshift	Absent	Not included in Stage 2
c.993_994insA	Frameshift	Absent	Not included in Stage 2
c.EX2del	Large deletion	Absent	Not included in Stage 2
p.Arg404del	Small deletion	Blank	Not included in Stage 2
p.G395_F396ins	Small insertion	Absent	Not included in Stage 2

^a fabry-database.org classification field “blank” (not classified) or classification “absent” (*GLA* variant not listed).

^b Preliminary classification based on workgroup member’s personal clinical observations made in Fabry patients with *GLA* variants included in the research, on evaluation of published scientific and medical literature and, if possible, on review of the variant’s potential deleterious impact on the *GLA* gene and/or α -galactosidase function in light of established molecular mechanisms. Phenotypes associated with ‘pathogenic’ *GLA* variants include ‘classic’ and ‘later-onset’ phenotypes. Reasons for not including variants in Stage 2 were insufficient 2-point scoring data (angiokeratomas or cornea verticillata) for a particular variant or categorization of variant as ‘likely benign’ or ‘benign’ not warranting in-depth investigation (see Methods section).

^c *GLA* variants may be identical.

*, translation termination codon.

Supplementary table 2. Comparison of workgroup consensus phenotype classifications with results from disease databases and prediction algorithms

GLA variants (n=33)	Variant type	Fabry Disease Genotype-Phenotype Workgroup Phenotype classification	dbfpg disease database Phenotype classification	ClinVar disease database Phenotype classification	LOVD disease database		PolyPhen-2 prediction algorithm		SIFT prediction algorithm		MutationTaster prediction algorithm	
					Phenotype classification	Affecting GLA function (as reported)	Score, range 0-1 (missense variants only)	Prediction	Score, cut-off <0.05 (missense variants only)	Prediction	Score, range 0-1 (missense variants only)	Prediction
p.Gln283*	Nonsense	Classic	Classic	Pathogenic	NR	Affects function	NA	NA	NA	NA	1	Disease causing
p.Trp162*	Nonsense	Classic	Classic	Pathogenic	NR	Affects function	NA	NA	NA	NA	1	Disease causing
p.Trp44Cys	Missense	Classic	Classic	Uncertain	NR	Effect unknown	1.000	Probably damaging	0.000	Damaging	0.999	Disease causing
p.Met284Thr	Missense	Classic	Classic	NR	NR	Affects function	1.000	Probably damaging	0.001	Damaging	0.999	Disease causing
p.Ser345Pro	Missense	Classic	Classic	NR	NR	Affects function	0.972	Probably damaging	0.059	Tolerated	0.865	Disease causing
p.Ala156Thr	Missense	Classic	Classic	Pathogenic	NR	Affects function	1.000	Probably damaging	0.003	Damaging	0.999	Disease causing
p.Thr194Ile	Missense	Classic	Likely classic	NR	NR	Affects function	1.000	Probably damaging	0.000	Damaging	0.999	Disease causing
p.Ala15Glu	Missense	Classic	Classic	NR	NR	NR	0.532	possibly damaging	NR	NR	0.999	Polymorphism
p.P70259A>V	Missense	Classic	Classic	Pathogenic	NR	Affects function	1.000	Probably damaging	0.000	Damaging	0.999	Disease causing
p.Trp340A>E	Missense	Classic	Classic	Likely pathogenic	NR	Affects function	1.000	Probably damaging	0.000	Damaging	0.999	Disease causing
p.Thr410Ile	Missense	Classic	Classic	Likely pathogenic	NR	Affects function	1.000	Probably damaging	0.002	Damaging	0.998	Disease causing
p.Ile198Thr	Missense	Later-onset	Later-onset	Conflicting interpretations: Likely pathogenic (n=2); Uncertain (n=1)	NR	NR	1.000	Probably damaging	0.002	Damaging	0.999	Disease causing
p.Ser238Asn	Missense	Later-onset	Later-onset	Pathogenic	NR	Affects function	1.000	Probably damaging	0.021	Damaging	0.999	Disease causing
p.Arg363His	Missense	Later-onset	Later-onset	Pathogenic	NR	Affects function	0.406	Benign	0.465	Tolerated	0.999	Polymorphism
p.Ala143Thr	Missense	zyJUS*	Benign	Conflicting interpretations: Pathogenic (n=2); Likely pathogenic (n=3); Uncertain (n=7)	NR	Affects function	1.000	Probably damaging	0.004	Damaging	0.999	Disease causing
p.Asp322Glu	Missense	Later-onset	Classic	Pathogenic	NR	NR	0.999	Probably damaging	0.000	Damaging	0.975	Disease causing
p.Ile117Ser	Missense	Later-onset	Classic	NR	NR	NR	0.999	Probably damaging	NR	NR	0.999	Disease causing
c.370-2A>G	Splice site	Classic	Classic	Pathogenic	NR	Affects function	NA	NA	NA	NA	NA	NA
c.802-3_802-2del	Splice site	Classic	Classic	Pathogenic	NR	Affects function	NA	NA	NA	NA	NA	NA
c.548-1G>A	Splice site	Classic	Classic	Pathogenic	NR	Affects function	NA	NA	NA	NA	NA	NA
c.999+2T>C	Splice site	Classic	Classic	Pathogenic	NR	Affects function	NA	NA	NA	NA	NA	NA
c.777del	Frameshift	Classic	Classic	NR	NR	Affects function	NA	NA	NA	NA	NA	NA
c.1042dup	Frameshift	Classic	Classic	NR	NR	Affects function	NA	NA	NA	NA	NA	NA
c.57_82del	Frameshift	Classic	Not available	NR	NR	NR	NA	NA	NA	NA	NA	NA
c.365_371del	Frameshift	Classic	Not available	NR	NR	NR	NA	NA	NA	NA	NA	NA
c.1188_1189insT	Frameshift	Classic	Not available	NR	NR	NR	NA	NA	NA	NA	NA	NA
c.568del	Frameshift	Classic	Classic	NR	NR	Affects function	NA	NA	NA	NA	NA	NA
p.Ser65_Tyr123del	Large deletion	Classic	Not available	NR	NR	NR	NA	NA	NA	NA	NA	NA
c.1212_1214del	Small deletion	Classic	Classic	NR	NR	Affects function	NA	NA	NA	NA	NA	NA
p.Glu358del	Small deletion	Classic	Classic	Pathogenic	NR	NR	NA	NA	NA	NA	NA	NA
c.639+4A>T	Intronic	Classic	Classic	Uncertain	NR	Affects function	NA	NA	NA	NA	NA	NA
c.1000-10G>A	Intronic	Classic	Not available	NR	NR	NR	NA	NA	NA	NA	NA	NA
c.639+919G>A	Intronic	Later-onset	Later-onset	Conflicting interpretations: Pathogenic (n=3); Uncertain (n=1)	NR	NR	NA	NA	NA	NA	NA	NA

*Genetic variant of unknown significance; experts' opinion in favour of a likely benign GLA variant

dbfpg, International Fabry Disease Genotype-Phenotype Database; LOVD, Leiden Open Variation Database; NA, not applicable; NR, no result available; PolyPhen-2, Polymorphism Phenotyping v2; SIFT, Scale-Invariant Feature Transform