Comparison of *GLA* variant profile in newborn screening confirmatory testing and diagnostic testing for Fabry disease

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1 INTRODUCTION

Fabry disease (FD) is an X-linked progressive multisystemic lysosomal disease caused by pathogenic variants in the *GLA* gene encoding alpha-galactosidase A, affecting both males and heterozygous females. In the US, FD was not recommended for inclusion in the Recommended Uniform Screening Panel (RUSP) due to concerns about variable clinical presentation, possible late disease onset, and the risk of serious symptoms not discernible in newborns. Nevertheless, newborn screening (NBS) for FD has now been implemented in several states and around the world. It is critical to study the current NBS data to improve early diagnosis and better evaluate the risks and benefits of NBS. This study focuses on comparing reported *GLA* variant profiles between the NBS cohort and the diagnostic cohort.

2 METHODS

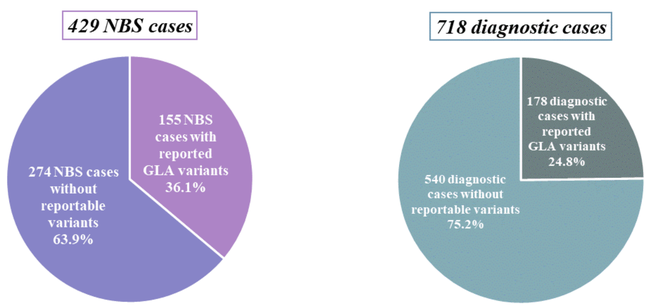
The NBS cohort comprises 429 cases with deficient alpha-galactosidase enzyme activity detected by NBS. The diagnostic cohort comprises 718 cases, ranging from ages 2 to adulthood, with clinical suspicion and/or family history of FD. Next-generation sequencing of the coding exons and flanking regions of the *GLA* gene was performed in all cases. Variant interpretation and classification were based on ACMG/AMP criteria, with pathogenic/likely pathogenic (P/LP) variants and variants of uncertain significance (VUS) reported.

3 RESULTS

Comparison of the cases with reported GLA variants between the NBS cohort and the diagnostic cohort

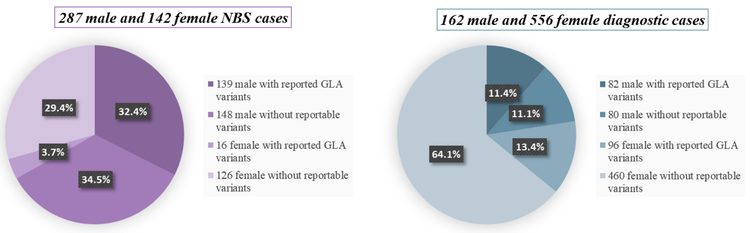
Variants in the *GLA* gene were reported in 36.1% (155/429) NBS cases compared to 24.8% (178/718) of the diagnostic cases.

Figure 1A



In the NBS cohort, 66.9% (287) of cases were male, with 139 cases having reportable findings, while 33.1% (142) were female, with 16 having reportable findings. In contrast, in the diagnostic cohort, 22.6% (162) were male, with 82 having reportable findings, and 77.4% (556) were female, with 96 having reportable findings.

Figure 1B



Comparison of the reported GLA variant profiles between the NBS cohort and the diagnostic cohort

Among cases with reported *GLA* variants, P/LP variants were identified in 76.1% (118) of NBS cases and 82.0% (146) of diagnostic cases, while VUSs were detected in 20.0% (31) of NBS cases and 14.0% (25) of diagnostic cases. It's worth noting that the c.937G>T(p.Asp313Tyr) variant (a.k.a. D313Y) is commonly considered the only pseudodeficiency *GLA* allele.

Figure 2

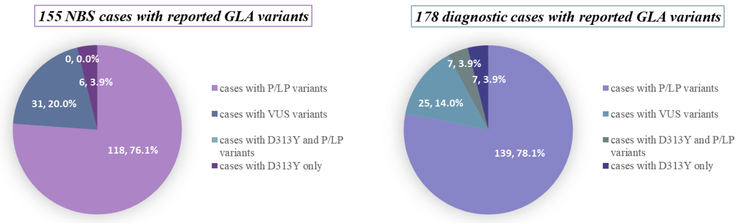


Table 1 *Reported GLA variant profiles in NBS cohort and diagnostic cohort*

Number of reported *GLA* variants	in NBS cohort	in diagnostic cohort
pathogenic/likely pathogenic	23	85
variant of uncertain significance	20	16
likely pseudodeficiency allele	1	1
missense	38	62
nonsense	3	12
frameshift	2	15
splicing	1	6
in-frame	0	4
start-loss	0	1
synonymous	0	1
copy number loss	0	1
total	44	102

Commonly identified GLA variants differ in frequency between the NBS cohort and the diagnostic cohort

Table 2 presents the most frequently reported *GLA* variants, identified in more than five cases within the total cohort. Additionally, their allele frequencies in the NBS cohort, diagnostic cohort, and general populations (gnomAD) are provided.

Notably, the allele frequencies of all listed variants, in both the NBS and diagnostic cohorts, surpass the highest population allele frequencies observed in the general population (gnomAD).

The c.427G>A(p.Ala143Thr) variant, associated with mild, late-onset, or non-classic Fabry disease, is known to be identified at a high frequency in newborn screening. It was detected in 11.2% (48) of NBS cases but only in 1.8% (13) of diagnostic cases. Similarly, the c.593T>C(p.Ile198Thr) variant, another common variant, was found in 6.8% (29) of NBS cases compared to only 0.3% (2) of diagnostic cases. Although both variants are currently classified as likely pathogenic, the observed clinical variability and reduced penetrance suggest a need for careful consideration.

Missense variants of uncertain significance, such as c.352C>T(p.Arg118Cys), c.619T>C(p.Tyr207His), and c.1067G>A(p.Arg356Gln), were notably more prevalent in NBS cases than in diagnostic cases. Given the absence of clinical symptoms in newborns, interpreting these novel variants identified by NBS poses a challenge.

The c.937G>T(p.Asp313Tyr) variant is recognized as the only common pseudodeficiency *GLA* allele; however, its interpretation remains debated. This variant was detected in six NBS cases and in 14 diagnostic cases, including seven diagnostic cases with another pathogenic/likely pathogenic *GLA* variant.

4 CONCLUSION

Comparison of *GLA* variants identified in NBS and diagnostic cases reveals different variant profiles. Genetic testing following newborn biochemical enzyme screening, coupled with the absence of clinical manifestation during infancy, resulted in a higher percentage of reports with VUS. In contrast, diagnostic testing prompted by clinical suspicion of disease and/or family history resulted in a higher percentage of reports with P/LP variants.

The increased incidence of VUS identified by NBS raises the possibility of variants being associated with pseudodeficiency, yielding abnormal biochemical test results without corresponding clinical symptoms, or being linked to later-onset and/or atypical Fabry disease. This highlights the necessity for evaluating these variants through long-term follow-up to monitor biochemical and clinical manifestations.

Examining two cohorts with different clinical scenarios provides additional insights into characterizing individual *GLA* variants, particularly those with controversial clinical evidence and interpretation in the literature. The information from this study can aid in genetic variant (re)classification and further the understanding of phenotype prediction, contributing to comprehensive clinical management of Fabry disease.

cDNA	Protein	Classification	NBS cohort				Diagnostic cohort				Global allele frequency % (gnomAD v4.0.0)	Highest allele frequency % (gnomAD v4.0.0)
			Number of male cases	Number of female cases	Numbe of total cases	Allele frequency %	Number of male cases	Number of female cases	Number of total cases	Allele frequency %		
c.427G>A	p.Ala143Thr	Likely Pathogenic	44	4	48	8.406	4	9	13	1.020	0.058	0.073
c.593T>C	p.Ile198Thr	Likely Pathogenic	29	0	29	5.079	1	1	2	0.157	0.001	0.002
c.679C>T	p.Arg227Ter	Pathogenic	1	1	2	0.350	8	12	20	1.570	0.000	0.007
c.937G>T	p.Asp313Tyr	likely pseudodeficiency allele	2	4	6	1.051	6	8	14	1.099	0.378	0.627
c.1088G>A	p.Arg363His	Pathogenic	10	0	10	1.751	1	4	5	0.392	0.003	0.037
c.644A>G	p.Asn215Ser	Pathogenic	4	1	5	0.876	5	4	9	0.706	0.003	0.004
c.1067G>A	p.Arg356Gln	Uncertain Significance	3	2	5	0.876	1	1	2	0.157	0.001	0.006
c.352C>T	p.Arg118Cys	Uncertain Significance	3	1	4	0.701	0	2	2	0.157	0.061	0.076
c.1078G>C	p.Gly360Arg	Likely Pathogenic					2	4	6	0.471		
c.619T>C	p.Tyr207His	Uncertain Significance	5	0	5	0.876					0.001	0.012
c.335G>A	p.Arg112His	Pathogenic	2	0	2	0.350	2	1	3	0.235	0.002	0.002
c.602C>T	p.Ser201Phe	Likely Pathogenic					4	1	5	0.392		

Table 2 *Most reported GLA variants in NBS cohort and diagnostic cohort, and their allele frequencies in the two cohorts and general populations*

5 REFERENCES

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