Genomic and Biochemical Profile of Pseudodeficiency in Lysosomal Storage Disorders

Xiangwen Chen-Deutsch, Taraka Donti, Zhili Lin, Christin Collins, Madhuri Hegde

INTRUDUCTION

Pseudodeficiency is well recognized phenomenon of lysosomal storage disorders (LSD), which refers to reduced enzyme activity in vitro due to decreased specificity toward an artificial substrate used in laboratory assays. More recently, pseudodeficiency is also known as a true partial reduction of enzyme activity but not to a level that results in disease-causing accumulation of substrates that are used as biomarkers. Genetic sequencing and evaluation of biomarkers are important in further clarifying pseudodeficiency from actual disease. Genetic and biochemical profile of pseudodeficiency in four LSDs, Pompe disease, Krabbe disease, Mucopolysaccharidosis type I (MPS I), and Fabry disease, are investigated in this study. Cases of newborn screening referral and single gene testing for clinical suspicion are included in this cohort.

METHODS

Genetic testing and analyses of four LSD-related genes, *GAA* (Pompe disease), *GALC* (Krabbe disease), *IDUA* (MPS I) and *GLA* (Fabry disease), were performed respectively in individuals. The targeted gene exons and flacking regions were enriched from genomic DNA and sequenced using next-generation sequencing. Thirteen well recognized pseudodeficiency (PD) alleles of four genes (***Table 1***), as well as pathogenic/likely pathogenic (P/LP) variants and variants of uncertain significance (VUS) were reported in all the cases of this cohort. Enzyme activity for GAA, GALC, IDUA and GLA was determined by LC-MS/MS assay. Biomarkers for Krabbe disease (Psychosine), MPS I (GAGs) and Fabry disease (Lyso-GB3) were also analyzed by LC-MS/MS based assay.

Table 1: *Common pseudodeficiency alleles of four LSD-related genes*

Disorder	Gene	Reference Transcript	Variant	Global MAF % (gnomAD v2.1.1)	Highest MAF % (gnomAD v2.1.1)
Pompe disease	***GAA***	NM_000152.3	c.271G>A (p.Asp91Asn)	2.049	3.291 in European (non-Finnish)
			c.664G>A (p.Val222Met)	0.06931	0.5393 in South Asian
			c.1726G>A (p.Gly576Ser)	1.700	14.27 in East Asian
			c.2065G>A (p.Glu689Lys)	5.524	23.55 in East Asian
			c.[1726G>A;2065G>A] (p.[Gly576Ser;Glu689Lys])		
Krabbe disease	***GALC***	NM_000153.4	c.550C>T (p.Arg184Cys)	4.183	6.993 in European (Finnish)
			c.742G>A (p.Asp248Asn)	12.56	15.71 in European (Finnish)
			c.1685T>C (p.Ile562Thr)	43.90	60.55 in African/African American
Mucopolysaccharidosis type I	***IDUA***	NM_000203.5	c.235G>A (p.Ala79Thr)	0.3083	3.141 in African/African American
			c.246C>G (p.His82Gln)	0.2906	0.4769 in European (non-Finnish)
			c.667G>A (p.Asp223Asn)	0.05247	0.5652 in African/African American
			c.965T>A (p.Val322Glu)	0.09359	0.8796 in African/African American
Fabry disease	***GLA***	NM_000169.2	c.937G>T (p.Asp313Tyr)	0.3040	0.6915 in Ashkenazi Jewish

RESULTS AND DISCUSSION

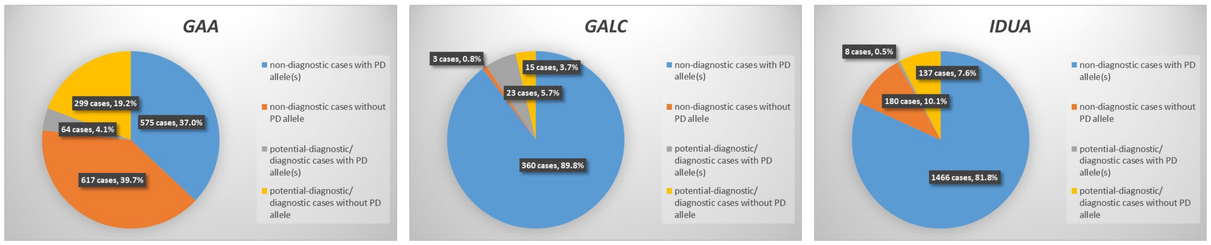
Pseudodeficiency alleles in autosomal recessive LSD-related genes

The genetic profile of the three common autosomal recessive LSD-related genes was categorized. Combinations of the detected pseudodeficiency alleles and different types of molecular diagnostic findings (potential-diagnostic/diagnostic and non-diagnostic) were investigated. Potential-diagnostic/diagnostic cases are defined as the identification of at least two alleles of P/LP and/or VUS variants. Non-diagnostic cases include non-detection of the P/LP/VUS variant, and carrier status with only one allele of identified P/LP/VUS variant. Comparing these three genes, the detection rate of pseudodeficiency alleles is much higher in the *GALC* and *IDUA* cases than the *GAA* cases; while the potential-diagnostic/diagnostic detection rate is lower in the *GALC* and *IDUA* cases than the *GAA* cases (***Table 2*** and ***Figure 1***).

Table 2: *The detection rates of pseudodeficiency (PD) alleles and potential-diagnostic/diagnostic cases in autosomal recessive LSD-related genes*

Gene	Cases with detected PD allele(s)	Potential-diagnostic/diagnostic cases
GAA	41.1% (639/1553)	23.3% (363/1555)
GALC	95.5% (383/401)	9.5% (38/401)
IDUA	82.3% (1474/1791)	8.1% (145/1791)

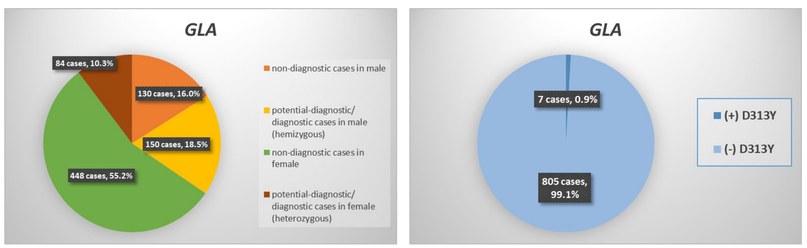
Figure 1: *Percentages of the cases with combination of detected pseudodeficiency (PD) alleles and different types of diagnostic findings in autosomal recessive LSD-related genes*



Pseudodeficiency allele in X-linked LSD-related gene

In the *GLA* gene testing cases, the detection rate of potential-diagnostic/diagnostic variants is higher in male than in female (***Figure 2***). The p.Asp313Tyr (D313Y) variant is currently the only well studied potential pseudodeficiency allele in the X-linked *GLA* gene, however the clinical consequence of this variant in the adult-onset neurological conditions is debatable. The detection rate of the *GLA* p.Asp313Tyr variant is 0.9% (7/812 cases, ***Figure 2***). Only in one female individual amongst seven p.Asp313Tyr positive cases, another likely pathogenic variant was also identified.

Figure 2: *The detection rates of different types of diagnostic findings and D313Y in GLA cases*



Biochemical profile of pseudodeficiency in four LSDs

Table 3: *Normal ranges of enzyme activities and biomarkers in four LSDs*

Disorder	Gene	Enzyme	Normal enzyme activity range (μmol/L/hr)	Biomarker
Pompe disease	***GAA***	Acid alpha-glucosidase	≥ 2.10	
Krabbe disease	***GALC***	Galactocerebrosidase	≥ 0.55	Psychosine (normal ≤ 1.50 nM)
Mucopolysaccharidosis type I	***IDUA***	Alpha L-iduronidase	≥ 1.80	Glycosaminoglycans (GAGs)
Fabry disease	***GLA***	Alpha-galactosidase	≥ 1.10	Lyso-GB3 (normal ≤ 1.11 ng/ml)

In the instances where enzyme activity and biomarker analysis were performed, none of the cases with pseudodeficiency alleles had an enzyme activity within the normal range, however biomarkers helped in differentiating the cases with pseudodeficiency alleles from the disease cases. Psychosine was significantly elevated in 100% of the cases with pathogenic *GALC* alleles. Out of 401 total cases, two cases with a pathogenic *GALC* allele and a pseudodeficiency allele had psychosine elevated along with deficient enzyme activity. GAGs were elevated in 100% of cases with pathogenic *IDUA* alleles. Out of 1791 total cases, 18 cases with pseudodeficiency alleles had elevated GAGs. Lyso-GB3 was elevated in 100% of cases with pathogenic *GLA* alleles.

CONCLUSION

These studies of correlation between genomic variants, enzyme activities and biomarkers can help to more accurately rule out false-positive cases caused by pseudodeficiency, especially in the newborn screening setting. The results may also help to further understand the ultimate clinical consequences of currently defined pseudodeficiency alleles, especially in late-onset LSD-related conditions. Since the enzyme activity levels in cases with pseudodeficiency alleles are indistinguishable from disease cases, incorporating biomarker analysis will substantially optimize laboratory analysis pipeline and reduce the burden on the laboratory and clinical management.

References

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