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ORIGINAL ARTICLE

Long-Term Health Outcomes of Individuals With Pseudodeficiency Alleles in *IDUA* May Inform Newborn Screening Practices for Mucopolysaccharidosis Type I

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ABSTRACT

Mucopolysaccharidosis type I (MPS I), a lysosomal disorder caused by variants in *IDUA*, was added to the Recommended Uniform Screening Panel for newborn screening in 2016. Positive screening results for MPS I are commonly due to variants known as "pseudodeficiency alleles," which decrease in vitro alpha-L-iduronidase enzyme activity but are thought to provide sufficient in vivo activity. Despite the historic assumption that these variants are biologically benign, the possibility that they could give rise to complex, multigenic, or attenuated phenotypes has not been systemically evaluated in adults. We completed a retrospective matched cohort study using a hospital-based biorepository with data from 65,309 participants, we identified 1803 individuals harboring homozygous *IDUA* pseudodeficiency alleles. Using electronic medical records (EMR), we compared the prevalence of features of MPS I in participants with homozygous pseudodeficiency alleles to a cohort of matched control participants. We found no clinically relevant significant differences between cases and controls nor genotype–phenotype associations across four alleles. These findings provide empiric support that adults with homozygous *IDUA* pseudodeficiency alleles are unlikely to develop mild symptoms of disease compared with controls. This study provides a proof-of-concept model for other nonclassical disease variants related to other inherited metabolic disorders, which is necessary as newborn screening expands.

Amel Karaa and Nina B. Gold contributed equally to this study.

1 | Introduction

Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder associated with neurologic, orthopedic, and other multi-systemic symptoms (Clarke et al. 2017). The spectrum of disease is typically divided into severe and attenuated Hurler syndrome, previously termed Hurler/Scheie or Scheie disease (Clarke et al. 2017). Individuals with severe disease typically develop progressive symptoms within the first year of life, while individuals with attenuated disease experience the onset later in childhood or very rarely as a young adult (Hampe et al. 2020). When diagnosed early, the progression of MPS I can be ameliorated by available therapeutics, including hematopoietic stem cell transplantation for infants with Hurler syndrome or early initiation of enzyme replacement therapy for people with attenuated disease (Hampe et al. 2020). Due to the availability of effective treatments that require early initiation, the Secretary of the Department of Health and Human Services suggested that MPS I be added to the Recommended Universal Screening Panel (RUSP) for newborn screening (NBS) in 2016 (Hampe et al. 2020).

NBS laboratories identify infants at risk for MPS I primarily via an assay of alpha-L-iduronidase enzyme activity in dried blood spots, using tandem mass spectrometry to measure products of the enzymatic reaction. The first NBS pilot study using a digital microfluidic fluorometric assay for enzyme activity measurement was performed in the Illinois Newborn Screening Laboratory, and has subsequently used in other states including Missouri and New York (Washburn and Millington 2020). Some states now employ a second-tier screen using genomic sequencing or genotype arrays for common *IDUA* variants, while others measure glycosaminoglycan levels on the dried blood spot (Herbst et al. 2023). Infants with positive screening results require a confirmatory blood-based IDUA assay, measurement of glycosaminoglycans in urine or blood, and often *IDUA* gene analysis (Clarke et al. 2017; Herbst et al. 2023).

Several alleles within the *IDUA* gene have been classified as "pseudodeficiency alleles" (Yu et al. 2020; Peck et al. 2020; Li, Wood, and Thompson 2002). These variants lead to low alpha-L-iduronidase enzyme levels on in vitro assays, but historically have been assumed to have normal affinity for the biological substrate and do not to lead to clinical features of MPS I (Donati et al. 2018; Yu et al. 2020; Pollard, Jones, and Wood 2013; Clarke et al. 2017). Pseudodeficiency alleles in *IDUA* are common and, across states, account for 20% to 61.5% of positive enzyme-based NBS for MPS I (Burton et al. 2020; Wasserstein et al. 2019; Bosfield et al. 2021). These variants, in particular p.Ala79Thr, p.Asp223Asn, and p.Gly409Arg, are enriched in individuals with African American ancestry, (Bosfield et al. 2021) leading to a disproportionate rate of positive NBS results in individuals who self-identify as Black (Blout Zawatsky et al. 2021). Although it is widely accepted that pseudodeficiency variants constitute "false positive" results on NBS, (Herbst et al. 2023) the possibility that they could give rise to complex, multigenic, or attenuated phenotypes has not been systemically evaluated in adults. As such, although parents of infants with these variants can be reassured that they will not develop classic symptoms of disease, further prognostication related to these variants has remained challenging (Pollard, Jones, and Wood 2013).

In this study, we utilized data from a hospital-based biobank linked to electronic medical record (EMR) data to identify if clinical features of MPS I were enriched among adults with homozygous pseudodeficiency alleles compared with matched controls.

2 | Methods

2.1 | Biobank Participants

The Massachusetts General Brigham Biobank (MGBB) is an EMR-linked biorepository based at an academic medical center in Boston, Massachusetts (Blout Zawatsky et al. 2021). At the time of analysis, 65,309 biobank participants had exome sequencing completed using a custom capture library from TWIST Biosciences (approximately 37 Mb target) with sequencing on the Illumina NovaSeq using 150 bp paired reads. Hybrid selection libraries were utilized for 85% of exonic targets at 20×, which is ~55× mean coverage (Blout Zawatsky et al. 2021). Informed consent for use of samples and data, including but not limited to genomic sequencing, EMR review, and publication of results was obtained at the time of enrollment for both cohort and control MGBB participants. EMR review for this study was approved by the Institutional Review Board of Massachusetts General Hospital.

We queried the MGBB for participants with 8 homozygous pseudodeficiency alleles in *IDUA*: p.Ala79Thr, p.His82Gln, p.Asp223Asn, p.Ala300Thr, p.Val322Glu, p.Ala361Thr, p.Gly409Arg, and p.Ser586Phe (Clarke et al. 2017; Chuang et al. 2018; Li, Wood, and Thompson 2002; Taylor et al. 2019; Yu et al. 2020). Demographic information, including self-reported sex, race, and the number of inpatient and outpatient hospital encounters during the last 5 years were collected for all participants. The frequency of these variants in gnomAD was also assessed (Karczewski et al. 2020).

2.2 | Matched Cohort Analysis

A subset of MGBB participants without homozygous pseudodeficiency alleles (controls) were matched 1:1 to participants with homozygous pseudodeficiency *IDUA* alleles (cases) (Figure S1). Matching was based on age at the time of data collection, biological sex, race, and the number of inpatient or outpatient encounters with a clinician at one of the hospitals associated with the primary academic medical center in the last 5 years. The number of visits was grouped into categories of 0 visit, 1–3 visits, 4–15 visits, 16–40 visits, and 41+ visits. At an alpha of 0.05 with 95% power, a sample size of at least 76 individuals would be needed to detect a difference between participants with homozygous pseudodeficiency alleles and matched control participants.

We created a list of ICD9 and ICD10 diagnostic codes corresponding to disease manifestations that have been previously reported in severe and attenuated MPS I (Table S1). These disease

manifestations, represented in parentheses, were grouped by clinical domain: joint and bone disease (joint pain, arthritis, back pain, kyphosis, scoliosis, carpal tunnel syndrome, reduced range of joint motion, short stature); cardiac disease (cardiomyopathy, mitral valve regurgitation); eye disease (corneal clouding, glaucoma); psychiatric (anxiety, depression); infectious disease (middle ear infections, respiratory infections); and neurologic (learning disability, hydrocephalus, abnormal brain MRI). The EMR of every MGBB participant with homozygous pseudodeficiency alleles and each matched control participant was queried for codes associated with these clinical findings (both individually, and aggregated across each clinical category) and, if present at least once, was defined as being present in that individual.

Chi-square or Fisher's exact test was used to compare the frequency of each of the individual and grouped disease manifestations of MPS I between cases and controls. Additionally, to investigate the presence of genotype–phenotype associations, Fisher's exact test was used to compare the frequency of clinical manifestations across participants who were homozygous for each of the pseudodeficiency alleles. In-depth chart review was completed to further investigate all statistically significant findings. To compare the distribution of race categories between the groups of individuals with homozygous pseudodeficiency alleles and all MGBB participants, a Monte Carlo estimation of exact p values was used. All statistical tests were two-sided with $p < 0.05$ considered statistically significant. Data were analyzed using SAS, version 9.4 (SAS Institute Inc. Cary, NC).

3 | Results

Among 65,309 MGBB participants who had previously undergone exome sequencing, 1803 participants (2.8%) were homozygous for one of the eight pseudodeficiency alleles in *IDUA* that were queried. In the MGBB, we identified 1780 (2.73%) p.Ala361Thr homozygotes, 16 (0.02%) p.Gly409Arg homozygotes, 4 (0.01%) p.His82Gln homozygotes, and 3 (0.05%) p.Ala79Thr homozygotes. No participants were found to be homozygous for the p.Asp223Asn, p.Ala300Thr, p.Val322Glu, or p.Ser586Phe variants in *IDUA*. The frequencies of the variants in the MGBB were comparable to those found in the gnomAD database. The cumulative frequency in gnomAD v4 of the variants queried is 2.8% (22,927) homozygotes of 807,162 total participants (Karczewski et al. 2020). Among 807,162 participants in gnomAD, 30 (0.004%) are homozygous for p.Ala79Thr, 32 (0.004%) for p.His82Gln, 3 (0.0004%) for p.Asp223Asn, 0 (0%) for p.Ala300Thr, 2 (0.0002%) for p.Val322Glu, 22,735 (2.8%) for p.Ala361Thr, 125 (0.015%) for p.Gly409Arg, and 0 (0%) for p.Ser586Phe (Karczewski et al. 2020).

The mean age of the individuals with homozygous pseudodeficiency alleles in *IDUA* was 60.3 years and they had a median of 16.0 hospital encounters, providing a substantial amount of EMR data for each participant. A statistically significant difference was found between the distribution of self-reported race among cases (participants with homozygous pseudodeficiency alleles) and all MGBB participants ($p < 0.0001$) (Table 1). In particular, the proportions of Black, Hispanic, and Asian participants were greater among cases.

There were no statistically significant clinically relevant differences between the proportion of homozygous *IDUA* cases and matched control participants with each of the manifestations of MPS I (Figure 1a). There were no significant differences for the presence of joint and bone disease, cardiac disease, eye disease, mental health, infections, hernia, hearing loss, hepatosplenomegaly, or sleep apnea between cases and controls. Participants with homozygous pseudodeficiency alleles were found only to have a statistically significant higher incidence of abnormal brain MRIs compared to biobank-matched controls. All 16 participants with diagnostic codes associated with abnormal brain MRIs were homozygous for the p.Ala361Thr variant. Retrospective reviews of these participants' EMR did not show any of the expected clinical features of MPS I. For example, two participants had pituitary adenomas, and one each with a ring-enhancing lesion and a cerebral hemorrhage.

TABLE 1 | Demographic information from participants in the total MGB biobank, compared with the subset of MGB biobank participants with homozygous pseudodeficiency alleles in *IDUA*.

Race	*N* in MGBB (%)	*N* in homozygous cohort (%)
American Indian or Alaskan	176 (0.27)	0 (0)
Asian	1863 (2.85)	106 (5.87)
Black or African American	3272 (5.01)	180 (9.98)
Hispanic or Latino	26 (0.04)	32 (1.77)
Native Hawaiian	18 (0.02)	0 (0)
Spanish	1 (0.00)	0 (0)
White	55,002 (84.2)	1378 (76.43)
Other	2712 (4.15)	57 (3.16)
Declined	621 (0.95)	0 (0)
Unknown	1618 (2.48)	50 (2.77)
Total	65,309	1803

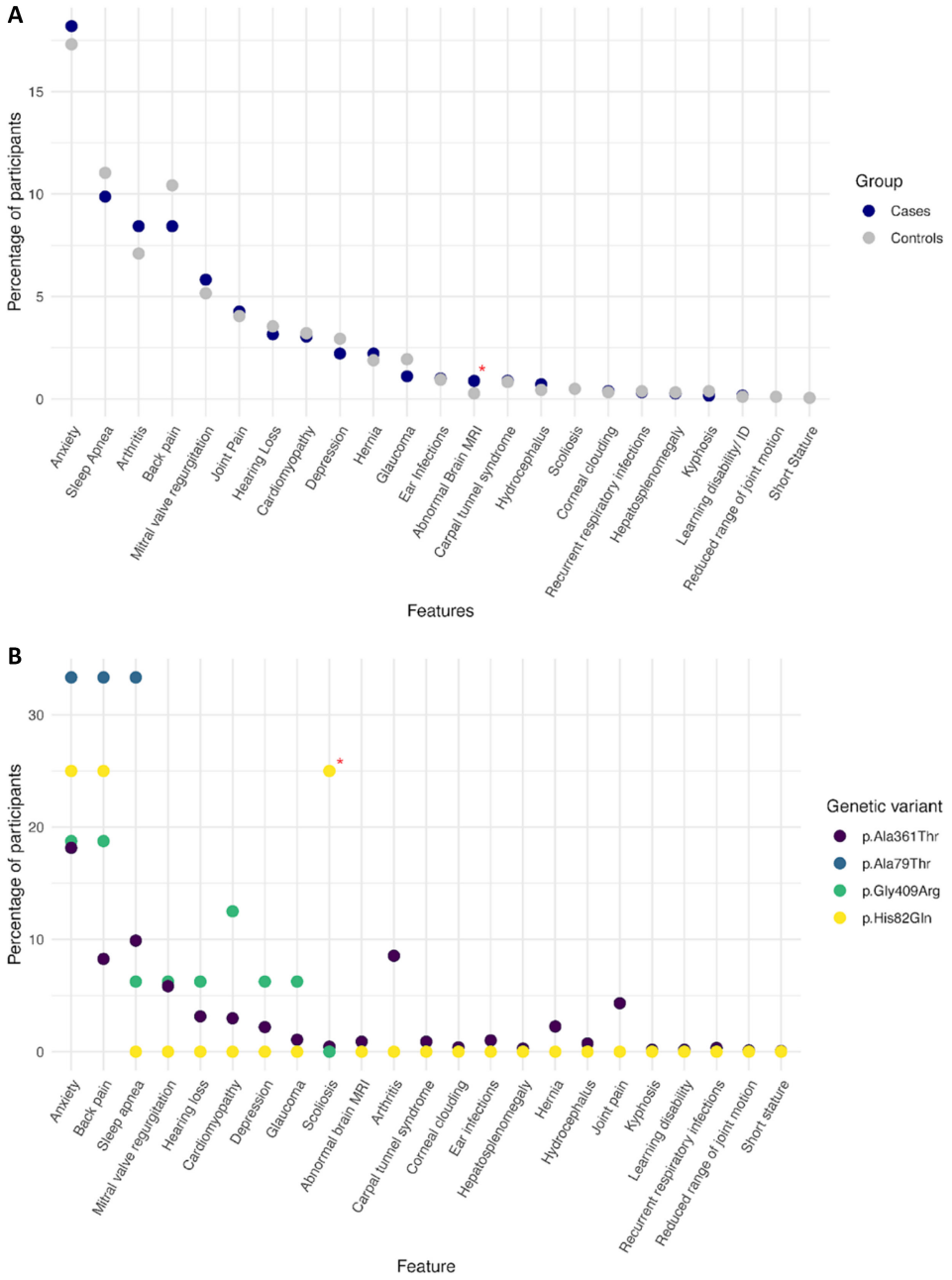

FIGURE 1 | (a) Comparison of presence of diagnoses associated with hallmark clinical features of MPS I between participants with pseudodeficiency alleles in *IDUA* and matched controls. (b) Genotype–phenotype associations for presence of clinical features associated with MPS I among cases by each individual pseudodeficiency allele in *IDUA*.

Genotype–phenotype associations for MPS I-related clinical features by individual pseudodeficiency alleles were assessed (Figure 1b). Across individuals with all homozygous pseudodeficiency alleles, scoliosis was significantly more common among participants with homozygous p.His82Gln alleles (25%) than all other homozygous genotypes ($p = 0.037$). No other genotype–phenotype associations were found.

4 | Discussion

The inclusion of enzyme assays for alpha-L-iduronidase in NBS has been lifesaving for infants at risk for severe or attenuated MPS I. The presence of pseudodeficiency alleles in *IDUA*, however, has led to false positive biochemical screening results in some newborns (Clarke et al. 2017), which may result in unnecessary family stress and healthcare utilization (Prosser et al. 2008). Additionally, pseudodeficiency alleles are more commonly found in infants of non-European descent and therefore may lead to unnecessary medical care and anxiety among families of infants who are non-white. In this matched cohort study, we found that over 1800 adults with a median age of over 60 years in a hospital-based cohort with homozygous pseudodeficiency alleles in *IDUA* did not have documented symptoms of MPS I at a higher frequency than control participants. Supporting historic assumptions, we therefore suggest that infants with homozygous pseudodeficiency alleles, specifically p.Ala361Thr, are unlikely to develop long-term clinical

symptoms. Parents of infants with positive newborn screens for MPS I related to these variants should be entirely reassured about their child's prognosis, including into adulthood.

Consistent with prior studies, we found that pseudodeficiency alleles were enriched among participants who identified as Black or Hispanic, as well as Asian (Clarke et al. 2017; Bosfield et al. 2021). The receipt of false positive NBS results have been demonstrated to lead to increased stress and dysfunction in the parent–child relationship, increased financial costs, and may cause confusion among primary care pediatricians (Gennaccaro, Waisbren, and Marsden 2005; Prosser et al. 2008). It has previously been suggested that testing glycosaminoglycans from dried blood spots can reduce the false positive screening rate to nearly zero (Herbst et al. 2023), but this method also has the potential to screen out children at risk for attenuated disease who may benefit from surveillance aimed at the early initiation of enzyme replacement therapy. By recommending that the samples of all infants with low IDUA enzyme activity and abnormal glycosaminoglycans undergo reflexive testing for common pseudodeficiency alleles as a third-tier test for NBS, we hope to obviate the harms of false-positive results which may be disproportionately affecting non-white families. Of note, endogenous, non-reducing end glycosaminoglycan analysis is also likely sufficient to differentiate among newborns with severe and attenuated MPS I and pseudodeficiency of alpha-L-iduronidase (Herbst et al. 2020).

Referrals for false positive NBS results may also overwhelm an already strained genomics workforce (Maiese et al. 2019). The detection of Krabbe disease, another lysosomal storage disorder which has both severe and attenuated forms, is also complicated by the presence of pseudodeficiency alleles (Guenzel et al. 2020). Experts have argued that Krabbe disease should not be widely screened among newborns in the United States due to several potential harms, including that the number of false positive screening results may challenge the capacity of metabolic referral centers (Bailey 2023), which often have a wait time of months (Maiese et al. 2019). Due to the potential to identify children at risk for severe disease, Krabbe disease was recently added to the RUSP. We suggest that a similar analysis of adults with pseudodeficiency alleles associated with this disorder should be undertaken in the future to guide counseling and prognostication for affected infants.

The findings in this study are limited by several factors. The vast majority of participants with pseudodeficiency alleles were homozygous for p.Ala361Thr, consistent with observations in gnomAD. This study is best generalized to individuals who are homozygous for that variant. Additionally, EMR-linked data are known to be incomplete and may not include documentation of subtle or mild signs and symptoms associated with genetic disease. Finally, because the MGBB includes exomes of only probands, we were unable to phase variants using parental data and cannot comment on the clinical outcomes of individuals found to have compound heterozygous pseudodeficiency alleles nor are we able to study individuals who may be compound heterozygotes for a pathogenic *IDUA* allele and a pseudodeficiency allele. Identifying individuals who are compound heterozygous for a pseudodeficiency allele and a pathogenic or likely pathogenic variant in *IDUA* is an important direction for future research. These individuals often have positive NBS results and reduced alpha-L-iduronidase enzyme activity, however, their long-term health outcomes remain unclear, and it is uncertain whether they require ongoing clinical surveillance. Ideally, these individuals could be recontacted with the goal of collecting samples from their parents or siblings, which would help determine the phase of the variants, and their EMR data could be retrospectively analyzed, similar to the approach used in this study.

This study provides empiric evidence of the assumption that individuals with homozygous pseudodeficiency alleles in *IDUA* have no long-term clinical features of MPS I compared with matched controls. These data can be used to improve counseling for families whose infants receive positive NBS results related to pseudodeficiency alleles. Finally, this study provides a template for EMR-linked biobank data to be used to adjudicate pseudodeficiency alleles across other genetic disorders or for variants of uncertain significance in a wide range of genomic conditions.

Author Contributions

Conceptualization: L.O.G., H.Z., E.P., I.S., A.K., N.B.G. Data curation: L.O.G., H.Z. Formal analysis: L.O., E.Z., H.Z., N.B.G. Investigation: L.O.G., E.Z., H.Z., W.H., E.P., L.C., J.G., A.S., I.S., R.C.G., A.K., N.B.G. Methodology: L.O.G., E.Z., H.Z., W.H., E.P., L.C., J.G., A.S., I.S., R.C.G., A.K., N.B.G. Project Administration: L.O.G. Supervision: E.P., L.C., J.G., A.S., I.S., R.C.G., A.K., N.B.G. Visualization: L.O.G., N.B.G. Writing – original draft: L.O.G., N.B.G. Writing – review and editing: L.O.G., E.Z., H.Z., W.H., E.P., L.C., J.G., A.S., J.Y., I.S., R.C.G., A.K., N.B.G. Guarantor: N.B.G.

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Ethics Statement

This study was approved by the Institutional Review Board of Massachusetts General Hospital (Protocol #: 2020P004138).

Consent

All participants provided consent to participate in the Mass General Brigham Biobank at the time of enrollment.

Conflicts of Interest

Amel Karaa receives compensation for advising several companies including Sanofi and has received grant support from Sanofi. Emma Perez is a paid consultant for Allelica Inc. Robert Green has received compensation for advising the following companies: Allelica, Atria, Fabric and Juniper Genomics; and is a co-founder of Genome Medical and Nurture Genomics. Nina Gold occasionally consults for RCG consulting and has received honoraria from Ambry Genetics.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.