HGG Advances

REPORT

Low frequency of treatable pediatric disease alleles in gnomAD: An opportunity for future genomic screening of newborns

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Summary

Hematopoietic stem cell transplant (HSCT) can prevent progression of several genetic disorders. Although a subset of these disorders are identified on newborn screening panels, others are not identified until irreversible symptoms develop. Genetic testing is an efficient methodology to ascertain pre-symptomatic children, but the penetrance of risk-associated variants in the general population is not well understood. We developed a list of 127 genes associated with disorders treatable with HSCT. We identified likely pathogenic or pathogenic (LP/P) and loss-of-function (LoF) variants in these genes in the Genome Aggregation Database (gnomAD), a dataset containing exome and genome sequencing data from 141,456 healthy adults. Within gnomAD, we identified 59 individuals with a LP/P or LoF variant in 15 genes. Genes were associated with bone marrow failure syndromes, bleeding disorders, primary immunodeficiencies, osteopetrosis, metabolic disorders, and epidermolysis bullosa. In conclusion, few ostensibly healthy adults had genotypes associated with pediatric disorders treatable with HSCTs. Given that most of these disorders do not have biomarkers that could be cheaply and universally assessed on a standard newborn screen, our data suggest that genetic testing may be a complementary approach to traditional newborn screening methodology that has the potential to improve mortality and is not expected to lead to a high burden of false-positive results.

Hematopoietic stem cell transplant (HSCT) and gene therapy prevent progression of symptoms in several severe childhood-onset monogenic diseases. These disorders include bone marrow failure syndromes, primary immunodeficiency disorders, inborn errors of metabolism, genetic forms of hemophagocytic lymphohistiocytosis, hemoglobinopathies and bleeding disorders, disorders of the skin (i.e., epidermolysis bullosa), and disorders of bone metabolism (i.e., osteopetrosis).[1–8] A genetic diagnosis of one of these conditions prior to the onset of symptoms provides an opportunity to initiate HSCT at an early stage of disease, before neuroregression, potential life-threatening infections, or other sequelae occur.[9,10]

Some conditions treated with HSCT or gene therapy, such as various types of severe combined immunodeficiency, mucopolysaccharidosis type I (MPS1, MIM: 607014), and X-linked (XL) adrenoleukodystrophy (MIM: 300100), have been added to the Recommended Uniform Screening Panel (RUSP) in the United States (U.S.).[11,12] Krabbe disease (MIM: 606890), which is also treated with early HSCT, has also been added to newborn screening panels in 10 states within the U.S.[13] Screening for these conditions includes biochemical or immunologic assays in combination with genetic testing. For example, MPS1 is screened using an enzymatic assay, but many states reflex to second-tier genetic testing for positive biochemical screening results. This second-tier testing greatly increases the positive predictive value of newborn screening for MPS1.[14] For actionable disorders that lack an efficient or accurate screening assay, such as spinal muscular atrophy type I (MIM: 253300), quantitative polymerase chain reactions have been introduced as a first-tier test in state newborn screening laboratories.[11]

Genomic screening of newborns has the potential to capture infants with a wider range of actionable childhood-onset genetic disorders than those that are currently included on the RUSP. Recent studies have demonstrated, however, that both false-positive and false-negative results are identified via genomic screening compared with standard newborn screening practices.[15–17] A better understanding of the phenotypic spectrum and penetrance of pathogenic genotypes associated with these disorders is needed, particularly when the appropriate treatments, like HSCT, are invasive, costly, and pose major health risks.

In this study, we used the Genome Aggregation Database (gnomAD), an aggregated dataset from unrelated individuals sequenced as part of various disease-specific and population genetic studies, to identify likely pathogenic or

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pathogenic (LP/P) and presumed loss-of-function (LoF) variants in genes associated with early-onset Mendelian diseases that are treatable with HSCT or gene therapy.[18] Given that gnomAD has been intentionally depleted of individuals with genetic disease, and that many individuals with severe forms of these disorders would not be expected to live until adulthood, these variants likely represent sequencing artifacts, non-penetrant alleles, or very attenuated phenotypes that may not require neonatal intervention.[18] In some cases, non-penetrant alleles may be due to mosaicism, as has been previously demonstrated in genes such as *NFKBIA*.[19] A higher-than-expected population frequency of disease variants could lead to low positive predictive value of screening and increase false-positive rates of individuals for whom preventive treatment would be unnecessary.

The objectives of this study were to (1) identify a list of genes associated with Mendelian disorders that can be treated with HSCT or gene therapy, and (2) assess of the number of individuals in gnomAD with disease-associated genotypes in these genes.

Institutional review board/research ethics committee review was not required for this study as it was conducted using anonymous genotype and phenotype data. gnomAD is an aggregated dataset of 125,748 exomes and 15,708 genomes from unrelated individuals sequenced as part of various disease-specific and population genetic studies.[18] The data in gnomAD were obtained primarily from the control groups of case-control studies of common adult-onset diseases, including cardiovascular disease, type 2 diabetes, and psychiatric disorders. Samples with inadequate consent for the release of aggregate data have been removed.[18] Attempts have been made to deplete this dataset of individuals known to be affected by severe pediatric disease, as well as their first-degree relatives.[18] Samples judged to have lower sequencing quality as well as samples from first- or second-degree relatives and samples with inadequate consent for the release of aggregate data have also been excluded.[18]

We identified a preliminary list of childhood-onset diseases treated with HSCT and gene therapy using medical literature and a commercial genetic panel for primary immunodeficiency disorders.[1–10] After evaluating gene-disease association validity by reviewing the number of disease-causing variants reported in ClinVar and the Human Gene Mutation Database (HGMD), genes with insufficient evidence were removed.[20,21] This list of genes was then distributed to content-area experts, who modified the list based upon their clinical experience, and included only genes and disorders that were childhood-onset, severe, and current or future targets of HSCT or gene therapy.

All gnomAD variants (v.2.1.1) that passed quality metrics from the selected genes were filtered to include only variants with implicated genotypes (i.e., for genes associated with an autosomal recessive (AR) disorder, only variants with ≥ 1 homozygous count were included, as the phase of heterozygous variants is unable to be determined). For genes associated with X-linked disorders, only variants with ≥ 1 homozygous count or ≥ 1 hemizygous count were included. This variant list was subsequently filtered to identify: (1) variants that have a minor allele frequency $< 5.0\%$ in gnomAD and have been classified as pathogenic or likely pathogenic from "single submitter, criteria provided" submitters in ClinVar; (2) predicted LoF variants (i.e., nonsense, frameshift, and $\pm$ 1,2 splice-site variants) with a minor allele frequency $\leq 1.0\%$ in gnomAD. Predicted LoF variants tagged as low confidence (LC LoF) were removed from analysis.

We identified 127 high-evidence genes associated with childhood-onset Mendelian disorders treated by HSCT (Table S1). This list includes genes associated with primary immunodeficiencies (n = 61), bone marrow failure disorders (n = 34), inborn errors of metabolism (n = 19), hemophagocytic lymphohistiocytosis (n = 4), bleeding disorders and hemoglobinopathies (n = 3), osteopetrosis (n = 3), and epidermolysis bullosa (n = 3). The majority of these disorders are associated with AR inheritance (n = 91), with a minority caused by autosomal dominant (AD) (n = 22) and X-linked recessive inheritance (n = 14).

Within gnomAD, we identified 59 individuals (0.04%) with 36 distinct LP/P variants. Assuming that no individuals within gnomAD have severe Mendelian childhood-onset disease, the false-positive rate of genomic screening in this population for individuals with homozygous variants would be 0.04%.

Variants were identified in 15 genes (Table 1). Associated genetic conditions included immunodeficiency disorders (n = 20), bone marrow failure syndromes (n = 18), hemoglobinopathies (n = 16), and inborn errors of metabolism (n = 5) (Figure 1). Most risk variants occurred in only one individual (n = 26), although several variants were identified in multiple individuals (n = 10). The highest number of individuals had variants in *ELANE* (n = 15), which is associated with AD congenital neutropenia (MIM: 202700).

Overall, individuals had variants that occurred predominantly in genes that confer disease in an AD pattern of inheritance (n = 24). A minority of individuals had homozygous variants in genes associated with AR conditions (n = 6) and hemizygous variants associated with X-linked recessive conditions (n = 6).

Within a large database of genome and exome sequence information, we identified only a small percentage of apparently healthy adults who harbor risk variants in a subset of genes associated with treatable Mendelian conditions. While issues regarding sensitivity of genomic sequencing versus traditional biochemical screening have been explored for inherited metabolic disorders, the reciprocal issue of specificity and positive predictive value of pathogenic genotypes has not been explored in as much detail. Incomplete penetrance or wide expressivity of childhood-onset conditions may lead to challenges in the screening and diagnosis of pre-symptomatic individuals. However, our data suggest that this issue is likely rare in the general population for the subset of genes

Table 1. Individuals (n = 59) with variants in genes associated with severe pediatric-onset disease treatable with HSCT or gene therapy identified in gnomAD

Gene	Variant	Individuals with disease-associated genotypes	Phenotype
ABCD1	c.2106_2122del (GenBank: NM_000033.3) (p.Leu703AspfsTer25)	1	Adrenoleukodystrophy
CARD11	c.3261−2A>G (GenBank: NM_032415.4)	2	B cell expansion with NKFB and T cell anergy/immunodeficiency 11B with atopic dermatitis
CARD11	c.2704−1G>C (GenBank: NM_032415.4)	1	B cell expansion with NKFB and T cell anergy/immunodeficiency 11B with atopic dermatitis
CARD11	c.3260+1G>A (GenBank: NM_032415.4)	1	B cell expansion with NKFB and T cell anergy/immunodeficiency 11B with atopic dermatitis
CARD11	c.1518+1G>A (GenBank: NM_032415.4)	1	B cell expansion with NKFB and T cell anergy/immunodeficiency 11B with atopic dermatitis
CARD11	c.7+2T>G (GenBank: NM_032415.4)	1	B cell expansion with NKFB and T cell anergy/immunodeficiency 11B with atopic dermatitis
CARD11	c.2585delA (GenBank: NM_032415.4) (p.His862ProfsTer52)	1	B cell expansion with NKFB and T cell anergy/immunodeficiency 11B with atopic dermatitis
CARD11	c.2671C>T (GenBank: NM_032415.4) (p.Arg891Ter)	1	B cell expansion with NKFB and T cell anergy/immunodeficiency 11B with atopic dermatitis
CARD11	c.1876G>T (GenBank: NM_032415.4) (p.Glu626Ter)	1	B cell expansion with NKFB and T cell anergy/immunodeficiency 11B with atopic dermatitis
CARD11	c.1156C>T (GenBank: NM_032415.4) (p.Arg386Ter)	1	B cell expansion with NKFB and T cell anergy/immunodeficiency 11B with atopic dermatitis
CHD7	c.5051−2A>T (GenBank: NM_017780.3)	1	CHARGE syndrome
CHD7	c.401_402insAA (GenBank: NM_017780.3) (p.His134GlnfsTer78)	1	CHARGE syndrome
CHD7	c.395_399delAGAGG (GenBank: NM_017780.3) (p.Glu132AlafsTer153)	1	CHARGE syndrome
CHD7	c.8522C>A (GenBank: NM_017780.3) (p.Ser2841Ter)	1	CHARGE syndrome
CTLA4	c.255_256delTG (GenBank: NM_005214.4) (p.Ala86GlyfsTer8)	1	autoimmune lymphoproliferative syndrome, type V
ELANE	c.258_269dup (GenBank: NM_001972.2) (p.His87_Ser90dup)	1	neutropenia, severe congenital 1
ELANE	c.427C>T (GenBank: NM_001972.2) (p.Arg143Cys)	5	neutropenia, severe congenital 1
ELANE	c.573G>C (GenBank: NM_001972.2) (p.Arg191Ser)	2	neutropenia, severe congenital 1
ELANE	c.659G>A (GenBank: NM_001972.2) (p.Arg220Gln)	1	neutropenia, severe congenital 1
ELANE	c.628G>A (GenBank: NM_001972.2) (p.Gly210Arg)	6	neutropenia, severe congenital 1
F8	c.6935dupT (GenBank: NM_000132.3) (p.Val2313GlyfsTer72)	1	hemophilia A
F8	c.6089G>A (GenBank: NM_000132.3) (p.Ser2030Asn)	3	hemophilia A

(*Continued on next page*)

Table 1. ***Continued***

Gene	Variant	Individuals with disease-associated genotypes	Phenotype
F8	c.1834C>T (GenBank: NM_000132.3) (p.Arg612Cys)	3	hemophilia A
F9	c.316G>A (GenBank: NM_000133.3) (p.Gly106Ser)	3	hemophilia B
GAA	c.−32−13T>G (GenBank: NM_001079803.1)	1	glycogen storage disease II
GATA2	c.16_17insC (GenBank: NM_001145661.1) (p.Glu6AlafsTer179)	1	GATA2 syndromes
GBA	c.1226A>G (GenBank: NM_001005741.2) (p.Asn409Ser)	3	Gaucher disease
HBB	c.19G>A (GenBank: NM_000518.4) (p.Glu7Lys)	1	beta thalassemia major
HBB	c.79G>A (GenBank: NM_000518.4) (p.Glu27Lys)	1	beta thalassemia major
HBB	c.20A>T (GenBank: NM_000518.4) (p.Glu7Val)	4	beta thalassemia major
NFKBIA	c.875_876delAG (GenBank: NM_020529.2) (p.Glu292ValfsTer14)	1	ectodermal dysplasia, anhidrotic, with T cell immunodeficiency
NFKBIA	c.735_736delAG (GenBank: NM_020529.2) (p.Arg245SerfsTer39)	1	ectodermal dysplasia, anhidrotic, with T cell immunodeficiency
NFKBIA	c.438_493del (GenBank: NM_020529.2) (p.Pro147ValfsTer43)	1	ectodermal dysplasia, anhidrotic, with T cell immunodeficiency
RPS10	c.373_374delGA (GenBank: NM_001014.4) (p.Asp125TyrfsTer34)	1	Diamond-Blackfan anemia
SBDS	c.258+2T>C (GenBank: NM_016038.2)	2	Shwachman-Diamond syndrome
XIAP	c.758C>G (GenBank: NM_001167.3) (p.Ser253Ter)	1	lymphoproliferative syndrome, X-linked, 2

assessed. Overall, assuming that the individuals within gnomAD are free from severe Mendelian childhood-onset disease, the false-positive rate of genomic screening of newborns for diseases treatable with HSCT was calculated to be 0.04%, better than that of many enzymatic assays.[15]

At present, most Mendelian disorders treatable with HSCT are diagnosed after the onset of symptoms or due to the presence of a known affected family member. Presymptomatic screening for those with risk variants offers a potential opportunity to evaluate additional biomarkers of the related condition or initiate disease-modifying therapy prior to the emergence of symptoms. The individuals we identified with LP/P variants likely have non-penetrant alleles or highly attenuated symptoms of disease; alternatively, the variants are sequencing artifacts. While not all individuals with disease genotypes would immediately proceed to HSCT, early identification of genetic diagnoses would facilitate surveillance by appropriate clinicians and the opportunity to monitor early disease progression.

Our findings demonstrate that first-tier genomic screening for these disorders would not lead to a surplus of false positives, which could negatively impact patients and families or burden clinicians specializing in pediatric hematology-oncology, immunology, and other related specialties. We estimate that 0.04%, or 1 in 2,500 individuals, would be expected to have false-positive genomic results, equal to approximately 1,500 of the 3,747,540 infants born in 2019.[22] Prior analyses of current newborn screening techniques for multiple inborn errors of metabolism (phenylketonuria, galactosemia, and biotinidase deficiency) and endocrinopathies (congenital hypothyroidism and congenital adrenal hyperplasia) have identified a positive predictive value of only 0.5%–6.0%, generating more than 50 false-positive results for every true-positive result on newborn screening in the United States. For phenylketonuria alone, there were 8,867 false-positive results generated during 1994.[23]

This study builds on previous analyses of the Exome Aggregation Consortium (ExAC) database that identified individuals with homozygous variants in genes associated with childhood-onset disease.[24] Other descriptive studies of large-scale exome datasets have identified healthy individuals with pathogenic and likely pathogenic genetic variants.[24,25] Our results reinforce prior observations regarding incomplete penetrance of some disease-related genotypes, although we found that in the genes of interest,

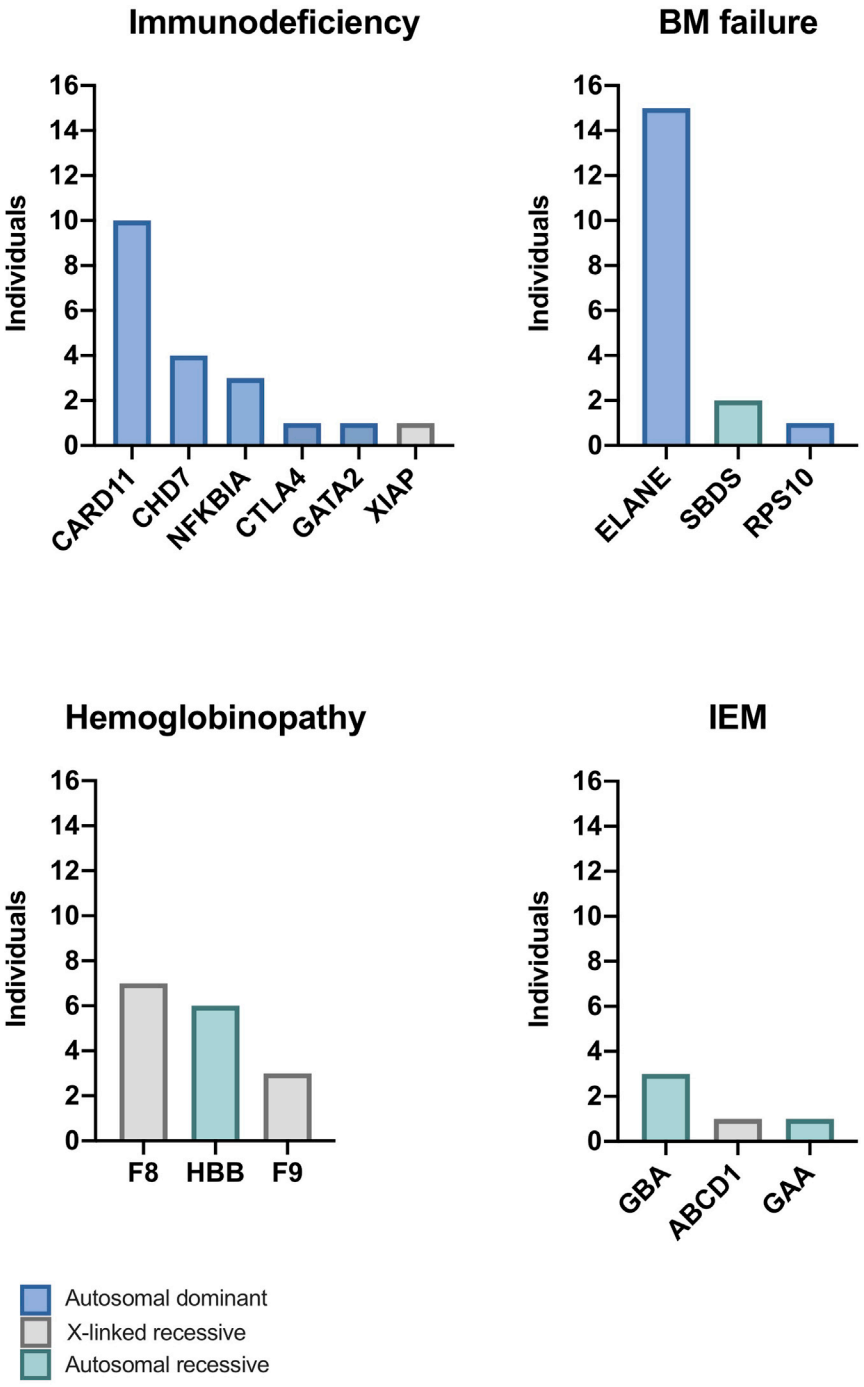

Figure 1. Variants in genes associated with disorders treatable by HSCT in gnomAD. (Abbreviations: BM, bone marrow; IEM, inborn errors of metabolism.)

incomplete penetrance or attenuated phenotypes were less common than previously reported.[24,25]

In general, the majority of disease-associated genotypes that we identified in ostensibly healthy adults were found in *ELANE*. A recent study utilizing CRISPR gene editing has found that variants in early exons of *ELANE* elicited nonsense-mediated decay, while terminal exon frameshift alleles escaped nonsense-mediated decay. Additionally, −1 frame insertions or deletions impeded neutrophil maturation and were associated with congenital neutropenia, whereas −2 frame late exon insertions and deletions supported neutrophil maturation.[26] It is likely that this more detailed information regarding the mechanisms underlying pathogenicity in *ELANE* will lead ClinVar submitters to reclassify many of the variants identified in gnomAD. Additionally, asymptomatic individuals harboring pathogenic *ELANE* variants exhibit mutant variant mosaicism in all examined cell types except neutrophils, which may provide the mechanism of decreased penetrance in the studied patient population.[27] For asymptomatic individuals with pathogenic variants in *ELANE*, there may be a need for the development of management guidelines to determine the frequency with which complete blood counts should be followed by a primary care doctor or hematologist.

This study provides a preliminary model for assessing the specificity of genomic screening, but it has several limitations due to the constraints of the available data. First, we cannot rule out that the gnomAD variants have been identified in error or that prior variant classifications as recorded in ClinVar are flawed.[28] Next, we cannot be certain that the individuals whose data are included in gnomAD are free from Mendelian disease and could benefit from appropriate treatment. Given the phenotypic heterogeneity of associated with some genes included in this study (i.e., hemoglobinopathies), it is also possible that some individuals may have mild or subclinical symptoms of disease, which would not require HSCT. Importantly, because biallelic variants cannot be ascertained in the gnomAD database, our analysis focused only on heterozygous and hemizygous variants associated with AD and X-linked disorders, as well as homozygous variants associated with AR conditions. We did not include compound heterozygotes, as these individuals cannot be definitively ascertained without parental data and, as such, may increase rates of false positivity. Additionally, structural variants were not a target of this analysis and should be included in future work. This analysis could be replicated using other datasets, such as dbGaP, or genetic information aggregation

tools, such as AnVil.[29,30] Finally, while this analysis assesses the specificity of genomic sequencing, further investigations of the sensitivity of sequencing must be pursued to determine the suitability of this test for population-wide screening in newborns. Future analyses, including compound heterozygous variants and structural variants, should aim to improve the sensitivity of analyses.

Given these limitations, we suggest that identification of individuals with heterozygous and hemizygous LP/P variants associated with AD and X-linked disorders, as well as homozygous LP/P variants associated with AR conditions, might be an initial target of genomic screening of newborns. A stepwise approach toward including biallelic variants should be considered in the future. However, it should be noted that screening of biallelic variants may give rise to downstream bioethical challenges, as a newborn with two parents available for genetic testing stands to benefit from this screening information more than a newborn who only has one available parent. The overall clinical utility, costs, scalability, and equity of genomic screening are important future research directions.

New and effective therapies for Mendelian disorders are being rapidly developed. Many of these therapies are most efficacious if begun before clinical symptoms emerge. Our findings suggest that first-tier genomic screening for disorders treated with HSCT may be of value for affected newborns and in some circumstances would not lead to an undue burden of false-positive results.

Data and code availability

Source data for this study are available at gnomAD. This published article includes the filtered gnomAD dataset analyzed during this study. While this manuscript analyzes anonymized data from a publicly available database, any materials or data produced during this study will be made available upon request

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.xhgg.2021.100059.

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Declaration of interests

R.C.G. receives compensation for advising AIA, Genomic Life, OptumLabs, Plumcare, Verily, and VibrentHealth and is co-founder of Genome Medical, Inc. S.M.H. is an employee of Ambry Genetics. E.F. is employed by Keros Therapeutics. All other authors declare no competing interests.

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Web Resources

gnomAD, https://gnomad.broadinstitute.org/
OMIM, https://omim.org/

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