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# Estimating the relative frequency of leukodystrophies and recommendations for carrier screening in the era of next-generation sequencing

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AUTHOR CONTRIBUTIONS

Johanna, L. Schmidt, Amy Pizzino, Guy Helman, and Adeline Vanderver made substantial contributions to conception and design, acquisition of data, analysis, and interpretation of data. Jessica Nicoll and Allison Foley made substantial contributions to conception and design, as well as acquisition of data. Yue Wang, Jill A. Rosenfeld, Lindsey Mighion, Lora Bean, Cristina da Silva, Megan T. Cho, Rebecca Truty, John Garcia, Virginia Speare, Kirsten Blanco, Zoe Powis, Grace M. Hobson, Susan Kirwin, Bryan Krock, Hane Lee, Joshua L. Deignan, Maggie A. Westemeyer, Ryan L. Subaran, Isabelle Thiffault, Ellen A. Tsai, and Terry Fang have made substantial contributions to the acquisition and interpretation of data. All authors were involved in drafting the manuscript and/or revising it critically for important intellectual content. Each author has given final approval of the version to be published and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST

A. F. is a coordinator in clinical trials sponsored by BioMarin Pharmaceuticals. Y. W. reports that the Department of Molecular & Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing conducted by Baylor Genetics. Y. W. receives salary support from Baylor Genetics. J. A. R. reports that the Department of Molecular & Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing conducted by Baylor Genetics. J. A. R. receives salary support from Baylor Genetics. L. M., L. B., Cd. S. are employees of Emory Genetics Lab. M. T. C. at the time of this work, was an employee of GeneDx, a wholly owned subsidiary of OPKO Health, Inc. R. T. and J. G. are employees and stockholders of Invitae. MW is an employee and stockholder of Natera. V. S., K. B., and Z. P. are employees of Ambry Genetics. J. E. H. is an employee of Qiagen. J. R. P. is an employee of Tempus. E. A. T. and T. C. F. are employed by Biogen. A. V. receives research support from Biogen, Gilead, Ionis, Illumina, Eli Lilly, and Shire/Takeda. The remaining authors do not report any conflicts of interest.

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## Abstract

Leukodystrophies are a heterogeneous group of heritable disorders characterized by abnormal brain white matter signal on magnetic resonance imaging (MRI) and primary involvement of the cellular components of myelin. Previous estimates suggest the incidence of leukodystrophies as a whole to be 1 in 7,000 individuals, however the frequency of specific diagnoses relative to others has not been described. Next generation sequencing approaches offer the opportunity to redefine our understanding of the relative frequency of different leukodystrophies. We assessed the relative frequency of all 30 leukodystrophies (associated with 55 genes) in more than 49,000 exomes. We identified a relatively high frequency of disorders previously thought of as very rare, including Aicardi Goutières Syndrome, *TUBB4A*-related leukodystrophy, Peroxisomal biogenesis disorders, POLR3-related Leukodystrophy, Vanishing White Matter, and Pelizaeus-Merzbacher Disease. Despite the relative frequency of these conditions, carrier-screening laboratories regularly test only 20 of the 55 leukodystrophy-related genes, and do not test at all, or test only one or a few, genes for some of the higher frequency disorders. Relative frequency of leukodystrophies previously considered very rare suggests these disorders may benefit from expanded carrier screening.

### Keywords

carrier screening; frequency; leukodystrophy; next-generation sequencing

## 1 | INTRODUCTION

Leukodystrophies are a group of genetic disorders associated with molecular defects affecting primarily the structural components of the central nervous system white matter (van der Knaap & Bugiani, 2017). Molecular advances, in particular next-generation

sequencing (NGS), have increased the number of leukodystrophy diagnoses made (Kevelam et al., 2016; van der Knaap & Bugiani, 2017; Vanderver et al., 2015).

To better assess the frequency of leukodystrophy diagnoses and to understand the extent to which carrier screening laboratories are identifying carrier individuals, we obtained data from carrier-screening laboratories and commercial and university-hospital based diagnostic testing companies using NGS across the United States.

Our goal was to determine which leukodystrophies are diagnosed most frequently and to determine if carrier screening laboratories are offering screening for the most commonly seen conditions. To ascertain the most comprehensive relative frequency data, we included diagnostic results from single gene, panel, and exome sequencing (ES) from clinical laboratories in the United States. From carrier screening laboratories, we obtained data on the leukodystrophy genes that are regularly screened in those laboratories to determine which conditions are included and how frequently carriers are identified using carrier screening methods. We report our findings of this national study over a 5-year period.

## 2 | METHODS

### 2.1 | Laboratory inclusion

The study received approval from the Institutional Review Board at the Children's Hospital of Philadelphia (IRB#14-011236). We identified the largest (volume and number of genes) carrier screening laboratories that included at least one leukodystrophy gene in their carrier screening panel. By surveying of members of the Global Leukodystrophy Initiative, a consortium including clinician scientists focused on the leukodystrophies, we identified molecular diagnostic laboratories that have been performing ES and/or using NGS for specific leukodystrophy/neurology genes between September 30, 2012 and September 30, 2017.

### 2.2 | Molecular diagnostic laboratory data and availability

Eight molecular testing laboratories elected to participate: six provided data from ES and targeted sequencing across the time period of the study or from when they began implementing ES in their testing options, and two provided data from single gene or panel-based testing. We provided each diagnostic laboratory with a list of 55 leukodystrophy-associated genes, causing 30 disorders, according to an established case description (Vanderver et al., 2015). Laboratories were asked to provide data on any pathogenic, likely pathogenic, or variants of unknown significance (VUS) reported in a clinical setting, by methodology used (single gene, panel-based testing, or ES). We obtained variant level data (position, coding sequence change, amino acid/protein change, transcript ID, and zygosity), number of patients identified with variant(s) in those genes, and how those variants were classified/reported. Variants were evaluated against the American College of Medical Genetics and Genomics criteria for variant classification, and variants with a benign/likely benign classification were removed (Richards et al., 2015).

### 2.3 | Relative frequency determination

Aggregate data for each gene were combined from clinical diagnostic testing laboratories. The relative frequency of different leukodystrophies identified by exome sequencing was calculated by dividing the sum of diagnosed individuals with each diagnosis by the total number of exomes sequenced. We expanded data collection to include singlegene testing or gene panels including our target list of genes to generate an overall diagnosis and calculated overall relative frequency by all methods of data collection (Vanderver et al., 2015). A true incidence rate of individual leukodystrophies could not be calculated due to the ascertainment bias in the genetic screening cohort, which does not represent the entire population from which these cases were derived.

### 2.4 | Carrier frequency determination

Data from five carrier screening laboratories were included in this study. Aggregate data for each gene were combined from all testing laboratories to determine carrier frequency. Ethnicity data were not available, so analysis both excluding and including the presence of known founder mutations in *ARSA*, *ASPA*, *CYP27A1*, *SAMHD1*, *and SUMF1* was performed (Wallace & Lora, 2018). This approach was intended to limit the bias of populations with known founder mutations more likely to seek out carrier testing (Kraft, Duenas, Wilfond, & Goddard, 2019). To derive the carrier frequency from commercial carrier screening laboratories, the total number of heterozygous individuals with a pathogenic variant as defined above was divided by the number of individuals for whom that gene was screened.

## 3 | RESULTS

### 3.1 | Relative frequency determination

From laboratories testing via ES, we found 332 cases of leukodystrophy were diagnosed with pathogenic/likely pathogenic variants out of 49,805 total exome sequencing tests for any indication (1/150 individuals tested). Aicardi Goutières Syndrome (AGS) (18.07%), *TUBB4A*-related leukodystrophy (9.04%), Peroxisomal biogenesis disorders (Zellweger) (7.23%), POLR3-related Leukodystrophy (6.93%), vanishing white matter (VWM) (6.63%), and Pelizaeus-Merzbacher Disease (PMD) (6.02%) represent the most frequently diagnosed leukodystrophies by ES (Table 1, Figure 1a).

Using an ES-based ascertainment generally excludes disorders which are predominantly tested for by biochemical testing or single gene sequencing performed on affected individuals due to highly recognizable phenotypes. These include conditions such as X-linked Adrenoleukodystrophy (X-ALD), Cerebrotendinous Xanthomatosis, Metachromatic Leukodystrophy (MLD), and Krabbe disease. These conditions are typically diagnosed through the use of testing of very long chain fatty acids, cholestanol levels, or lysosomal enzyme screening, respectively. When a biochemical diagnosis is made, clinicians often obtain molecular testing as well, usually via single gene or targeted panel, to confirm the diagnosis and/or to provide this molecular information to families. While not every case diagnosed by biochemical testing also receives a molecular diagnosis, we wanted to include those cases that did. Therefore, to calculate the overall relative burden of molecular

diagnoses during the study time period, we combined data from single gene, targeted panel and ES testing. This identified 664 leukodystrophy diagnoses among 52,648 (1/79) tests, identifying X-ALD (14.61%), MLD (10.24%), and AGS (9.79%) as the most frequently diagnosed leukodystrophies (Table 1, Figure 1b).

### 3.2 | Carrier frequency determination

We then queried testing rates for the 55 leukodystrophy associated genes in carrier screening laboratories. Across all five carrier screening laboratories, only 20 of the 55 leukodystrophy-related genes were tested (Table 2). Data were available from approximately 116,000 to more than 200,000 screened individuals, depending on the gene. The remaining 28 genes in our case description were not assessed in any laboratories.

Results from carrier screening predict high carrier frequencies for Globoid cell leukodystrophy, also known as Krabbe disease (*GALC*; 1/105), Polyglucosan Body disease and its biochemical variants (*GBE1*; 1/152) and Peroxisomal biogenesis disorder caused by *PEX6* (*PEX6*; 1/153) (Table 2). Of note, *GBE1* is also associated with glycogen branching disorder, however imperfect genotype–phenotype correlation precluded exclusion of cases not predicted to present with a leukodystrophy phenotype. Excluding founder variants commonly seen in the Ashkenazi population reduced the numbers of carriers for Canavan disease (*ASPA*; from 1/173 to 1/867) and Polyglucosan Body disease (*GBE1*, from 1/152 to 1/225), as well as disease due to *PEX2* variants (from 1/794 to 1/2,343), and multiple sulfatase deficiency (*SUMF1*; from 1/838 to 1/1,359).

## 4 | DISCUSSION

In this study, we use an established case description for leukodystrophies (Vanderver et al., 2015) and modern sequencing data to assess the relative burden of leukodystrophy diagnoses. We also assess current carrier screening approaches as they relate to leukodystrophy carrier identification.

We were able to identify the conditions with the highest relative molecular diagnostic frequency in the US. Using ES only, these included AGS (most often *RNASEH2B* variants), *TUBB4A*-related leukodystrophy, and VWM (*EIF2B5*). Across all types of molecular diagnostic testing, including single gene testing, the most common leukodystrophies included X-ALD, MLD, and AGS. The relatively high frequency of AGS may be, in part, due to the existence of the recurring p.Ala177Thr variant (19/38 alleles in this cohort). However, the fact that 70% of affected individuals had mutations in one of the six other known genes causative this disorder suggests that this may also be related to the number of overall genes associated with AGS. AGS has previously been considered to be a very rare condition; however, these findings support that it is more common than previously known.

Carrier screening has been rapidly expanding with improvements in genomics. Importantly, fewer than 50% of genes classically associated with recessive leukodystrophies are screened at the time of this study. Screening for VWM (*EIF2B5*) was being done at three out of the five participating laboratories. It is important to note that some autosomal recessive disorders with the highest frequency in our study were not included in carrier screening panels at

the time of analysis. In particular, *RNASEH2B*, associated with AGS (2.86%) was seen at a high rate in this cohort. Indeed, the only AGS-related gene screened by any laboratory was *SAMHD1*, which was screened at two laboratories. Similarly, *POLR3A*, associated with a hypomyelinating leukodystrophy known as 4H Syndrome (Hypomyelination with hypodontia and hypogonadotropic hypogonadism) (3.16%) (Table 3), was absent from carrier screening panels and yet seen at relatively high frequency in this cohort. Based on their relative frequency in this cohort of diagnosed cases, these disorders may warrant inclusion in carrier screening panels.

However, decisions around inclusion of disorders in carrier screening are also dependent on their pattern of inheritance. Conditions caused predominantly by de novo variants such as Alexander Disease or *TUBB4A*-related leukodystrophy would not be included in carrier screening panels (Vanderver et al., 2015). Of note, *ADAR1* and *IFIH1*, associated with Aicardi Goutieres syndrome, were also seen at relatively high frequency (2.56 and 1.36% respectively), but, their inclusion in carrier screening would be complicated by autosomal dominant inheritance.

### 4.1 | Limitations

There are several limitations to our methods. The phenotypic spectrum of many of these disorders is still incompletely understood, and predictions of disease incidence from molecular diagnoses is challenging. For example, upon closer examination of these variants through routine screening, previously discovered pathogenic variants may have incomplete penetrance. An individual with pathogenic variants may not present with disease until adulthood or may never present with classic disease features. This may partially explain the difference between disorders predicted to be most frequent based on carrier frequency and the relative frequency seen in our cohort, an example being Krabbe disease, which has an adult-onset form. Some genes (*GBE1*, *POLR1C*, *and CSF1R*) have allelic conditions that do not typically present as a leukodystrophy. Some disorders may be underrepresented, such as PMD, where gene dosage changes are common and are not analyzed on clinical ES (Inoue, 2005). Our case definition may also be too narrow as more gene-disease associations are established (Vanderver et al., 2016). Finally, our analysis was based only on clearly pathogenic or likely pathogenic variants as identified in clinical testing, thus, not taking into account private variants considered to be VUS when applying ACMG guidelines.

### 4.2 | Conclusion

In conclusion, we determined the relative frequency of the various leukodystrophies in clinical practice. Using ES frequency rates, the following leukodystrophies may be more common than previously understood: AGS, VWM, POLR3-related Leukodystrophy, and PMD. As expanded carrier screening becomes more commonplace, inclusion of more leukodystrophy-related genes will become more feasible, and our data support the addition of more leukodystrophy disorders in carrier screening panels.

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## DATA AVAILABILITY STATEMENT

De-identified data are available at the request of a qualified investigators.

## REFERENCES

Inoue K. (2005). PLP1-related inherited dysmyelinating disorders: Pelizaeus-Merzbacher disease and spastic paraplegia type 2. Neurogenetics, 6(1), 1–16. 10.1007/s10048-004-0207-y [PubMed: 15627202]

Kevelam SH, Steenweg ME, Srivastava S, Helman G, Naidu S, Schiffmann R, … van der Knaap MS (2016). Update on Leukodystrophies: A historical perspective and adapted definition. Neuropediatrics, 47(6), 349–354. 10.1055/s-0036-1588020 [PubMed: 27564080]

Kraft SA, Duenas D, Wilfond BS, & Goddard KAB (2019). The evolving landscape of expanded carrier screening: Challenges and opportunities. Genetics in Medicine, 21(4), 790–797. 10.1038/s41436-018-0273-4 [PubMed: 30245516]

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, … Committee, A. L. Q. A. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in Medicine, 17(5), 405–424. 10.1038/gim.2015.30 [PubMed: 25741868]

van der Knaap MS, & Bugiani M (2017). Leukodystrophies: A proposed classification system based on pathological changes and pathogenetic mechanisms. Acta Neuropathologica, 134(3), 351–382. 10.1007/s00401-017-1739-1 [PubMed: 28638987]

Vanderver A, Prust M, Tonduti D, Mochel F, Hussey HM, Helman G, … Consortium, G. (2015). Case definition and classification of leukodystrophies and leukoencephalopathies. Molecular Genetics and Metabolism, 114(4), 494–500. 10.1016/j.ymgme.2015.01.006 [PubMed: 25649058]

Vanderver A, Simons C, Helman G, Crawford J, Wolf NI, Bernard G, … Taft RJ (2016). Whole exome sequencing in patients with white matter abnormalities. Annals of Neurology, 79(6), 1031–1037. 10.1002/ana.24650 [PubMed: 27159321]

Wallace SE, & Lora JHB (2018). Resources for genetics professionals — Common founder variants: Genetic disorders associated with founder variants common in the Ashkenazi Jewish population. In MP AHA, Pagon RA, et al. (Eds.), GeneReviews® (pp. 1993–2019). Seattle (WA): University of Washington, Seattle.

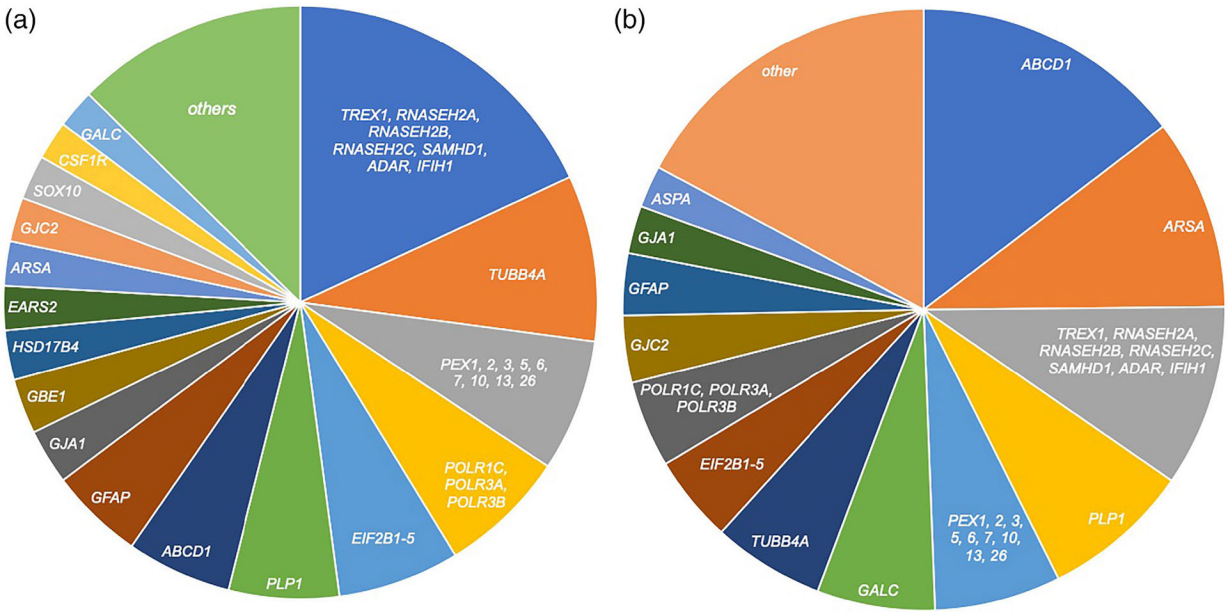


**FIGURE 1.**

(a) Relative frequency of cases identified in exome sequencing cohorts. The remainder of the genes (*SLC17A5*, *CYP27A1*, *SUMF1*, *PSAP*, *DARS2*, *HEPACAM*, *MLC1*, *PSAP*, *RNASET2*, *ALDH3A2*, *ASPA*, *FUCA1*, *DARS*, *FAM126A*, *ACOX1*, *LMNB1*, *CLCN2*, *SCP2* in decreasing order of frequency) each represented less than 2% of the total population. (b) Relative frequency of cases identified in exome, gene panel and single gene sequencing cohorts. The remainder of the genes (*LMNB1*, *GBE1*, *HSD17B4*, *EARS2*, *CYP27A1*, *SUMF1*, *SOX10*, *CSF1R*, *SLC17A5*, *HEPACAM*, *DARS2*, *MLC1*, *ALDH3A2*, *PSAP*, *FUCA1*, *FAM126A*, *PSAP*, *RNASET2*, *DARS*, *ACOX1*, *CLCN2*, *SCP2* in decreasing order of frequency) each represented less than 2% of the total population

## TABLE 1

Relative frequency of leukodystophies by all molecular testing methodologies

| Disorder | Gene | OMIM | Relative frequency solved by all methods (*n* = 664) | *n* | Relative frequency solved by exome sequencing (*n* = 332) | *n* |
|---|---|---|---|---|---|---|
| Adrenoleukodystrophy | *ABCD1* | 300371 | 14.61% | 97 | 5.72% | 19 |
| Metachromatic leukodystrophy | *ARSA* | 607574 | 10.24% | 68 | 2.41% | 8 |
| Aicardi Goutières syndrome | *TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR, IFIH1* | 606609,606034, 610326,610330, 606754,146920, 606951 | 10.00% | 65 | 18.07% | 60 |
| Pelizaeus-Merzbacher disease | *PLP1* | 300401 | 7.98% | 53 | 6.02% [a] | 20 |
| Peroxisomal biogenesis disorder | *PEX1, PEX2, PEX3, PEX5, PEX6*[b], *PEX7, PEX10, PEX13, PEX26* | 602136,170993, 603164,600414, 601498,601757, 602859,601789, 608666 | 6.78% | 45 | 7.23% | 24 |
| Krabbe disease | *GALC* | 606890 | 6.33% | 42 | 2.11% | 7 |
| TUBB4A-related leukodystrophy | *TUBB4A* | 602662 | 6.02% | 40 | 9.04% [c] | 30 |
| Vanishing white matter disease | *EIF2B1, EIF2B2, EIF2B3, EIF2B4, EIF2B5* | 606686,606454, 606273,606687, 603945 | 4.67% | 31 | 6.63% | 22 |
| POLR3-related leukodystrophy | *POLR1C, POLR3A, POLR3B* | 610060,614258, 614366 | 4.67% | 31 | 6.93% | 23 |
| Leukodystrophy hypomyelinating, 2 | *GJC2* | 608803 | 3.61% | 24 | 2.41% | 8 |
| Alexander disease | *GFAP* | 137780 | 3.31% | 22 | 5.12% | 17 |
| Oculodentodigital dysplasia | *GJA1* | 121014 | 2.56% | 17 | 3.01% | 10 |
| Canavan disease | *ASPA* | 608034 | 2.26% | 15 | 0.3% | 1 |
| Leukodystrophy adult onset | *LMNB1* | 15034 | 1.66% | 11 | 0 | 0 |
| Megalenchephalic leukoencephalopathy with subcortical cysts | *HEPACAM, MLC1* | 611642, 605906 | 1.66% | 11 | 2.1% | 7 |
| Polyglucosan body disease, adult | *GBE1* | 607839 | 1.66% | 11 | 3.01% | 10 |
| D-bifunctional protein deficiency | *HSD17B4* | 601860 | 1.51% | 10 | 2.71% | 9 |
| Combined oxidative phosphorylation deficiency 12 | *EARS2* | 612799 | 1.36% | 9 | 2.41% | 8 |
| Cerebrotendinous xanthomatosis | *CYP27A1* | 606530 | 1.20% | 8 | 1.51% | 5 |
| Multiple sulfatase deficiency | *SUMF1* | 607939 | 1.20% | 8 | 1.51% | 5 |
| PCWH syndrome | *SOX10* | 602229 | 1.20% | 8 | 2.41% | 8 |
| Hereditary diffuse leukoencephalopathy with spheroids | *CSF1R* | 164770 | 1.20% | 8 | 2.11% | 7 |

| Disorder | Gene | OMIM | Relative frequency solved by all methods (*n* = 664) | *n* | Relative frequency solved by exome sequencing (*n* = 332) | *n* |
|---|---|---|---|---|---|---|
| Sialic acid storage disorder, infantile | *SLC17A5* | 604322 | 1.05% | 7 | 1.81% | 6 |
| Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation | *DARS2* | 610956 | 0.75% | 5 | 1.20% | 4 |
| Sjogren-Larsson syndrome | *ALDH3A2* | 609523 | 0.75% | 5 | *0.60%* | 2 |

*Note:* Disease due to variants in other leukodystrophy genes make up the rest of the diagnoses across all methods; these include atypical Krabbe due to PSAP variants (*n* = 4 cases), FUCA1 (*n* = 2), FAM126A (*n* = 2), atypical MLD due to PSAP (*n* = 2 cases), RNASET2 (*n* = 2), DARS (*n* = 1), and ACOX1 (*n* = 1). No cases were found with disease due to variants in *CLCN2*, *EIF2B1*, *PEX3*, or *SCP*.

[a]Second most commonly detected individual genotype on exome-sequencing of leukodystrophies patients.

[b]Most commonly disease associated within this disease entity.

[c]Most commonly detected individual genotype on exome sequencing of leukodystrophies patients.

**TABLE 2**

Carrier screening rates across all data sets

| Gene | Number of carriers identified | Number of individuals screened | Carrier frequency in available clinical testing | Number of carriers excluding founder mutations | Revised carrier frequency |
|---|---|---|---|---|---|
| *GALC* | 1,731 | 182,245 | 0.0094982 | 1,731 | 0.0094982 |
| *GBE1* | 849 | 128,651 | 0.00659925 | 570 | 0.00443059 |
| *PEX6* [a] | 838 | 128,348 | 0.00652912 | 838 | 0.00652912 |
| *ARSA* | 865 | 137,882 | 0.00627348 | 865 | 0.00627348 |
| *ASPA* | 1164 | 202,053 | 0.00576086 | 233 | 0.00115316 |
| *CYP27A1* | 619 | 136,531 | 0.00453377 | 619 | 0.00453377 |
| *PEX1* | 829 | 201,959 | 0.00410479 | 829 | 0.00410479 |
| *HSD17B4* | 700 | 182,097 | 0.00384411 | 700 | 0.00384411 |
| *MLC1* | 424 | 189,003 | 0.00224335 | 424 | 0.00224335 |
| *SLC17A5* | 334 | 181,304 | 0.00184221 | 334 | 0.00184221 |
| *ALDH3A2* | 283 | 181,289 | 0.00156104 | 283 | 0.00156104 |
| *PEX7* | 315 | 201,954 | 0.00155976 | 315 | 0.00155976 |
| *PEX2* | 174 | 138,261 | 0.00125849 | 59 | 0.00042673 |
| *SUMF1* | 227 | 190,263 | 0.00119309 | 140 | 0.00073582 |
| *EIF2B5* | 127 | 127,891 | 0.00099303 | 127 | 0.00099303 |
| *PEX10* [a] | 98 | 118,299 | 0.00082841 | 98 | 0.00082841 |
| *SAMHD1* [a] | 88 | 118,287 | 0.00074395 | 75 | 0.00063405 |
| *ABCD1* | 45 | 116,504 | 0.00038625 | 45 | 0.00038625 |
| *PSAP* [a] | 32 | 118,279 | 0.00027055 | 32 | 0.00027055 |
| *ACOX1* | 14 | 120,495 | 0.00011619 | 14 | 0.00011619 |

[a]Analysis performed in two or fewer labs.

**TABLE 3**

Relative frequency of AGS, VWM, and POLR3-related leukodystrophy genes

| Condition | Gene | Locus | OMIM | % of | Number of patients (*n* = 664) |
|---|---|---|---|---|---|
| Aicardi Goutieres syndrome | *TREX1* | 3p21.31 | 606609 | 1.36% | 9 |
| Aicardi Goutieres syndrome | *RNASEH2A* | 19p13.13 | 606034 | 0.45% | 3 |
| Aicardi Goutieres syndrome | *RNASEH2B* | 13q14.3 | 610326 | 2.86% | 19 |
| Aicardi Goutieres syndrome | *RNASEH2C* | 11q13.1 | 610330 | 0.90% | 6 |
| Aicardi Goutieres syndrome | *SAMHD1* | 20q11.23 | 606754 | 0.30% | 2 |
| Aicardi Goutieres syndrome | *ADAR* | 1q21.3 | 146920 | 2.56% | 17 |
| Aicardi Goutieres syndrome | *IFIH1* | 2q24.2 | 606951 | 1.36% | 9 |
| POLR3-related leukodystrophy | *POLR1C* | 6p21.1 | 610060 | 0.45% | 3 |
| POLR3-related leukodystrophy | *POLR3A* | 10q22.3 | 614258 | 3.16% | 21 |
| POLR3-related leukodystrophy | *POLR3B* | 12q23.3 | 614366 | 1.05% | 7 |
| Vanishing white matter | *EIF2B1* | 12q24.31 | 606686 | 0.00 | 0 |
| Vanishing white matter | *EIF2B2* | 14q24.3 | 606454 | 1.05% | 7 |
| Vanishing white matter | *EIF2B3* | 1p34.1 | 606273 | 0.45% | 3 |
| Vanishing white matter | *EIF2B4* | 2p23.3 | 606687 | 0.30% | 2 |
| Vanishing white matter | *EIF2B5* | 3q27.1 | 603945 | 2.86% | 19 |