Genetic testing of leukodystrophies unraveling extensive heterogeneity in a large cohort and report of five common diseases and 38 novel variants

Nejat Mahdieh1,2, Mahdieh Soveizi2, Ali Reza Tavasoli3, Ali Rabbani1, Mahmoud Reza Ashrafi3, Alfried Kohlschütter4 & Bahareh Rabbani1,5

This study evaluates the genetic spectrum of leukodystrophies and leukoencephalopathies in Iran. 152 children, aged from 1 day to 15 years, were genetically tested for leukodystrophies and leukoencephalopathies based on clinical and neuroradiological findings from 2016 to 2019. Patients with a suggestive specific leukodystrophy, e.g. metachromatic leukodystrophy, Canavan disease, Tay-Sachs disease were tested for mutations in single genes (108; 71%) while patients with less suggestive findings were evaluated by NGS. 108 of 152 (71%) had MRI patterns and clinical findings suggestive of a known leukodystrophy. In total, 114 (75%) affected individuals had (likely) pathogenic variants which included 38 novel variants. 35 different types of leukodystrophies and genetic leukoencephalopathies were identified. The more common identified disorders included metachromatic leukodystrophy (19 of 152; 13%), Canavan disease (12; 8%), Tay-Sachs disease (11; 7%), megalencephalic leukodystrophy with subcortical cysts (7; 5%), X-linked adrenoleukodystrophy (8; 5%), Pelizaeus–Merzbacher-like disease type 1 (6; 5%), Sandhoff disease (6; 4%), Krabbe disease (5; 3%), and vanishing white matter disease (4; 3%). Whole exome sequencing (WES) revealed 90% leukodystrophies and genetic leukoencephalopathies. The total diagnosis rate was 75%. This unique study presents a national genetic data of leukodystrophies; it may provide clues to the genetic pool of neighboring countries. Patients with clinical and neuroradiological evidence of a genetic leukoencephalopathy should undergo a genetic analysis to reach a definitive diagnosis. This will allow a diagnosis at earlier stages of the disease, reduce the burden of uncertainty and costs, and will provide the basis for genetic counseling and family planning.

Background

Leukodystrophies and genetic leukoencephalopathies are a large heterogeneous group of genetic diseases affecting the white matter of the central nervous system. The single diseases are rare, but overall they affected 1 per 7663 live births, in a US American study1; the estimated prevalence of leukodystrophies is about 1–2/100,000 live births in Germany2. Most of these diseases are associated with severe progressive functional losses of motor and cognitive abilities, helplessness and early death. Their causes are either related to primary defects of myelin synthesis and myelin stability, but myelin damage may also be secondary to disturbances outside this structure3. Some mitochondrial and lysosomal storage disorders, organic acidemias, other inborn errors of metabolism and vascular disorders are also categorized under genetic leukoencephalopathies4.

1Growth and Development Research Center, Tehran University of Medical Sciences, Tehran, Iran. 2Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran. 3Myelin Disorders Clinic, Pediatric Neurology Division, Children's Medical Center, Pediatrics Center of Excellence, Tehran University of Medical Sciences, Tehran, Iran. 4Department of Pediatrics, University Medical Center Hamburg Eppendorf, Hamburg, Germany. 5Iranian Comprehensive Hemophilia Care Center, Tehran, Iran. 6email: baharehrabbani@yahoo.com
Leukodystrophies are clinically and genetically heterogeneous disorders; their diagnosis is challenging and nearly half of the patients will remain undiagnosed, putting a high economical and psychological burden on the society and the affected families. Many known genes have been recognized to cause these diseases, though there are many with unknown genetic etiology. Advances in gene sequencing procedures and whole exome sequencing (WES) unravel the genetic causes of leukodystrophies. Genetic testing confirms the diagnosis and may offer a chance for disease-specific palliative treatment or experimental therapies of some diseases (e.g. metachromatic leukodystrophy (MIM 250100), Alexander disease (MIM 203450), and Krabbe disease (MIM 611722)). In addition, molecular genetic analysis would help for family screening and reproductive decisions. Most of the pediatric disorders follow an autosomal recessive pattern of inheritance and come from consanguineous marriages which are prevalent in Iran and the Middle East. Despite advances in molecular technologies and the high frequency of genetic diseases in Iran as the crossroads of the Middle East, there is no comprehensive study on genetics of pediatric white matter disorders in this region of the world. The genetic composition of different parts of Iran could be representative of the respective neighbors.

Here, we have evaluated the genetic spectrum of subjects clinically diagnosed with leukodystrophies referred to a tertiary pediatric center in Iran. The purpose of the study was to determine the common types of leukodystrophies and genetic leukoencephalopathies, neurological findings in the patients, and ethnical distribution of the disease.

Results

Patients’ demographic data and clinical diagnoses. A total of 152 patients, including 94 (62%) males and 58 females, aged from 1 day to 15 years old, has been clinically diagnosed with leukodystrophy or genetic leukoencephalopathy. The distribution of the more common referred diseases among the patients was as follows: 25 patients clinically diagnosed with MLD, 13 CD, 10 PMLD, 6 PMD or PMLD, 12 TSD, 10 X-ALD, 8 SHS, 8 MLC, 3 AxD, 3 KD, 4 hypomyelination and congenital cataract (HCC; Hypomyelinating Leukodystrophy 5; HLD5; MIM 253260), 1 Sialic disease, 1 RNase T2 deficient leukoencephalopathy (MIM 612951), and 2 biotinidase deficiency (MIM 253260).

Totally, 108 of 152 patients (71%) had defined MRI patterns (not available) and were clinically diagnosed with a known leukodystrophy. Measurements of lysosomal enzymes in MLD, KD, TSD and SHS were performed for diagnosis. Urinary sulfatides (for e.g. MLD), plasma very long chain fatty acids (for e.g. X-ALD) were also available (Supplementary Table 2).

Demographic, clinical and genetic evaluation of patients confirmed genetically. Thirty-five different leukodystrophies and genetic leukoencephalopathies were identified in this study (Table 1). The clinical characteristics of the most common genetically confirmed patients are summarized in Table 1 and Fig. 1A.

The main clinical manifestation was motor regression and neurological complaints including dystonia, hypotonia, developmental delay, ataxia, tremor, seizure, macrocephaly, nystagmus, cognition and learning impairment (Table 1 and Supplementary Table 2).

114 (75%) patients were confirmed based on genetic testing. Male consist of 73 of 114 (64%) of patients. The mean age of onset was 5yrs and 1 m ± 18yrs and 11 m. 94 of 114 (82. 5%) cases were born in a consanguineous family. The ethnicity of these patients is compared in Fig. 1B. The ethnic distribution showed higher incidence in Fars 32%; other ethnic distribution included 27% in Turk, Arab 13%, Lur 8%, Kurd 7%, Mazani 4%, Gilak 3%, and the rest Baloch, Afghan, Lak, and Turkeman (Fig. 1B). Based on age of onset of disease, 47 infantile (41%, I), 17 late infantile (15%, LI), 29 early juvenile (25%, EJ), 19 late juvenile (17%, LJ) and 2 adults (A) were available (Supplementary Table 2).

Totally, 2 of 152 (25%) patients were not genetically confirmed based on genetic analysis. Some candidates of single gene analysis were not tested for panel based analysis because the parents were not satisfied for the test performance (Fig. 2). In addition, panel negative patients did not perform WES.

Single gene analyses. Sixteen patients had mutations in the ARSA gene (MLD), 8 in ABCD1 gene (X-ALD), 12 in ASPA gene (CD), 3 in GALC (GLB), 7 in MLC1 gene (MLC), 1 in GEAP gene, 1 in PLP1 gene (PMD), 6 in GJC2 gene (PMLD), 11 in HEXA, 5 in HEXB, 2 in L2HGDH, 1 in RTD and 1 in SCL17A5 gene (Table 1). Totally, 74 out of 108 (69%) patients were genetically diagnosed based on single gene analysis (Fig. 2).

Next generation sequencing: gene-panel and WES. Gene-panel and WES identified 40 of 44 (90%) patients having leukodystrophies and leukoencephalopathies (Table 1, Supplementary Table 2). Four cases did not show any variants with multigene panel analysis of leukodystrophies (Fig. 2).

Frequency of lysosomal, peroxisomal, mitochondrial and errors of intermediary metabolism. Fifty of 114 patients were diagnosed as lysosomal disorders (29 lysosomal LD and 21 lysosomal gLE) (Table 1, Fig. 2 and Supplementary Table 2).

Eleven patients were diagnosed as peroxisomal disorders which eight of them were X-ALD (Table 1, Fig. 2 and Supplementary Table 2).

Forty patients diagnosed as errors of intermediary metabolism, consisted of 12 CD, 8 PMLD and 7 MLC (Table 1). CD as the most common degenerative cerebral diseases, due to abnormal amino acid/organic acid metabolism, accounted for the second most common disease in our population (Table 1, Fig. 2 and Supplementary Table 2).
| No | Name of disease | Alternative designation, abbreviation | MIM # | Gene | Location of protein | No of families (%) | Genetic testing | Phenotypes |
|----|----------------|---------------------------------------|-------|------|--------------------|-------------------|----------------|------------|
|    |                |                                       |       |      |                    |                   |                |            |
| 1   | Metachromatic leukodystrophy | MLD | 250100 | ARSA | ER, Lysosome       | 19 (16.7)         | 16 3          | AG:2, MR:15, DD:2, CI:1, speech problem:6 |
| 2   | Krabbe Disease | KD | 245200 | GALC | Lysosome           | 5 (4.4)           | 3 2          | Hypotonia:1, speech problem:2, Spasticity:2, AG:2, Seizure:2, MR:5, DD:2 |
| 3   | Fucosidosis   | MSD | 230000 | FUCAI | Lysosome          | 2 (1.8)           | 0 2          | Hypotonia:1, Dental germination:1, skin lesions:1, AG1, DD:2 |
| 4   | Salla Disease | SD | 604369 | SLC17A5 | Lysosomal and cell membrane | 1 (0.9)         | 1 0         | speech problem, Seizure, DD, MD |
| 5   | Multiple sulfatase deficiency | MSD | 272200 | SUMF1 | ER               | 1 (0.9)           | 0 1         | dried skin, spasticity, incapable to walk and talk, R, mental retardation, coarse facial feature |
| 6   | RNAse T2 deficiency | 612944 | RNASET2 | ER, Lysosome, Extracellular | 1 (0.9)       | 0 1         | Hypotonia, DD |
| 7   | X-linked adrenoleukodystrophy | X-ALD | 300100 | ABCD1 | Membrane of ER, Mitochondrion, peroxisome and lysosome | 8 (7)       | 8 0         | Hypotonia:2, Vision problem:1, Feeding problem:2, AG:3, Seizure:2, MR:2, LD:1, CI:4 |
| 8   | Rhizomelic chondrodysplasia punctata | RCDP | 601757 | PEX7 | Peroxisome       | 1 (0.9)           | 0 1         | coarse facial feature, cataract, digestive problem, DD, MR |
| 9   | Zellweger Spectrum | ZS | 614883 | PEX13 | Peroxisome membrane | 1 (0.9)          | 0 1         | Hypotonia, Seizure, MR, feeding problem |
| 10  | D-bifunctional protein deficiency | DBPD | 601860 | HSD17B4 | Peroxisome | 1 (0.9) | 0 1 | swelling problem, walking difficulty, speech problem, MR |
| 11  | Canavan Disease | CD | 271900 | ASPA | Nucleus, Cytoplasm | 12 (10.5) | 12 0 | Hypotonia:8, Nystagmus and eye problem:5, Macrocephaly:9, Spasticity:3, Irritability:6, Seizure:3, R:9, DD:7 |
| 12  | Pelizaeus–Merzbacher-like disease type | PMLD | 260600 | GJC2 | Cell membrane, gap junction | 8 (7)       | 6 2         | Macrocephaly:7, Dystonia:2, AG:4, Seizure:2, MD:2, MR:5 |
| 13  | Malignant leukaencephalopathy with subcortical cysts | MLC | 604004 | MLCL | ER and cell membrane | 7 (6.1)       | 7 0         | Macrocephaly:7, Dysontia:2, AG:4, Seizure:2, MD:2, MR:5 |
| 14  | Vanishing white matter disease | vWM | 606273 | EIF2B3 | Cytosol | 1 (0.9) | 0 1 | MR:4, Hypotonia:3, Tremor:2, AG:2, Seizure:2, speech problem:1 |
| 15  | Hypomyelinating-hypogonadotropic hypogonadism-hypodontia | 4H | 614366, 614381 | POLR3A, POLR3B | Nucleus, Cytoplasm | 1 (0.9) | 0 1 | Hypotonia:2, speech problem:2, Tremor:1, Ataxia:2, AG:2, Seizure:2, MR:1, DD:1, nystagmus:1 |
| 16  | hypomyelination and congenital cataract | HCC | 610532 | FAM126A | Cytosol | 1 (0.9) | 0 1 | congenital cataract |
| 17  | Pelizaeus–Merzbacher disease | PMD | 312080 | PLP1 | Cell (myelin) membrane | 1 (0.9) | 1 0 | MR, Hypotonia, nystagmus |
| 18  | Alexander disease | AxD | 203450 | GFAP | Cytoplasm | 1 (0.9) | 1 0 | Seizure, R, DD, hypotonia |
| 19  | Infantile neuroaxonal dystrophy/atypical neuroaxonal dystrophy | INAD | 603604 | PLA2G6 | Peripheral membrane | 1 (0.9) | 0 1 | Hypotonia, bristling head, Seizure |
| 20  | Hypomyelinating leukodystrophy-9 | HLD9 | 616140 | RARS | Cytosol | 1 (0.9) | 0 1 | Spasticity, hypotonia, MD |

Continued
Thirteen patients diagnosed with mitochondrial genetic leukoencephalopathies (Table 1, Fig. 2 and Supplementary Table 2).

Common variants. Five common diagnosed leukodystrophies accounted for 51% (58 of 114 patients) in our studied patients included 19 of 58 (33%) MLD, 12 CD, 11 TSD, 8 X-ALD and 8 PMLD (Fig. 2). Six common mutations were found including p.Gly311Ser (in 6 MLD patients), c.465 + 1G > A (in 3 MLD patients), c.634 + 1G > T (in 6 CD patients), c.237_238insA (in two homozygous and one compound heterozygous CD patients), c.1528C > T (in 4 TSD patients) and c.449_455delTCC TGC T (two homozygous and one heterozygous MLC patients).

Novel variants. Thirty-eight novel variants were identified in 40 patients (Table 2). Each of ABCD1 and GJC2 showed four novel variants. Following genes had each two novel variants: ASPA, FUCA, GALC, HEXA, L2HGDH and MLC1 (Table 2). The variants were classified according to ACMG guideline; 11 variants met the criteria for being pathogenic, 17 and 10 variants were likely pathogenic and VUS, respectively.

Discussion
Genetic diagnosis of childhood leukodystrophies is rapidly increasing throughout the past years in Iran and worldwide; approximately, 30 leukodystrophies and more that 60 disorders have been classified as genetic leukoencephalopathies. This study provides a comprehensive spectrum of leukodystrophies and other genetic leukoencephalopathies in Iran as referred to a tertiary pediatric center. Totally, 35 types of leukodystrophies were determined in the studied population. Based on pattern of brain MRI and single gene analysis, approximately 69% (74 of 108) of the referred patients were confirmed by direct Sanger sequencing. Clinical diagnosis reduced the number of genes to be evaluated. Panel based analysis also confirmed leukodystrophies in 90% (40 of 44) of the cases. Our diagnostic rate of panel-based analysis was comparable to other studies. Four patients were

Table 1. The distribution of the leukodystrophies and genetic leukoencephalopathies based on single gene analysis and WES/panel based gene sequencing in 114 positive patients in the studied population. DD: Developmental delay; LD: learning difficulties; CI: Cognitive impairment; MR/R: Motor regression/retardation; MD: motor delay; MD: AG: Abnormal gait; ER: Endoplasmic reticulum.
genetically undiagnosed with panel-based/WES studies and WGS is needed to define the causes. Consequently, we had 25% (38 of 152) unsolved genetic cases and the diagnostic rate was 75% (114 of 152) of leukodystrophies and genetic leukoencephalopathies in the study. Various novel variants identified, show that a high rate of allelic heterogeneity exists among our patients. A specific composition of population living in Iran complicates this picture; different ethnicities with specific cultural customs demand to run more specific investigations on each population.

MLD was the most common cause of leukodystrophies in our population. The next diseases were CD, TSD, PMLD, X-ALD and then MLC. MLC is the most common (6 of 23) among Turk patients while PMLD may be common among Arab population in our study. Moreover, ten common diseases of this study, compromise 70% of all recognized patients (80 of 114) (Table 1). A recent study showed that peroxisomal disorders are identified to be common. Although other common disorders including Aicardi Goutières Syndrome, TUBB4A-related leukodystrophy, POLR3-related Leukodystrophy and Pelizaeus–Merzbacher Disease were not found in our study with a high frequency. ABCD1 had the highest relative frequency in their study while ARSA was the most common in our population.

Clinically, we had unsolved cases due to variable phenotypic features or overlapping neurological manifestations which were candidates of gene-panel and/or WES analysis. Despite we had patients with no genetic diagnosis even though they had undergone panel-based analysis. This could be due to intronic variants, copy number variations, unknown gene defects, and multigenic effect. Therefore, more genetic analysis should be performed for these cases and they could benefit from reanalysis of exome sequencing data, genome sequencing and transcriptomics. For rare diseases genetic analysis, NGS may unravel more genes relating to leukodystrophies in patients with unsolved genetics.

Lysosomal diseases had 43% incidence in our studied population which could be managed at earlier age of diagnosis. Individuals with known causal variants benefit from unexpected clinical presentations, prognosis, palliative treatment and avoiding unnecessary treatments. Hematopoietic stem cell transplantation (HSCT) has been used for lysosomal storage diseases. Some of our patients might potentially have benefitted from HSCT at early stages of the disease. However, patients’ follow up for HSCT is out of the scope of this study.

Some have an ethnic-specific distribution, e. g. TSD in Ashkenazi Jewish population, GM1 gangliosidosis in Rudari isolate and MLD in Western Navajo Nation. MLD patients were from western part of Iran. Four of our TSD patients were from northern parts of Iran.

The peroxisomal disorders, as a heterogeneous group, occur due to a defect in function (e. g. X-ALD) and biogenesis (e. g. Zellweger spectrum) of peroxisomes. X-ALD is the most common peroxisomal disorder caused by mutation in the ABCD1 gene co-expressed with HSD17B4 gene. Patients with X-ALD could benefit from HSCT or hematopoietic stem-cell gene therapy.

CD is the second frequent disease in our study. It is the most common disease during infancy and has been observed mainly in Ashkenazi Jews while in our study patients were from various ethnicities. Various experimental therapies for Canavan patients are under investigation. Patients with known genetic etiology may benefit from such experimental therapies.

PMLD is responsible for 8% of hypomyelinating leukodystrophy patients. In this study 7% of the patients had the disease. In addition to GJC2, mutations in other genes such as a Myelin-associated glycoprotein (MAG) gene have been reported to cause PMLD. GJC2 is co-expressed with PLP1 and interacts with products of FAM1256A, POLR3A and EIF2B5 genes. Our results highlighted that PMLD may have a higher frequency than...
11% of patients diagnosed with mitochondrial genetic leukoencephalopathies; Leigh syndrome and L-2-HGA accounted for 4 and 3 of them, respectively. Leigh spectrum was due to abnormal amino acid/organic acid metabolism, accounted for the second most common disease in our population. PMD and PMLD are disorders of myelin genes. 4 patients had vWM, 2 patients with hypomyelination-hypogonadotropic-hypopituitarism, 1 hypomyelination and congenital contract, 1 PMD, 1 AxD, 1 infantile neuroaxonal dystrophy/atypical neuroaxonal dystrophy, 1 hypomyelination leukodystrophy 9 (HLD9, MIM 616140), 1 Cockayne syndrome CS, MIM 133540), and 1 biotinidase deficiency. Thirteen patients diagnosed with mitochondrial genetic leukoencephalopathies; Leigh syndrome and L-2-HGA accounted for 4 and 3 of them, respectively.

**Analysis of founder effect and hotspot mutations.** Ancestral or founder effect or a genetic signature within an ethnicity usually leads to a high frequency and homozygosity of a mutation in that cohort; in contrast, if a specific mutation is distributed uniformly among many ethnicities, it is known as a mutational hotspot. Haplotype analysis is used to define recognized that a mutation is a hotspot or a founder one. The studied mutations of ABCD1 (c. 1415_1416delAG), ASPA (c. 634 + 1G > T and c. 237_238insA) and HEXA (c. 1528C > T) show a wide distribution around the world, especially c. 634 + 1G > T in ASPA gene has been reported from Turkey for the first time and we found it in patients from Fars, Afghani, Lur and Arab ethnicities. These mutations are
considered as hotspots i.e. they are mutated in many populations. Contrarily, mutations of MLC1 (c. 177 + 1G > T and c. 449_455delTCC TGC T) may have ancestors in Turk population. Especially, the c. 449_455delTCC TGC T variant was observed in three families; it may be originated from a founder ancestor in Turk population and it previously has been reported from Turkey21.

**Challenges and limitations.** We have not included all the affected patients in our registry, only the patients referred to our center for genetic testing were accounted in this study. In addition, Children’s Hospital is a tertiary center in Tehran and some patients around the country may have not been registered and/or died previously before registration. Therefore, a multicenter registry is needed. The incidence of the disease in this part of the world may be different due to consanguineous marriages. Ethnical background had higher incidence in Fars and Turk; however, the population of these ethnicities is also high in Iran.

| No | Nucleotide change | AA change | Gene | no. of patients | Zygosity | ACMG | MutationTaster | Polyphen-2 | CADD |
|----|------------------|-----------|------|----------------|----------|------|----------------|------------|------|
| 1  | c. 2099A > C     | p. Asn700Thr | POLR3B | 1              | Hom      | Likely pathogenic (2) | DC         | PD 0.998 | 27.2 |
| 2  | c. 786A > C     | p. Gln262Asp | SLC17A5 | 1              | Hom      | Likely pathogenic (2) | DC         | PD 1.000 | 24.2 |
| 3  | c. 904_905delINSAT | p. Glu302Met | ABCD1   | 1              | Hemi     | Likely pathogenic (2) | DC         | NA      | 26.8 |
| 4  | c. 1628C > G    | p. Pro543Arg  | ABCD1   | 1              | Hemi     | Likely pathogenic (2) | DC         | PD 1.000 | 23.8 |
| 5  | c. 2002A > G + c. 1021G > T | p. Thr668Ala + p. Ala-341Ser | ABCD1   | 1              | Hemi     | Likely pathogenic (2) | DC         | PD 0.761 | 23.7 |
| 6  | c. 836G > C     | p. Arg280Pro  | ABCD1   | 1              | Hemi     | Likely pathogenic (2) | DC         | PD 1.000 | 32   |
| 7  | c. 233C > A     | p. Ser78Ter   | RNASET2 | 1              | Hom      | Pathogenic (1)       | DC         | NA      | 36   |
| 8  | c. 437_449delCTCTGGCTCCACT | p. Ser146TyrfsX7 | ASPA   | 1              | Hom      | Pathogenic (1)       | DC         | NA      | 34   |
| 9  | c. 359C > T     | p. Ser120Phe  | ASPA   | 1              | Hom      | Uncertain significance (3) | DC         | PD 1.000 | 29.1 |
| 10 | c. 866G > A     | p. ser289ile  | EIF2B4  | 1              | Hom      | Uncertain significance (3) | DC         | B 0.002 | 22.9 |
| 11 | c. 422G > T     | p. Gly141Val  | FUC1    | 1              | Hom      | Likely pathogenic (2) | DC         | PD 1.000 | 28.8 |
| 12 | c. 82delG      | p. Val28CysfsX105 | FUC1    | 1              | Hom      | Pathogenic (1)       | DC         | NA      | 16.62 |
| 13 | c. 830G > A     | p. Ser277Asn  | GALC    | 1              | Hom      | Likely pathogenic (2) | DC         | PD 0.946 | 23.9 |
| 14 | c. 1942A > T    | p. Lys648Ter  | GALC    | 1              | Hom      | Uncertain significance (3) | DC         | NA      | 36   |
| 15 | c. 408 + 1G > C | -           | L2HGDH  | 1              | Hom      | Pathogenic (1)       | DC         | NA      | 34   |
| 16 | c. 1213A > G    | p. Arg405Gly  | L2HGDH  | 1              | Hom      | Uncertain significance (3) | DC         | PD 1.000 | 22.7 |
| 17 | c. 183C > A     | p. Cys61Ter   | MLC1    | 1              | Hom      | Pathogenic (1)       | DC         | NA      | 37   |
| 18 | c. 819C > G     | p. Phe273Leu  | MLC1    | 1              | Hom      | Uncertain significance (3) | DC         | PD 0.990 | 24.1 |
| 19 | c. 571_572insC  | p. Thr195AspfsX69 | GIC2   | 1              | Hom      | Pathogenic (1)       | DC         | NA      | 17.5 |
| 20 | c. 118G > C     | p. Ala40Pro   | GIC2    | 2              | Hom      | Likely pathogenic (2) | DC         | PD 1.000 | 24.6 |
| 21 | c. 733 T > A    | p. Cys245Ser  | GIC2    | 2              | Hom      | Likely pathogenic (2) | DC         | PD 1.000 | 25.1 |
| 22 | c. 883C > T     | p. Gln295Ter  | GIC2    | 1              | Hom      | Likely pathogenic (2) | DC         | NA      | 38   |
| 23 | c. 529_531delAAA| p. Lys177del  | PEX13   | 1              | Hom      | Pathogenic (1)       | DC         | NA      | 22.2 |
| 24 | c. 345C > G     | p. Ile115Met  | PEX14   | 1              | Het      | Uncertain significance (3) | DC         | PD 0.999 | 23.5 |
| 25 | c. 655_657delATT| p. Ile219del  | HEXB    | 1              | Hom      | Pathogenic (1)       | DC         | NA      | 20.3 |
| 26 | c. 754C > T     | p. Arg252Cys  | HEXA    | 1              | Hom      | Likely pathogenic (2) | DC         | PD 1.000 | 30   |
| 27 | c. 1147-1G > T  | -           | HEXA    | 1              | Hom      | Pathogenic (1)       | DC         | NA      | 28.3 |
| 28 | c. 46C > T      | p. Arg6Cys    | PLA2G6  | 1              | Hom      | Uncertain significance (3) | DC         | PD 0.994 | 25   |
| 29 | c. 416 T > A    | p. Leu139Gln  | GLB1    | 1              | Hom      | Likely pathogenic (2) | DC         | PD 1.000 | 29.3 |
| 30 | c. 997G > T     | p. Asp333Tyr  | SUCLA2  | 1              | Hom      | Likely pathogenic (2) | DC         | PD 1.000 | 31   |
| 31 | c. 3482 + 6C > T| -           | POLG    | 1              | Hom      | Uncertain significance (3) | DC         | NA      | 9.6  |
| 32 | c. 29A > C      | p. Gln10Pro   | SDHAF1  | 1              | Hom      | Uncertain significance (3) | DC         | PD 1.000 | 27   |
| 33 | c. 808_812delGAGCA | p. Gln270SerfsX20 | SURF1  | 1              | Hom      | Pathogenic (1)       | DC         | NA      | 35   |
| 34 | c. 362 + 5G > A | -           | PPT1    | 1              | Hom      | Pathogenic (1)       | DC         | NA      | 21.9 |
| 35 | c. 659A > C     | p. Tyr220Ser  | CLN6    | 1              | Hom      | Uncertain significance (3) | DC         | PD 0.986 | 32   |
| 36 | c. 392C > A     | p. Thr131Lys  | HSJD1B4 | 1              | Hom      | Likely pathogenic (2) | DC         | PD 0.985 | 33   |
| 37 | c. 1285G > A    | p. Val429Met  | NDUF51  | 1              | Hom      | Likely pathogenic (2) | DC         | PD 0.971 | 28.8 |
| 38 | c. 415G > A     | p. Asp139Asn  | NDUF57  | 1              | Hom      | Likely pathogenic (2) | DC         | PD 1.000 | 25.5 |

Table 2. Novel variants identified in this study. DC: disease causing, PD: probably damaging, Hom = homozygous, B = benign, NA = not available.
Conclusion
In conclusion, five common disorders are responsible for more than fifty percent of leukodystrophies in this region. Considering Iran as the crossroad of the Middle East is composed of more than 15 ethnicities\textsuperscript{22}, it may reflect the distribution of leukodystrophies in the Middle East especially its neighboring populations. For instance, PMDL may be common among Arab countries while MLC may have a high frequency in Turkish countries. Genetic analysis provides diagnostic confirmation of the disease, and physicians are allowed for prognosis and management of patients and affected families. Genetic testing following counseling decreases further worry of the family about the diagnosis and further costs. The mortality rate in affected families is very high and it underscores the necessity of genetic testing in the country. Moreover, this study provides information to help for future therapeutic planning's in the country. This will allow a diagnosis at earlier stages of the disease, reduce the burden of uncertainty and costs, and will provide the basis for genetic counseling and family planning.

Methods
Patients. Clinically diagnosed patients with white matter deterioration were enrolled in the study from different ethnicity of Iran between 2016 and 2019. Clinical characteristics of leukodystrophies and leukoencephalopathies were approved by pediatric neurologists. Demographic data, medical and family history, physical evaluations, neurological examinations, magnetic resonance imaging (MRI), and laboratory testing of each patient were recorded for each patient. The study was approved by the ethical committee of Iran University of Medical Sciences. Informed consent was obtained for genetic testing from the parents of patients. All experimental protocols were approved by Growth and Development Research Center, Tehran University of Medical Sciences and performed in accordance with relevant guidelines and regulations.

Study strategy. Single gene analysis based on clinical diagnosis. Patients with a strongly suspected cause of their leukodystrophy were genetically analyzed for the respective relevant gene. These studies included the genes of metachromatic leukodystrophy (MLD), Canavan disease (CD, MIM 271900), X-linked adrenoleukodystrophy (X-ALD, MIM 300100), Alexander disease (AxD), Tay-Sachs disease (TSD, MIM 272800), Sandhoff disease (SHS, MIM 268800), Krabbe disease (KD), megalencephalic leukodystrophy with subcortical cysts (MLC, MIM 604004), Sialic acid storage disease (SD, MIM 269920), Pelizaeus–Merzbacher disease (PMD, MIM 312080), and Pelizaeus–Merzbacher-like disease type 1 (PMLD1, MIM 608804).

DNA was extracted and amplified by using specific designed primers for coding regions (exons and exon–intron boundaries). The selected genes associated with leukodystrophy were classified to inherited autosomal recessive diseases: ARSA (NM_000487.5; 605908), GALC (NM_001515.3; 606890), MLC1 (NM_015666.3; 605908), BTD (NM_000060.4; ), GFAP (NM_020255.4; 137780), GJC2 (NM_020435.3; 608030), HEXB (NM_000521.3; MIM 606873), HEXA (NM_000520.5; MIM 606869), ASPA (NM_000049.2; 608034) and SLC17A5 (NM_012434.5 MIM, 603422), FAM126A (NM_032581.3; 610531), and X-linked recessive ABCD1 (NM_000033.3; 300371), and PLP1 (NM_001128834.2; 300401), respectively. Direct sequencing was performed by BigDye termination method ABI 3500 (Applied BioSystems, US).

Next generation sequencing: gene-panel and whole exome sequencing (WES). Those patients (n = 41) with indefinite clinical diagnosis or overlapping symptoms and neurological findings underwent panel gene analysis to detect the genetic cause. Panel based gene analysis was performed for cases for 59 genes involving in leukodystrophy, leukoencephalopathy and vanishing matter white disease (Supplementary Table 1). The coding regions and exon–intron boundaries of the genes were enriched using NimbleGen kit (NimbleGen, Roche, Basel, Switzerland). Sequencing analysis was performed by Illumina, Hiseq2000 (Illumina, San Diego, California, USA). Reads were aligned using Burrows–Wheeler Aligner (BWA) on reference genome (hg19), called by SAMTools and annotated by GATK and ANNOVAR. Based on, 1000Genome and dbSNP database variant were selected for analysis. Coverage of target region with at least depth of 30X was 99%. In addition, WES was only performed for the patients with an average coverage depth of \( \approx \)100X for three patients. Sanger sequencing was done for the candidate variants in the affected families.

Variant categories. The sequence data were compared with public databases and filtered to find out the candidate variants according to published pipelines. The candidate variants were categorized as the previously reported pathogenic variants and novel variants. ACMG guideline criteria were used to interpret novel variants and classify them\textsuperscript{26}.

In silico analyses. Pathogenic effect. According to HGVS (http://varnomen.hgvs.org/hgvs.org/), novel variants were named as missense, nonsense, splice site, intronic, regulatory and indel. The following software tools were applied to predict the pathogenic effects of novel variants: polymorphism phenotyping (PolyPhen-2.v2.1)\textsuperscript{27}, combined annotation dependent depletion (CADD)\textsuperscript{28} and MutationTaster\textsuperscript{29}.

Ethics approval and patients' consent. Ethical approval was supported by Growth and development research center, Tehran University of Medical Sciences ID number 98–02–80–43,432 and Iran University of Medical Sciences (IR.IUMS.REC.1399.817). Informed consent was obtained from the parents of patients.

Consent for publication. All contributing authors have read the manuscript and given their consent for the publication of this study.
Data availability
There are no additional unpublished data.

Received: 7 November 2020; Accepted: 25 January 2021
Published online: 05 February 2021

References
1. Bonkowsky, J. L. et al. The burden of inherited leukodystrophies in children. Neurology 75, 718–725. https://doi.org/10.1212/WNL.0b013e3181ebe4eb (2010).
2. Heim, P. et al. Leukodystrophy incidence in Germany. Am. J. Med. Genet. 71, 475–478 (1997).
3. Kohlschutter, A. & Eichler, F. Childhood leukodystrophies: a clinical perspective. Expert Rev. Neurother. 11, 1485–1496. https://doi.org/10.1586/ern.11.135 (2011).
4. Vanderver, A. et al. Case definition and classification of leukodystrophies and leukoencephalopathies. Mol. Genet. Metab. 114, 494–500. https://doi.org/10.1016/j.ymgme.2015.01.006 (2015).
5. van der Knaap, M. S., Breiter, S. N., Naidu, S., Hart, A. A. & Valk, J. Defining and categorizing leukoencephalopathies of unknown origin: MR imaging approach. Radiology 213, 121–133. https://doi.org/10.1148/radiology.213.1.996e01121 (1999).
6. Vanderver, A. et al. Whole exome sequencing in patients with white matter abnormalities. Ann. Neurol. 79, 1031–1037. https://doi.org/10.1002/ana.24650 (2016).
7. Boelens, J. J. & van Hasselt, P. M. Neurodevelopmental outcome after hematopoietic cell transplantation in inborn errors of metabolism: current considerations and future perspectives. Neuropediatrics 47, 285–292. https://doi.org/10.1055/s-0036-1584602 (2016).
8. Eichler, F. et al. Hematopoietic stem-cell gene therapy for cerebral adrenoleukodystrophy. N. Engl. J. Med. 377, 1630–1638. https://doi.org/10.1056/NEJMoal700554 (2017).
9. Mahdieh, N. et al. Novel disease-causing variants in a cohort of Iranian patients with metachromatic leukodystrophy and in silico analysis of their pathogenicity. Clin. Neurosci. Neurosurg. 201, 106448. https://doi.org/10.1016/j.clineuro.2020.106448 (2020).
10. Schmidt, J. L. et al. Estimating the relative frequency of leukodystrophies and recommendations for carrier screening in the era of next-generation sequencing. Am. J. Med. Genet. Part A 182, 1906–1912. https://doi.org/10.1002/ajmg.a.61641 (2020).
11. Helman, G. et al. Genome sequencing in persistently unsolved white matter disorders. Ann. Clin. Transl. Neurol. 7, 144–152. https://doi.org/10.1002/acn3.50957 (2020).
12. Platt, F. M., d’Azzo, A., Davidson, B. L., Neufeld, E. F. & Tifft, C. J. Lysosomal storage diseases. Nat. Rev. Dis. Primers 4, 27. https://doi.org/10.1038/s41572-018-0025-4 (2018).
13. Miller, W. P. et al. Outcomes after allogeneic hematopoietic cell transplantation for childhood cerebral adrenoleukodystrophy: the largest single-institution cohort report. Blood 118, 1971–1978. https://doi.org/10.1182/blood-2011-01-329235 (2011).
14. Pleasure, D. et al. Pathophysiology and treatment of canavan disease. Neurochem. Res. 45, 561–565. https://doi.org/10.1007/s11064-018-2693-6 (2020).
15. Hennenke, M. et al. GJA12 mutations are a rare cause of Pelizaeus–Merzbacher-like disease. Neurology 70, 748–754. https://doi.org/10.1212/01.wnl.0000098428.84464.35 (2008).
16. Lossos, A. et al. Myelin-associated glycoprotein gene mutation causes Pelizaeus–Merzbacher disease-like disorder. Brain J. Neurol. 138, 2521–2536. https://doi.org/10.1093/brain/avv204 (2015).
17. Kemp, S. et al. Identification of a two base pair deletion in five unrelated families with adrenoleukodystrophy: a possible hot spot for mutations. Biochem. Biophys. Res. Commun. 202, 647–653. https://doi.org/10.1016/0006-291X(94)90636-9 (1994).
18. Rady, P. L., Penzien, J. M., Vargas, T., Tyring, S. K. & Matakon, R. Novel splice site mutation of aspartoacylase gene in a Turkish patient with Canavan disease. Eur. J. Paediatr. Neurol. 4, 27–30. https://doi.org/10.1016/j.ejpn.1999.0256 (2000).
19. Elpeleg, O. N. & Shaag, A. The spectrum of mutations of the aspartoacylase gene in Canavan disease in non-Jewish patients. J. Inherit. Metab. Dis. 22, 531–534. https://doi.org/10.1023/a:10055123524957 (1999).
20. Kaya, N. et al. GM2 gangliosidosis in Saudi Arabia: multiple mutations and considerations for future carrier screening. Am. J. Med. Genet. Part A 155A, 1281–1284. https://doi.org/10.1002/ajmg.a.33932 (2011).
21. Leegwater, P. A. et al. Identification of novel mutations in MLC1 responsible for megalencephalic leukoencephalopathy with subcortical cysts. Hum. Genet. 110, 279–283. https://doi.org/10.1007/s00439-002-0682-x (2002).
22. Mahdieh, N. & Rabbani, R. Beta thalassemia in 31734 cases with HBB gene mutations: pathogenic and structural analysis of the common mutations; Iran as the crossroads of the Middle East. Blood Rev. 30, 493–508. https://doi.org/10.1016/j.blre.2016.07.001 (2016).
23. Richards, S. et al. 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. Genet. Med. 17, 405–424. https://doi.org/10.1038/gim.2015.30 (2015).
24. Adzhubei, I. A. et al. A method and server for predicting damaging missense mutations. Nat. Methods 7, 248–249. https://doi.org/10.1038/nmeth0410-248 (2010).
25. Rentzsch, P., Witten, D., Cooper, G. M., Shendure, J. & Kircher, M. CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res. 47, D866–D894. https://doi.org/10.1093/nar/gky1016 (2019).
26. Schwartz, J. M., Cooper, D. N., Schuelke, M. & Seydow, D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat. Methods 11, 361–362. https://doi.org/10.1038/nmeth.2890 (2014).

Acknowledgements
The study was supported in part by Growth and Development Research Center, Tehran University of Medical Sciences and performed in accordance with relevant guidelines and regulations.

Author contributions
N.M.: Study design, data analysis, project administrator, writing and review editing. M.S.: Data extraction, data validation, data analysis, review editing. A.R.T.: Clinical evaluation. A.R.: Clinical evaluation. M.R.A.: Clinical evaluation. A.K.: Review editing. B.R.: Study design, Data validation, Data analysis, Writing and review editing.

Funding
This work has no funding support and approved by Tehran University of Medical Sciences.
Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-82778-0.

Correspondence and requests for materials should be addressed to B.R.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021