Genetic Testing of Leukodystrophies Unraveling Extensive Heterogeneity in a Large Cohort: The Role of Five Common Diseases and Report of 38 Novel Variants

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Research

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Abstract

Background: This study evaluates the genetic spectrum of leukodystrophies and leukoencephalopathies in Iran.

Methods: 152 children, aged from 1day 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.

Results: 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 (8; 5%), Sandhoff disease (6; 4%), Krabbe disease (5; 3%), and vanishing white matter disease (4; 3%). Whole exome sequencing (WES) revealed 26% leukodystrophies and genetic leukoencephalopathies. The total diagnosis rate was 75%.

Conclusions: 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 study[8]; the estimated prevalence of leukodystrophies is about 1-2/100 000 live births in Germany[4]. 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 structure[10]. Some mitochondrial and lysosomal storage disorders, organic acidemias, other inborn errors of metabolism and vascular disorders are also categorized under genetic leukoencephalopathies[4].

Leukodystrophies are clinically and genetically heterogeneous disorders; their diagnosis is challenging and nearly half of the patients will remain undiagnosed[1], 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[6]. 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, Alexander disease, and Krabbe disease). 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.

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 Children's Hospital, Tehran University of Medical Sciences. Informed consent was obtained for genetic testing.

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), X-linked adrenoleukodystrophy (X-ALD), Alexander disease (AxD), Tay-Sachs disease, Sandhoff disease, Krabbe disease (KD), megalencephalic leukodystrophy with subcortical cysts (MLC), Sialic acid storage disease, Pelizaeus-Merzbacher disease (PMD), and Pelizaeus-Merzbacher-like disease type 1 (PMLD1).

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), *GALC*(NM_000153. 3), *MLC1*(NM_015166. 3),

BTD(NM_000060. 4), *GFAP*(NM_002055. 4), *GJC2*(NM_020435. 3), *HEXB*(NM_000521. 3), *HEXA*(NM_000520. 5), *ASPA*(NM_000049. 2) and *SLC17A5*(NM_012434. 5), *FAM126A*(NM_032581. 3), and X-linked recessive *ABCD1*(NM_000033. 3), and *PLP1*(NM_001128834. 2), 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 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) and annotated by SAMTools. Based on, 1000Genome and dbSNP database variant were selected for analysis. Coverage of target region with at least depth of 30X was 99. 78%. In addition, whole exome sequencing (WES) was performed with an average coverage depth of ≈100X. 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[10]. 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[5].

In silico analyses

Pathogenic effect

According to HGVS (http://varnomen. 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-2v2. 1)[4], combined annotation dependent depletion (CADD)[D1] and MutationTaster[4].

Protein interaction

STRING as a database for protein-protein interactions was used to figure out the interactions among proteins and co-expression of the studied proteins in human and other vertebrates. The studied proteins were investigated the co-expression in Homo sapiens to determine the function of proteins in cellular machinery by ProteomeHD[D1].

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 year 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, 2 PMD or PMLD, 12 Tay-Sachs disease, 10 X-ALD, 8 Sandhoff disease, 8 MLC, 3 AxD, 3 KD, 4 hypomyelination and congenital cataract (HCC), 1 Sialic disease, 1 RNAse T2 deficient leukoencephalopathy, and 2 biotinidase deficiency .

Totally, 108 of 152 patient (71%) had defined MRI patterns (not available) and were clinically diagnosed with a known leukodystrophy. Measurements of lysosomal enzymes in MLD, KD, Tay-Sachs disease, Sandhoff disease were performed for diagnosis. Urinary sulfatides (for e. g. MLD), plasma very long chain fatty acids (for e. g. X-ALD) were also tested to help the diagnosis. These patients were candidates for single gene analysis.

44 of 152 patients (29%) had no definite MRI pattern and no definite biochemical or single gene test could be performed for them. They were candidates for panel gene analysis or WES.

Demographic, clinical and genetic evaluation of patients confirmed genetically

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 1m ± 18yrs and 11m.

94 of 114 (82. 5%) cases were born in a consanguineous family. The ethnicity of these patients is compared in figure-1A. The ethnical distribution showed higher incidence in Fars 32%; other ethnical distribution included 27% in Turk, Arab 13%, Lur 8%, Kurd 7%, Mazani 4%, Gilak 3%, and the rest Balooch, Afghan, Lak, and Turkeman(Figure-1A).

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).

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 figure 1B. 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).

Single gene analyses

Sixteen patients had mutations in the *ARSA* gene (MLD), 8 in *ABCD1* gene (X-ALD), 12 in *ASPA* gene (CD), 2 in *GALC*(GLB), 7 in *MLC1* gene (MLC), 1 in *GFAP* gene, 1 in *PLP1* gene (PMD), 8 in *GJC2* gene (PMLD), 11 in *HEXA*, 5 in *HEXB,* and 1 in *SCL17A5* gene (Table 1). Totally, 74 out of 152 (49%) patients were genetically diagnosed based on single gene analysis. The diagnostic rate was 69% (74 of 108), based on the clinical diagnosis (brain MRI) and single gene analysis.

Next generation sequencing: gene-panel and WES

Gene-panel and WES identified 40 of 152 (26%) patients having leukodystrophies and leukoencephalopathies (Table-1, Supplementary Table-2). Four cases did not show any variants with multigene panel analysis of leukodystrophies.

38 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.

Categorization of patients based on protein location

Lysosomal disorders

Forty nine of 114 patients were diagnosed as lysosomal disorders (28 lysosomal LD and 21 lysosomal gLE). Forty-one patients genetically were confirmed for MLD, TSD, SD and KD (Table-1).

Peroxisomal disorders

Eleven patients were diagnosed as peroxisomal disorders which eight of them were X-ALD. One patient had peroxisomal single enzyme beta oxidation deficiency, and two patients had peroxisomal biogenesis disorders (Table-1).

Errors of intermediary metabolism and other leukoencephalopathies

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. PMD and PMLD are disorders of myelin genes. 4 patients had vWM, 2 patients with hypomyelination-hypogonadotropic-hypogonadism-hypodontia, 1 hypomyalination and congenital contract, 1 PMD, 1 AD, 1 infantile neuroaxonal dystrophy/atypical neuroaconal dystrophy, 1 hypomyelination leukodystrophy 9 (HLD9), 1 Cockayene syndrome, and 1 biotinidase deficiency (Table-1 and supplementary table-2)

Mitochondrial diseases

Thirteen patients diagnosed with mitochondrial genetic leukoencephalopathies; Leigh syndrome and L-2-HGA accounted for 4 and 3 of them, respectively. Mutations in *POLG* gene encoding DNA polymerase subunit gamma-1 were responsible in two patients.

Analysis of common genes and mutations

The distribution of the most common diagnosed leukodystrophies accounted for 75% (86 of 114 patients) in our studied patients included 19 of 86 (22%) MLD, 12 CD, 11 Tays-Sachs disease, 8 MLC, 8 X-ALD, 8 PMLD, 6 Sandhoff disease, 4 VWM (vanishing white matter leukoencephalopathy) and 3 L-2-hydroxyglutaric aciduria disease (Figure-1B).

12 mutations observed in at least two unrelated patients which were as follow: 6 MLD patients had Gly311Ser and 3 patients had c. 465+1G>A in ARSA gene (data under publication), two X-ALD patients had c. 1415_1416delAG in *ABDC1*, six CD patients showed c. 634+1G>T in ASPA, three patients (two homozygotes and one compound heterozygote) had c. 237_238insA in *ASPA*, each c. 118G>C(p. Ala40Pro) and c. 733T>A(p. Cys245Ser) variant in *GJC2* were observed in two PMLD patients which were novel variants, c. 1528C>T in *HEXA* observed in 4 of 11 TSD patients (2 were from Turkish, 1 from Fars, 1 from Mazani ethnicity), and c. 509G>A in *HEXA* was found in two Gilak patients, two of six Sandhoff disease patients had c. 833C>T and three (two homozygotes and one heterozygote) MLC patients had c. 449_455delTCCTGCT and two MLC patients had c. 177+1G>T.

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; 12 variants met the criteria for being pathogenic, 18 and 11 variants were likely pathogenic and VUS, respectively.

One X-ALD patient had two variants as a haplotype because his mother was heterozygous for both (case5). These two variants considered as one haplotype, although they were classified as likely pathogenic variants.

In silico analyses

Protein interaction analysis predicted that the studied proteins had physical and functional relationships except proteins encoded by *PLA2G6, RARS, SLC17A5, L2HGDH* and *MLC1* genes (Figure-2, supplementary table-3).

Functional association was predicted among different studied proteins. In co-expression prediction analysis (Data not shown), the following protein pairs such as POLG and SUCLA, in mitochondria were expressed together.

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[4]. 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% 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 26% of the cases. Our diagnostic rate of panel-based analysis was comparable to other studies[6]. Three patients were genetically undiagnosed with panel-based studies and WES/WGS is needed to define the causes. Consequently, we had 25% unsolved genetic cases and the diagnostic rate was 75% 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, Tays-Saches, 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).

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.

Our understanding of *in silico* protein analysis and prediction analysis showed that interaction of proteins are beyond single cell type or physiological condition; proteins are highly specific and could interact without binding e. g. transcription factors in expression regulation. The functional association predicted the interaction between two proteins to a joint biological function[D1]. Five proteins did not show any association with other proteins in leukodystrophies and leukoencephalopathies in our study. It is surprising that these proteins were not associated in leukodystrophies pathways and showed no functional interaction. To name, PLA2G6, MLC1, L2HGDH, RARS, SLC17A5 showed no association. All these proteins have relationship with metabolism and function of cells but did not show interaction in prediction analysis. Therefore, for rare diseases genetic analysis, WES 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, pallitative treatment and avoiding unnecessary treatments. Hematopoietic stem cell transplantation (HSCT) has been used for lysosomal storage diseases[5]. 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[1]. MLD patients were from western part of Iran (data under publication). 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 (Figure-2). Patients with X-ALD could benefit from HSCT[7] or hematopoietic stem-cell gene therapy[17].

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[3]. Patients with known genetic etiology may benefit from such experimental therapies.

PMLD is responsible for 8% of hypomyelinating leukodystrophy patients[10]. 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[Pt 9]. GJC2 is co-expressed with *PLP1* and interacts with products of *FAM1256A*, *POLR3A* and *EIF2B5* genes (Figure-2). Our results highlighted that PMLD may have a higher frequency than PMD in our population especially in Arab and Fars ethnicities.

Additionally, our prediction analysis showed that MLC1 protein had no interaction or expression with other studied proteins. Six of them were from Turk ethnicity; it may be a common disorder and limit to specific ethnicity e. g. from Turkey.

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 *SURF1*. Also, it was due to *NDUFS1, NDUFS7* and *SDHAF1* genes. *L2HGDH* encoding mitochondrial L-2-hydroxyglutarate dehydrogenase may be common in our ethnicities. Its protein showed no interaction with other proteins of our study, instead it has interaction with other proteins. The mechanism of leukodystrophy is very complicated and there may be proteins involved in disease progress which show overlapping phenotype but have no or unknown interaction with each other.

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[2,1,4,6]; 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[1]. 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_455delTCCTGCT) may have ancestors in Turk population. Especially, the c. 449_455delTCCTGCT variant was observed in three families; it may be originated from a founder ancestor in Turk population and it previously has been reported from Turkey[3].

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.

Conclusion

In conclusion, this study gains other studies in the distribution of genetics of leukodystrophies in Middle East. 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, treatments for some diseases at early stages are successful before initiation of presymptomatic stage. Enzyme replacement, metabolic correction, cell-based therapies at the right time increases the patient's life span. This study provides information to help for future therapeutic planning's in the country.

Declarations

Ethics approval and Patients' consent

Ethical approval was supported by Growth and development research center, Tehran University of Medical Sciences ID number 98-02-80-43432. Informed consent was obtained from the patients.

Consent for publication

All contributing authors have read the manuscript and given their consent for the publication of this study.

Availability of supporting data

There are no additional unpublished data. MLD data is under publication.

Competing interests

None of authors declared any conflict of interest.

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Author's contribution

N. M. : Study design, data analysis, project administrator, writing and review editing

M. S. : Data extraction, data validation, data analysis, review editing

A. T. : Clinical evaluation

A. R. : Clinical evaluation

M. A : Clinical evaluation

A. K : Review editing

B. R. : Study design, Data validation, Data analysis, Writing and review editing

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Tables

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.

No.	Name of Disease	Alternative designation, abbreviation	MIM #	Gene	Location of protein	No of families (%)	Genetic testing		Phenotypes
							Single gene	WES	
	Leukodystrophies								
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		230000	FUCA1	Lysosome	2 (1. 8)	0	2	Hypotonia:1, Dental germination:1, skin lesions:1, AG:1, 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	swallowing 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, Irritable:6, Seizure:3, R: 9, DD:7
12	Pelizaeus-Merzbacher-like disease type	PMLD	260600	GJC2	Cell membrane, gap junction	8 (7)	6	2	Hypotonia: 6,Nystagmus: 8, Ataxia: 4, Speech problem:6, DD:6
13	Megalencephalic leukoencephalopathy with subcortical cysts	MLC	604004	MLC1	ER and cell membrane	7 (6. 1)	7	0	Macrocephaly:7, Dystonia: 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
			603945	EIF2B5	Cytosol, nucleus	2 (1. 8)	0	2	
			606687	EIF2B4	Cytosol	1 (0. 9)	0	1	
15	Hypomyelination-hypogonadotropic hypogonadism-hypodontia	4H	614366,	POLR3A	Nucleus	1 (0. 9)	0	1	Hypotonia: 2, speech problem: 2, Tremor:1, ataxia:2, AG: 2, Seizure:2, MR:1, DD:1, nystagmus:1
			614381	POLR3B	Nucleus	1 (0. 9)	0	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
	Genetic Leukoencephalopathies								
21	Tay-Sachs Disease	TSD	272800	HEXA	Lysosome	11 (9. 6)	11	0	Vision problem and nystagmus:8, R:6, DD:4
22	Sandhoff disease	SHS	606873	HEXB	Lysosome	6 (5. 3)	5	1	Visual problem:2, Seizure:1, R:4, DD:4
23	Gangliosidosis	GM1	230500	GLB1	Lysosome	1 (0. 9)	0	1	
24	Neuronal Ceroid-Lipofuscinoses	NCL	204300	PPT1,	Extracellular, Lysosome	1 (0. 9)	0	1	Hypotonia:1, speech problem:2, AG: 2, Seizure:2, MR:2, DD:1
				CLN6	ER membrane	1 (0. 9)	0	1	
25	Mucopolysaccharidosis type IIIB	MPS IIIB	609701	NAGLU	Lysosome	1 (0. 9)	0	1	coarse facial feature, macrocephaly
26	Cockayne Syndrome	CS	609413	ERCC6	Nucleus	1 (0. 9)	0	1	Microcephaly, AG, MR/R
27	Biotinidase deficiency	BTD	253260	BTD	Extracellular	1 (0. 9)	1	0	Seizure
28	L-2-hydroxyglutaric aciduria	L-2-HGA	236792	L2HGDH	Mitochondrion	3 (2. 6)	2	1	Hypotonia:1, Macrocephaly:1, speech problem: 1, tremor: 1, AG: 1, Seizure: 1, DD: 3, LD:1, Mental retardation:3
29	Glutaric acidemia IIC	GAIIC	231680	ETFDH	Mitochondrion inner membrane	1 (0. 9)	0	1	Walking problem, speech problem, digestive problem, MR
30	Mitochondrial DNA depletion syndrome 5	MDDS5	612073	SUCLA2	Mitochondrion	1 (0. 9)	0	1	Dystonia, R, DD
31	Ataxia neuropathy spectrum	ANS	203700	POLG	Mitochondrion	2 (1. 8)	0	2	Speech difficulty:1, walking difficulty:1, vision problem:1, ataxia:1, Seizure:1, DD:2
32	Leigh syndrome	LS	185620	SURF1	Mitochondrion inner membrane	3 (2. 6)	0	3	Muscle weakness:3, walking problem:3, swallowing problem:2, R:3, DD:1
33	Mitochondrial complex I deficiency, nuclear type 5	MC1DN5	618226	NDUFS1	Mitochondrion inner membrane	1 (0. 9)	0	1	Walking problem, Seizure, MR
34	Mitochondrial complex I deficiency, nuclear type 3	MC1DN3	618224	NDUFS7	Mitochondrion	1 (0. 9)	0	1	Hypotonia, Seizure
35	succinate dehydrogenase complex assembly factor 1 deficiency	MCIID	252011	SDHAF1	Mitochondrion	1 (0. 9)	0	1	Speech problem, walking problem, R
Total						114 (75)	74 (49%)	40 (26%)	

DD: Developmental delay; LD: learning difficulties; CI: Cognitive impairment; MR/R: Motor regression/retardation; MD: motor delay, MD; AG: Abnormal gait; ER: Endoplasmic reticulum

Table 2:
Novel variants identified in this study

No.	Nucleotide change	AA change	Gene	no. of patients	Zygosity	ACMG	MutationTaster	Polyphen-2	CAD
1	c. 2099A>C	p. Asn700Thr	*POLR3B*	1	Hom	Likely pathogenic (2)	DC	PD 0. 998	27.
2	c. 786A>C	p. Gln262Asp	*SLC17A5*	1	Hom	Likely pathogenic (2)	DC	PD 1. 000	24.
3	c. 904_905delinsAT	p. Glu302Met	*ABCD1*	1	Hemi	Likely pathogenic (2)	DC	NA	26.
4	c. 1628C>G	p. Pro543Arg	*ABCD1*	1	Hemi	Likely pathogenic (2)	DC	PD 1. 000	23.
5	c. 2002A>G +c. 1021G>T	p. Thr668Ala+p. Ala341Ser	*ABCD1*	1	Hemi	Likely pathogenic (2) Likely pathogenic (2)	DC	PD 0. 761	23.
6	c. 839G>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.
10	c. 866G>A	p. ser289Ile	*EIF2B4*	1	Hom	Uncertain significance (3)	DC	B 0. 002	22.
11	c. 422G>T	p. Gly141Val	*FUCA1*	1	Hom	Likely pathogenic (2)	DC	PD 1. 000	28.
12	c. 82delG	p. Val28CysfsX105	*FUCA1*	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.
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.
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.
19	c. 571_572insC	p. Thr195AspfsX69	*GJC2*	1	Hom	Pathogenic (1)	DC	NA	17.
20	c. 118G>C	p. Ala40Pro	*GJC2*	2	Hom	Likely pathogenic (2)	DC	PD 1. 000	24.
21	c. 733T>A	p. Cys245Ser	*GJC2*	2	Hom	Likely pathogenic (2)	DC	PD 1. 000	25.

22	c. 883C>T	p. Gln295Ter	*GJC2*	1	Hom	Likely pathogenic (2)	DC	NA	38
23	c. 529_531delAAA	p. Lys177del	*PEX13*	1	Hom	Pathogenic (1)	DC	NA	22.
24	c. 345C>G	p. Ile115Met	*PEX14*	1	Het	Uncertain significance (3)	DC	PD 0. 999	23.
25	c. 655_657delATT	p. Ile219del	*HEXB*	1	Hom	Pathogenic (1)	DC	NA	20.
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.
28	c. 16C>T	p. Arg6Cys	*PLA2G6*	1	Hom	Uncertain significance (3)	DC	PD 0. 994	25
29	c. 416T>A	p. Leu139Gln	*GLB1*	1	Hom	Likely pathogenic (2)	DC	PD 1. 000	29.
30	c. 997G>T	p. Asp333Tyr	*SUCLA2*	1	Homo	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. Glu270SerfsX20	*SURF1*	1	Hom	Pathogenic (1)	DC	NA	35
34	c. 362+5G>A	-	*PPT1*	1	Hom	Pathogenic (1)	DC	NA	21.
35	c. 659A>C	p. Tyr220Ser	*CLN6*	1	hom	Uncertain significance (3)	DC	PD 0. 986	32
36	c. 392C>A	p. Thr131Lys	*HSD17B4*	1	Hom	Likely pathogenic (2)	DC	PD 0. 985	33
37	c. 1285G>A	p. Val429Met	*NDUFS1*	1	Hom	Likely pathogenic (2)	DC	PD 0. 971	28.
38	c. 415G>A	p. Asp139Asn	*NDUFS7*	1	Hom	Likely pathogenic (2)	DC	PD 1. 000	25.

DC: disease causing, PD: probably damaging, Hom= homozygous, B=benign, NA= not available

Figures

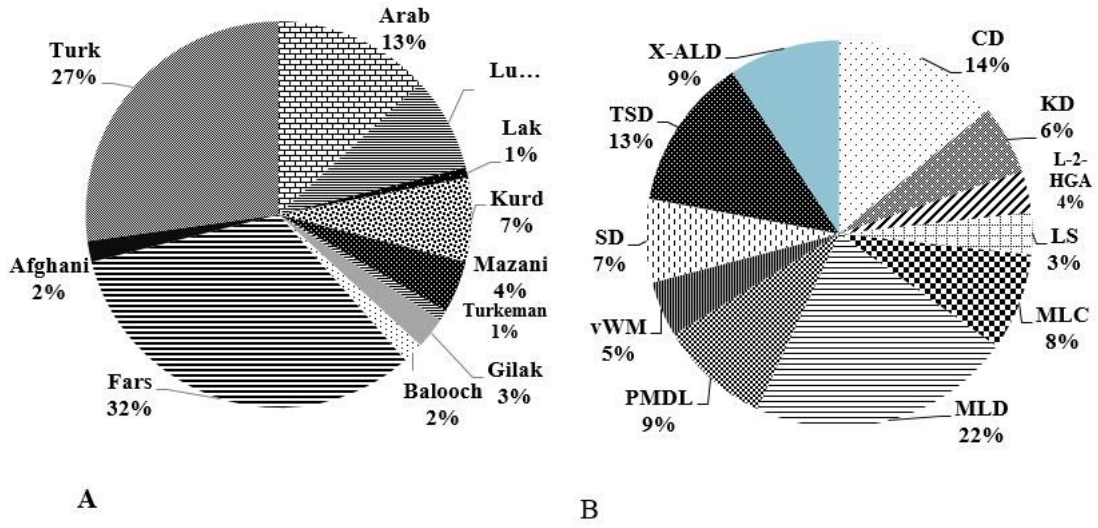

Figure 1

A) The distribution of 114 leukodystrophy genotype positive patients in Iran, based on ethnicity. B) The distribution of the most common diagnosed leukodystrophies (86 of 114 patients) in our studied patients include 19 of 86 (22%) metachromatic leukodystrophy (MLD), 12 Canavan (CD), 11 Tays-Sachs disease (TD), 7 megalencephalic leukodystrophy with subcortical cysts (MLC), 8 X-linked adrenoleukodystrophy (X-ALD), 8 Pelizaeus-Merzbacher-like disease type 1 (PMLD), 6 Sandhoff disease (SD), 4 VWM (Leukoencephalopathy with vanishing white matter) and 3 L-2-HGA(L-2-hydroxyglutaric aciduria disease).

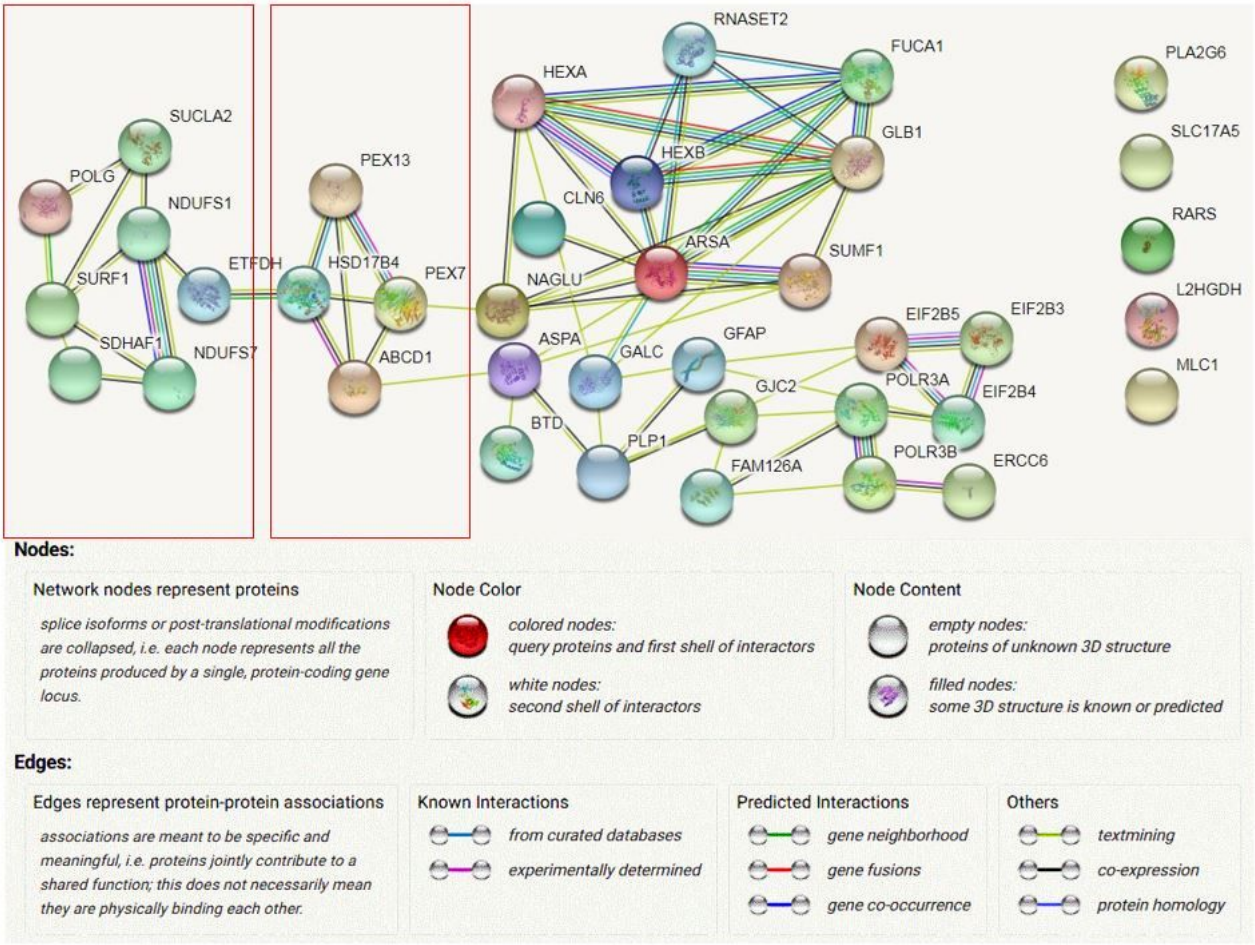

Figure 2

Protein interaction-interaction analysis. Mitochondrial (POLG, SUCLA2, NDUFS1, SURF1, ETFDH, SDHAF1, NDUFS7) and peroxisomal proteins (PEX13, HSD17B4, PEX7, ABCD1) are showed in rectangles. Other genes including the one in lysosomes are shown in middle panel. Some proteins show no interaction with other proteins (PLA2G6, MLC1, L2HGDH, RARS, SLC17A5). The Protein-protein interaction is categorized based on one of seven channels : 1-3) genomic context (neighborhood, fusion, gene co-occurrence), 4) co-expression, 5) text-mining, 6) biochemical/genetic data ('experiments') and 7) previously curated pathway and protein-complex knowledge ('databases').

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