Title: Expanding the Phenotype and Genotype Spectrum of a Novel Mutation in Hypomyelinating Leukodystrophie-5 with a Review of the Literature on 42 Cases

Running Title: A Novel Mutation in HLD-5 with a Comprehensive Literature Review

Authors: Sahar Bayat[1], Milad Gholami[2], Hamidreza Khodadadi[3], Mohammadreza Ghazavi[4], Jafar Nasiri[4,5], Majid Kheirollahi[1,*]

1. *Department of Genetics and Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.*
2. *Department of Biochemistry and Genetics, School of Medicine, Arak University of Medical Sciences, Arak, Iran.*
3. *Department of Biotechnology, School of Medicine, Lorestan University of Medical Sciences, Lorestan, Iran .*
4. *Department of Pediatric Neurology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.*
5. *Child Growth and Development Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.*

***Corresponding Author**: Majid Kheirollahi, Department of Genetics and Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: mkheirollahi@med.mui.ac.ir

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Abstract

Background: Hypomyelinating leukodystrophie-5 (HLD-5) is a rare multiple congenital anomaly with intellectual disability caused by an autosomal recessive mutation in the *FAM126A* gene and is characterized by bilateral congenital cataract, developmental delay, cerebellar ataxia, slowly progressive gait disturbance and cognitive impairment. This study aims to contribute to a better understanding of HLD-5 by reviewing previous patients and introducing a novel variant in a new case.

Methods and Results: We subjected a case with an initial diagnosis of HLD-5 in an Iranian family. To identify the possible genetic cause(s), whole exome sequencing (WES) was carried out to detect exon mutations and Sanger sequencing was performed to verify the DNA sequence variants and co-segregation analysis. We predicted the potential deleterious effects of the novel mutation using in silico predictive tools. WES identified a novel homozygous mutation (NM_032581: c.636_639del p.C213Dfs*7) in the *FAM126A* gene. The variant can cause premature termination of amino acid translation or affect mRNA expression.

Conclusions: In this study, the clinical manifestations and molecular findings of HLD-5 were explained. Additionally, we reported a novel variant and some rare clinical features, such as exophthalmos and strabismus, in our proband for the first time. Further research is needed to clarify the molecular mechanisms underlying HLD-5 pathogenesis.

Keywords: Hypomyelinating leukodystrophie-5, *FAM126A*, *DRCTNNB1A*, *HYCC1*, Whole exome sequencing

Abbreviations:

- HLD-5: hypomyelinating leukodystrophie-5
- HCC: hypomyelination and congenital cataract
- NGS: next generation sequencing
- WES: whole exome sequencing
- CNS: central nervous system
- PNS: peripheral nervous system
- PI4K: phosphatidylinositol 4-kinase
- PI4P: phosphatidylinositol 4-phosphate
- HYCC1: hyccin PI4KA lipid kinase complex subunit 1
- PI4KA: PI4KIII-alpha complex
- MRI: Magnetic resonance imaging
- EMG: Electromyography
- NCV: Nerve Conduction Studies
- VCF: variant call format
- GATK: Genome Analysis Toolkit
- BWA: Burrows Wheeler Aligner
- ACMG: American College of Medical Genetics
- PolyPhen-2: polymorphism phenotyping v2
- NMD: Nonsense-mediated mRNA decay
- ER: endoplasmic reticulum

Introduction

Myelin sheath around axons is formed by the spiral wrapping of the differentiated oligodendroglial cell plasma membrane in the central nervous system (CNS) and Schwann cell plasma membrane in the peripheral nervous system (PNS)(1). This myelin sheaths play an intricate role in the saltatory conduction and in protecting axons from cellular stresses(2). Hypomyelinating leukodystrophies (HLDs) are a group of genetic demyelinating or dysmyelinating diseases that affect the proper development of the myelin sheath in the CNS(3).

HLDs are generally rare, heterogeneous and challenging to diagnose because they have diverse clinical manifestations that most often include neurological dysfunction such as ataxia, motor ability, and intellectual disability(3). The emergence of next generation sequencing (NGS) revolutionized the potential to identifying multiple autosomal recessive HLD genes(4).

According to the Online Mendelian Inheritance in Man (OMIM) the number of HLDs and responsible genes identified in the last 10 years has increased (Table-1)

Among HLDs, HLD-5 is a rare genetic disorder caused by an autosomal recessive mutation in the *FAM126A* (formerly *DRCTNNB1A*) gene(4). The *FAM126A* gene also known as hyccin PI4KA lipid kinase complex subunit 1 (*HYCC1*) is located on chromosome 7p15.3, which contains 14 exons and 13 introns. This gene encodes a protein containing 521 amino acids (https://www.ncbi.nlm.nih.gov).

FAM126A expression is down regulated by beta-catenin which is an essential protein for myelin formation in the CNS and PNS (5).

The PI4KIII-alpha (PI4KA;600286) complex synthesize phosphatidylinositol 4-phosphate (PI4P), which is localized at the plasma membrane and essential for the formation of oligodendrocytes(6). Moreover, one component of the PI4KA complex, FAM126A or FAM126B (HYCC2), has been found to directly bind to TTC7B (620060) through its N-terminal portion. The FAM126A-TTC7B heterodimer then instantaneously bound to PI4KIII-alpha to form a ternary complex. (7) Therefore, PI4KIII-alpha complex plays a regulatory role in PI4P synthesis (7). Therefore, as previously described, FAM126A plays a key role in oligodendrocytes formation. The interaction of PI4KIII-alpha with TTC7B and FAM126A is responsible for catalytic activity and stabilization of PI4KIII-alpha.

On the other hand, FAM126A dysfunction may affect oligodendrocyte myelination mediated through lipid synthesis and lipid-associated signaling pathway. Transgenic mice expressing the FAM126 mutant were generated as HLD-5 model mice and showed significantly decreased expression of FAM126 in the corpus callosum and abnormal myelination (8). Also, the occurrence of mutations in the members of PI4KIII-alpha complex such as TTC7B and PI4K leads to similar diseases (9, 10). Consequently, the clinical symptoms and hypomyelination observed in HLD-5 patients are caused by the disruption of myelin production by this complex.

HLD-5 manifests with characteristic clinical conditions impacting the eyes, spine, muscles, CNS, PNS and remarkably congenital cataract (HCC). These clinical symptoms are bilateral congenital cataract, developmental delay, cerebellar ataxia, slowly progressive gait disturbance and cognitive impairment(11).

Herein, we report a case of HLD-5 variant in a 31-year-old male suffering from progressive neurological impairment and congenital cataract. WES revealed a pathogenic homozygous variant in proband, and both parents were carriers of the same genotype. In the present study, we recruited a family with consanguineous marriage from Eastern Iran and detailed clinical characteristics in affected individual. The clinical presentations together with the identification of a homozygous variant in the *FAM126A* gene, allowed us to attribute the disorder to HLD-5.So far, 16 different variants have been reported in the *FAM126A* gene.

In this study we conducted a retrospective review of our experience with HLD-5 and 42 previously published cases based on *FAM126A* gene. We reviewed the clinical and molecular characteristics of all reported patients with the *FAM126A* mutation and summarized them in Table 2.

Case presentation:

The proband was a 31-year-old Iranian man whose parents were double first-cousin- and his mother's relatives had a history of complete paralysis. The patient was examined by a neurologist. No history of hearing impairment was observed. Milestones of motor development were delayed. He presented with progressive gait disturbance at the age of 14 years .Motor impairments with gait unsteadiness; difficulties in both climbing up and down the stairs were also noted.

Magnetic resonance imaging (10) of the brain revealed bright signal changes in centrum semiovale, periventricular area that were compatible with myelin disorders and dysmyelogenesis at 11 years of age. Furthermore, brain MRI studies at age 29 showed moderate to severe diffuse brain atrophy and signal intensity changes in the white matter. Also basal ganglia, brain stem and thalamus have shown normal signal intensity.

Electromyography (EMG) and Nerve Conduction Studies (NCV) examination showed increased latency and decreased NCV in the right Median nerve. Also, no response was observed in stimulation of right superficial peroneal and anterior tibialis nerves which indicates a type of peripheral neuropathy. Finally, a patient with peripheral neuropathy, loss of ability to walk progressively, delayed motor development, mild Intellectual disability and congenital cataracts referred to the neurology service in our study. Both parents were non-symptomatic. We obtained written informed consent from legal representatives of the patient according to the established ethical protocol guidelines.

Methods

Literature review

A systematic literature search was carried out by searching relevant keywords “HLD-5”, “DRCTNNB1A”, “HYCC1” and “FAM126A” and MeSH terms in electronic databases PubMed and Google scholar. Inclusion criteria encompassed studies evaluating HLD-5, with a focus on clinical presentation and/or molecular genetic testing targeting the FAM126A gene. Studies without HLD-5 or HYCC1 mutation were excluded. Records were screened for eligibility and data were extracted from included articles by two independent reviewers. Demographic and clinical features of patients with pathogenic variants of HLD-5 and FAM126A are summarized in Table 2. Afterward, the data were evaluated to provide a broaden overview of the reported cases of HLD-5, encompassing both molecular outcomes and clinical manifestations.

Genomic DNA extraction and WES

Genomic DNA was extracted from the venous blood sample taken from the proband and his parents by salting-out method. Quantitative and qualitative analysis of DNA was performed using a NanoDrop 2000 spectrophotometer. The extracted DNA was fragmented, adapted, barcoded, and subjected to solution phase hybridization; so, exome libraries were constructed

using the Twist Exome 2.0. WES (WES) was carried out to detect exon mutations by the Illumina Novaseq 6000 sequencing platform. Sequencing data were obtained in FASTQ format, which were converted to BAM files, and then variant call format (vcf) files. The Genome Analysis Toolkit (GATK) was used to identify of INDEL and SNVs. Also the Annovar tool was also used to annotate genetic variant results in detail. Exomes was sequenced to target coverage of 100X. The raw sequence data were aligned to the human genome reference (GRCh37) using the Burrows-Wheeler Aligner (BWA).

We then filtered out variants with an allelic frequency greater than 1% in the databases, including the 1000 Genomes database, the dbSNP, the ExAC03, HapMap, ESP6500 and an in-house database. Candidate variants were classified based on the American College of Medical Genetics (ACMG) guidelines and ClinGen specifications (Richards et al., 2015; Zhang et al., 2020). Finally, Sorting of intolerance from tolerance (SIFT) and polymorphism phenotyping v2 (PolyPhen-2) analyzes were performed to determine the pathogenicity of candidate genes. Only disease-causing or disease-associated genetic variants are reported. PROVEAN and MAPP software were used to identify the structure or function and evolutionary conservation.

Sanger sequencing

Sanger sequencing was performed to verify the DNA sequence variants identified through exome sequencing within the proband and co-segregation analysis (Figure1). PCR amplification was performed using specific primers for the target region using Primer3 online software (http://bioinfo.ut.ee/primer3-0.4.0/) corresponding to FAM126A gene. The sequences of the primers were: 5′-TCTGTGTATCAACATACCTCAGCT-3′ and 3′-ACTGATTTTCATTCCGTCCTTGA-5′. PCR products with size of 596 bp were subjected to direct sequencing by ABI 3730XL DNA Analyzer (ThermoFisher Scientific) capillary sequencing and the results were analyzed by the chromas software. According to Franklin database, the p.C213Dfs*7 substitution is a novel frameshift null variant of the X-chromosomal FAM126A gene. The mentioned variant is predicted to undergo mRNA decay (NMD) because this premature termination codon-causing mutation is located in exon 8, which is not last exon or the last 50 bp of preliminary exon. However, it was classified as a Likely Pathogenic variant according to the ACMG criteria.

Bioinformatics analysis

We predicted the potential deleterious effects of the novel mutation using Mutation Taster (http://www.mutationtaster.org/).

Swiss-Model software (https://swissmodel.expasy.org/interactive) was used to make three-dimensional protein structure models, Figure 2.

Results and Literature review

We identified a novel homozygous mutation (NM_032581: c.636_639del p.C213Dfs*7) in the FAM126A gene using WES data analysis. This variant causes a shift in the reading frame starting at codon 213 and the patient's phenotype were consistent with autosomal recessive hypomyelinating leukodystrophy type 5. The mentioned variant can cause premature termination of amino acid translation or affect mRNA expression. Sanger sequencing was performed to confirm the presence of identified variant. Based on current scientific knowledge, the variant is considered as Likely Pathogenic variant. According to the ACMG standards for the classification of human gene variants, the p.C213Dfs*7 was identified as PVS1 (predicted null variant in a gene where LOF is a known mechanism of disease and predicted to undergo NMD) and PM2 (absent from controls in GnomAD, Exome Sequencing project, 1000 Genomes, or ExAC) with an ACMG score of 9. Thus, p.C213Dfs*7 was classified as a Likely Pathogenic variant.

Initially, 47 results were obtained from the PubMed search and 844 results from Google Scholar. Duplicate results were later removed from the searches. Articles in languages other than English or non-human studies were excluded based on predetermined criteria. After reviewing the full text of the remaining articles, 10 were included for data extraction. A total of 42 patients with a confirmed molecular diagnosis of HLD-5 or HHC, with homozygous variants in the *FAM126A* gene, were identified through database analysis (refer to Table 2). Our data indicated that homozygous exonic splicing mutations are the most common type of pathogenic variant (23 out of 43) reported so far in the *FAM126A* gene. The pathological variation c.414+1G>A was the most prevalent mutation, which was detected in 14 out of 43 patients (32.5%). Causative mutations in the *FAM126A* gene are predominantly protein truncating (11 of 15 variant types), including frameshift, deletion, and splice site mutations. Although missense variants and whole gene deletions have also been reported.

Common clinical manifestations in the literature and our case were developmental delay or intellectual disability (43/43), loss of walking (23/43), congenital cataract (35/43), nystagmus

(20/43), scoliosis (13/43), and seizures (9/43). Patients can have variable clinical manifestations and disease severity, suggesting the effect of modifier genes.

Discussion

In the present study, we conducted a comprehensive review of the literature on *FAM126A* mutations and HLD-5, with a focus on genotype-phenotype correlations. So far, 23 families and 42 patients have been reported. In the present study, we describe an Iranian HCC patient carrying a novel mutation in the *FAM126A* gene, c.636_639del (p.C213Dfs*7). Examination of the primary amino acid sequence of FAM126A reveals a highly conserved, structured N-terminal domain (residues 1-289) shared by both splice forms, and a poorly conserved, disordered C-terminal tail (residues 290-521)(7). Our variant is located in a highly conserved region, leading to disruption of the N-terminal domain of the FAM126A protein and elimination of the C-terminal domain. In addition to the mentioned symptoms, our case presented other rare symptoms such as exophthalmos, strabismus, mild osteoporosis, flat foot, and Coxa valga in the femur.

The *FAM126A* gene consists of 14 exons and codes for a 521-amino acid protein. Hypomyelination and Congenital Cataract (HCC) is a rare genetic disease caused by pathogenic variants in the *FAM126A* gene. FAM126A/Hyccin participates in the regulation of phosphatidylinositol 4-phosphate (PI4P) synthesis, which contributes to the pool of polyanionic lipids that define plasma membrane identity. Phosphatidylinositol 4-kinase is a three-component complex that has recently been characterized and consists of FAM126A, PI4KIIIα, and its adaptors TTC7 and EFR3B, which are conserved from yeast to mammals (7). This complex is anchored to the PM by a transmembrane protein EFR3, the mammalian homologues of yeast Efr3, which is crucial for the recruitment of PI4KIIIα to the PM. Although TTC7, the mammalian homologues of yeast Ypp1, is a shuttling protein and binds to EFR3 and PI4KA (12). Finally, FAM126A, which is present only in higher eukaryotes, and TTC7 form a tight heterodimer, with a large protein–protein interface and a conserved surface that increases PM recruitment of PI4KIIIα (7, 12).

PI4KA/TTC7/FAM126A heterotrimers probably promote PI4KA activity by stabilizing and orienting its active site toward the membrane. Additionally, the irregular C-terminus of FAM126A controls the PI4KA catalytic activity in vitro through an unknown mechanism. This implies that the localization, assembly, and activity of the PI4KA complex is tightly controlled, but the specific pathway involved in this process in mammals remains elusive. In the absence

of the *FAM126A* gene, a general destabilization and degradation of the PI4KIIIα complex components occurs (12).

Sequence variations in TTC7 and FAM126A are associated with a heterogeneous group of neurological (FAM126A) and immunological (TTC7A) disorders. Furthermore, biallelic variants in the PI4KA sequence are associated with neurological diseases, especially hypomyelinating leukodystrophy. Comparable organ-specific diseases, such as HLD and HCC, have been described in patients with FAM126A pathogenic variants. It is conceivable that the mentioned phenotypical outcomes arise from deficiencies in the specific functions of the PI4KIIIα-TTC7-FAM126 complex, due to alterations in catalytic activity or intra-complex functional interactions (13).

More than 10 mutations in the *FAM126A* gene have been described in patients with HLD-5/HCC, most of which are splicing, missense/nonsense, small deletions, small insertions, and gross deletions. Consistently, fibroblast cells from HCC patients with nonsense or missense mutations in the *FAM126A* gene had significantly reduced expression of PI4KIIIa, PI4P, EFR3A, TTC7A, and TTC7B (14). Thus, HCC has a common pathogenesis involving a disturbance in PI4P production in oligodendrocytes, whose specific function involves massive plasma membrane expansion and thus the generation of PI4P and downstream phosphoinositides (7).

To date, more than 15 *FAM126A* variants have been identified in diverse geographical populations, including Morocco, Turkey, Israel, Iran, India, Italy, Egypt, Chile, Tunisia, and Yemen. Therefore, HLD/HCC is common (81%) in some countries of the Mediterranean region, which tend to have high rates of consanguinity. Additionally, HLD/HCC mostly affects men (28 out of 43 cases). Differences in clinical phenotypes have been reported among unrelated individuals sharing the same alteration. For example, out of 9 probands carrying the c.414+1G>T mutation, 3 did not show congenital cataract.(15-17).

Similarly, probands with a large intragenic deletion involving 2 exons (c.531-439_743+348 del) developed cataracts at birth or in the first 9 years of life(18). Mutations affecting intron 4 (c.415-1 G > A) were associated with a severe phenotype (19), while mutations affecting c.414+1G>A were identified in probands with milder phenotypes.(20). The significant clinical variability suggests that other genetic or environmental factors likely modify the HLD/HCC phenotype (16).

Mutations affecting mRNA splicing are the most common molecular findings in patients with HLD-5. These variants are expected to significantly disrupt the acceptor/donor splice sites, leading to exon skipping or introduction of cryptic splice sites, resulting in a lack of protein production (Table 2).

Although most *FAM126A* variants lead to disruption of pre-mRNA splicing and consequent changes in protein conformation and activity, the Leu-53-to-Pro mutation can still result in the production of some hyccin protein. However, this variant can also lead to the accumulation of misfolded proteins in the endoplasmic reticulum (ER). In contrast, the wild-type hyccin protein mainly localizes in the cytoplasm. It is plausible that HLD-5-associated variants of *FAM126A* lead to a decline in the localization of FAM126A in plasma membranes (21).

On the other hand, Traverso (22) reported that patients with the p.Cys57Arg variant showed a 40% reduction in hyccin protein levels, while patients with the p.Leo53Pro variant experienced an approximately 70% decrease (22). Additionally, cells of patients with nonsense variants such as p.Arg217X completely lacked hyccin protein. Furthermore, reintroduction of GFP-tagged FAM126A into patient fibroblasts using recombinant lentivirus as a gene therapy vector was shown to partially rescue the phenotypes. All reported deletion variants, including c.100-101delAA, c.725del, c.274delA, 531-439_743+348del, and c.636_639del in FAM126A, cause frameshift mutations and create premature stop codons that it significantly disrupts the protein structure. These variants were located in exons 2, 3, 7, 8, and 9. Two studies by Ugur and Biancheri (7, 23) stated that cataracts are not invariably congenital or necessary for the diagnosis of HCC and can be seen later in life. Moreover, the onset of neurological conditions varied among cases. Therefore, even in the absence of cataracts or with atypical clinical manifestations, increased T2 signal from the pyramidal tracts in the mesencephalon and pons suggests the diagnosis of HCC, and molecular analysis of FAM126A should be the first diagnostic step (7).

Conclusion

In this study, clinical manifestations and molecular findings of HLD-5/HCC were explained. Additionally, we report for the first time a novel variant and some clinical features, such as exophthalmos and strabismus, in our proband. This broadens the pathologic spectrum in HLD-5/HCC patients and highlights the pathologic and clinical variability that can be associated with the same genetic variation, suggesting an important role for modifier genes in the pathogenesis of HLD-5/HCC.

Ethical Approval

Ethical approval was issued by the Iran National Committee for Ethics in Biomedical Research (IR.MUI.MED.REC.1402.398) for this study.

Informed consent

Written informed consent was obtained from the legal guardians of the patient for publication of this manuscript and any accompanying images.

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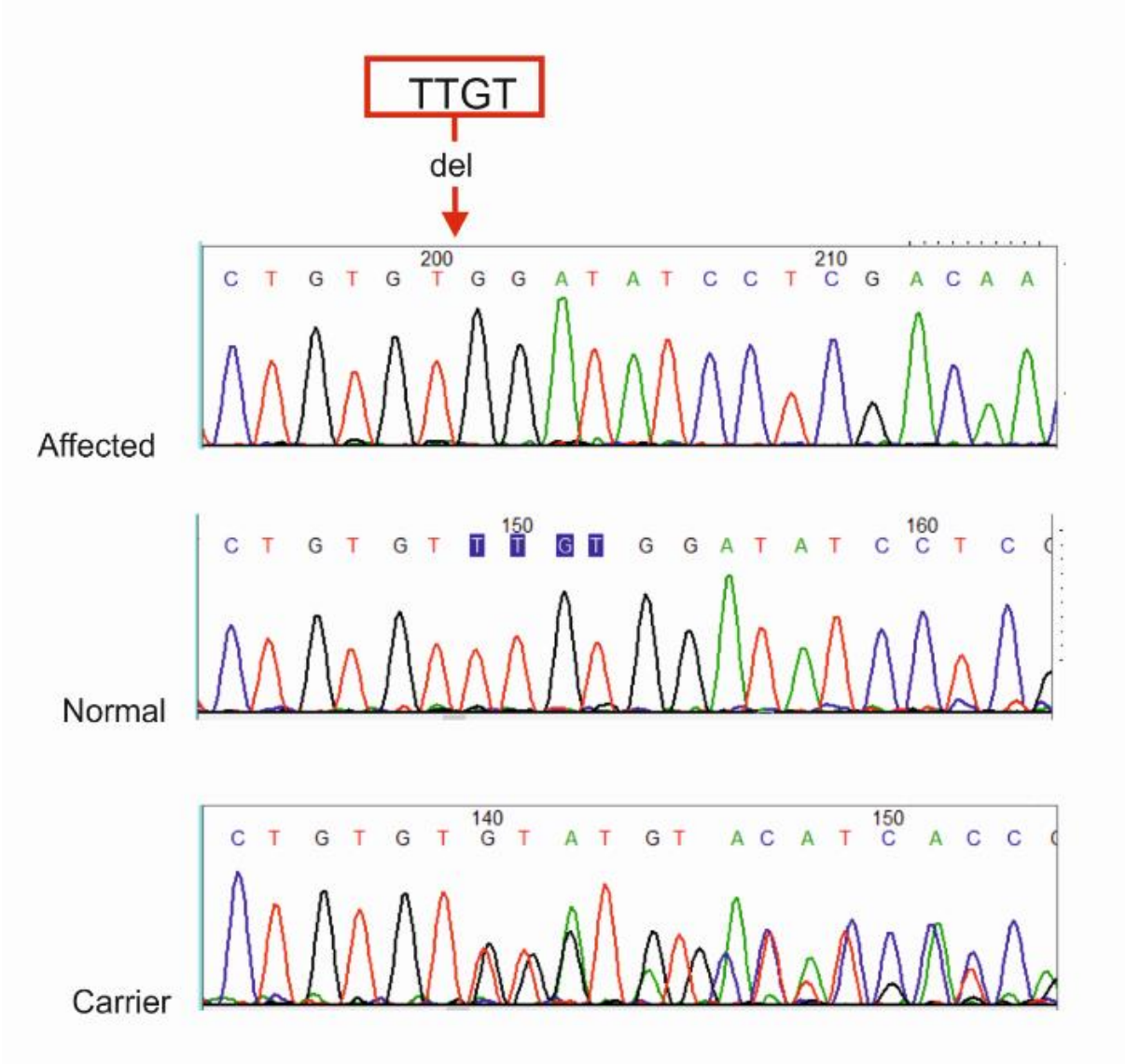

Figure 1. Sanger Sequencing. Partial DNA sequences in the FAM126A by Sanger sequencing of the proband and his parents. Arrows point to the mutation.

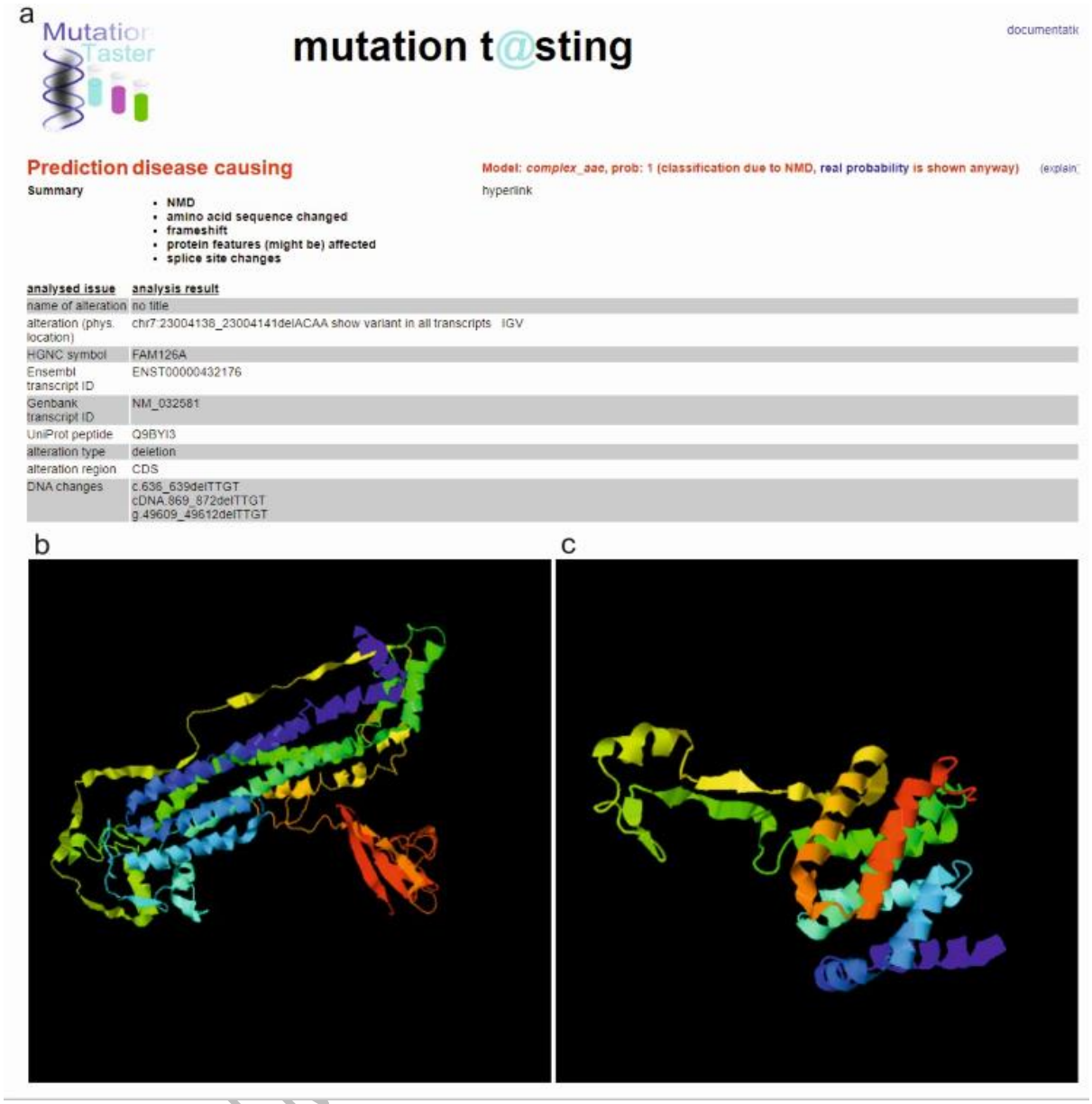

Figure-2. Swiss-model and Mutation Taser were used to predict the arrangement of the wild-type and mutant FAM126A proteins.

Table 1, The all types of HLDs and responsible genes. (https://www.omim.org, accessed on 3 April 2024).

Disorder	Phenotype mim number	Related Gene	Location
HLD1	312080	*PLP1*	Xq22.2
HLD2	608804	*GJC2/GJA12*	1q41
HLD3	260600	*AIMP1*	4q24
HLD4	612233	*HSPD1*	2q33.1
HLD5	610532	*FAM126A*	7p15
HLD6	612438	*TUBB4A*	19p13
HLD7	607694	*POLR3A*	10q22
HLD8	614381	*POLR3B*	12q23
HLD9	616140	*RARS*	٥q34
HLD10	616420	*PYCR2*	1q42
HLD11	616494	*POLR1C*	6p21
HLD12	616683	*VPS11*	11q23
HLD13	616881	*HIKESHI*	11q14
HLD14	617899	*UFM1*	13q13
HLD15	617951	*EPRS*	1q41
HLD16	617964	*TMEM106B*	7p21
HLD17	618006	*AIMP2*	7p22
HLD18	618404	*DEGS1*	1q42
HLD19	618688	*TMEM63A*	1q42
HLD20	619071	*CNP*	17q21
HLD21	619310	*POLR3K*	16p13
HLD22	619328	*CLDN11*	3q26
HLD23	619688	*RNF220*	1p34
HLD24	619851	*ATP11A*	13q34
HLD25	620243	*TMEM163*	2q21
HLD26	620269	*SLC35B2*	6p21
HLD27	620675	*POLR1A*	2p11

Table 2. Clinical and genetic findings of all HLD-5/HCC patients worldwide.

Reference	Family number	Patient number	Sex	Age	Nationality	Gene. DNA variant	Protein variant	codon	Region	ACMG classification	Consanguinity	Developmental delay or Intellectual disability	Loss of walking (1)	congenital cataract	nystagmus	scoliosis	Seizures	Other symptoms
(2)	1	1	M	12	Indian	CCA>TCA	Pro-Ser	80	Exon 4	Likely Pathogenic	+	+	_	+	+	NA	NA	oligodontia hirsutism synophrys ataxia
(3)	2	2	M	6.5	Italy	51+1G>A	skipping of exon 2, results in the lack of protein production.	51	Exon 2	Likely Pathogenic	_	Mild	NA	+	_	NA	+	
		3	M	14								Mild	8	+	_	+	_	
		4	M	27								Moderate	8	+	_	+	_	
		5	F	6								Mild	NA	+	_	_	_	
	3	6	M	25	Italy	51+1G>A	skipping of exon 2, results in the lack of protein production.	51	Exon 2		+	Moderate	9	+	_	+	+	
	4	7	M	13	Italy	51+1G>A	skipping of exon 2, results in the lack of protein production.	51	Exon 2		+	Mild	9	+	_	+	NA	
	5	8	F	16	Italy	414+1G>T	introduce a cryptic splice site in intron 5, leads to the insertion of a 20-bp intronic segment into mRNA, resulting in a premature stop codon at position 145	414	Exon 5	Likely Pathogenic	_	Moderate	8	+	_	_	NA	
		9	M	2							_	Mild	NA	+	_	_	NA	
		10	M	2							_	Mild	NA	+	_	_	+	
	6	11	M	20	Turkey	c.158 CTA>CCA	p.Leo53Pro without altering the structure of the mRNA.	53	Exon 3	VUS	+	Mild	NA	+	_	_	_	

(4)	7	12	F	8	Egypt	c.100-101delAA	p.Lys33Glufs⁄17	33	Exon 2		+	+	+	+	+	+	_	
		13	F	5				33			+	+	+	+	+	+	_	
	8	14	F	1.2	Morocco	c.169T > C	p.Cys57Arg	57	Exon 3	Likely Pathogenic	+	+	_	+	+	+	_	
(5)	9	15	M	21	Turkey	415-1 G > A	affecting the acceptor splice site of intron 5	139	Exon 5	Pathogenic	+	Sever	Never walked	+	+	+	+	
		16	F	13							+	Sever	Never walked	+	+	+	+	
	10	17	M	2.6	Israeli arab	c.649C > T	p.Arg217X	217	Exon 8	Pathogenic	+	NA	NA	+	_	_	+	primary hyperplastic vitreous, micrognathia, dolichocephaly, pectus excavatum, and muscle hypotonia
(6)	11	18	NA	NA	Iran	c.333 +1 G>C	it eliminates the splice donor site.	111	Intron 4	VUS	NA	NA	NA	+	NA	NA	NA	
(7)	12	19	M	15	Chile	c.414+1G>A	it eliminates the splice donor site.	138	Intron 5	Pathogenic	+	Moderate	Never walked	Bilateral cataract (3 mo)	_	+	_	central hypotonic syndrome not present dysmorphisms
		20	F	3mo		c.414+1G>A	it eliminates the splice donor site.	138	Intron 5	Pathogenic	+	NA	NA	+	_	_	_	
(8)	13	21	M	23	Morocco	c.414+1G>T		138	Exon5	Likely Pathogenic	+	mild	15	Bilateral cataract (7 mo)	+	NA	_	
	14	22	M	15	Morocco	414+1G>T		138	Exon5	Likely Pathogenic	+	Mild	3	Bilateral cataract (3 mo)	_	NA	_	hypotonia and feeding difficulties
	15	23	M	3	Italy	414+1G>T		138	Exon5	Likely Pathogenic	_	Mild	NA	Bilateral cataract (3 mo)	_	NA	_	mild opacity of the lens

	16	24	M	13	Morocco	414+1G>T		138	Exon5	Likely Pathogenic	+	Mild	NA	+	+	NA	_	
	17	25	M	15	Morocco	c.215_224dup	p.Leu76SerfsX25	72	Exon3	Likely Pathogenic	+	Mild	4	+	+	NA	_	
	18	26	F	2	Turkey	c.725del	(p.Thr242fs)	242	Exon7	Likely Pathogenic	+	Mild	_	+	_	NA	_	
	19	27	M	18	Turkey	c.274delA	p.Ser92AlafX15	92	Exon3	Likely Pathogenic	+	Mild	9	Bilateral cataract (5 mo)	+	NA	+	
		28	M	17		c.274delA	p.Ser92AlafX15	92	Exon3	Likely Pathogenic	+	Mild	11	+	+	NA	_	
	20	29	M	4	Turkey	c.274delA	p.Ser92AlafX15	92	Exon3	Likely Pathogenic	+	Mild	_	+	+	NA	_	
(9)	21	30	F	Died (2y)	Turkey	531-439_743+348del	Arg209fsTer213	214	Exon8 & Exon9	Likely Pathogenic	+	NA	_	+	NA	NA	NA	
		31	M	10								Moderate	Never walked	+	NA	NA	_	
		32	F	Died (12y)								Moderate	2	unilateral Congenital cataract	NA	NA	NA	
		33	M	16								Mild	6	+	NA	NA	_	
		34	M	13								Mild	6	Bilateral cataract (9 Y)	NA	NA	_	
(1)	22	35	F	3	Tunisia	c.414 +1G >T		138	Exon 5	Likely Pathogenic	+	Mild	NA	+	+	_	_	
		36	M	16								Mild	NA	+	+	+	_	
		37	F	5								Mild	NA	+	+	_	+	
		38	M	8								Moderate	Never walked	+	+	+	_	
		39	F	2								Mild	NA	+	+	_	+	
(10)	23	40	M	9	Yemen	homozygote complete gene deletion	No product	complete gene		Pathogenic	+	+	2	Soon after birth	+	+	_	Hypotonia, muscle weakness, bilateral Babinski sign, swallowing difficulties.
		41	F	5		homozygote complete gene deletion	No product					Mild	NA	+	+	_	NA	Hypotonia, muscle weakness,

																	bilateral Babinski sign,
		42	F	4		homozygote complete gene deletion	No product				+	Never walked	+	+	_	NA	hypotonia, increased muscle tonus, bilateral Babinski sign.
Our report	24	43	M	31	Iran	c.636_639del	p.C213Dfs*7	212	Exon7	Likely Pathogenic	+	Mild	14y	+	_	+	_	Exophthalmos Strabismus Mild osteoporosis Flat foot Coxa valga in femur

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