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Identification of novel mutations by targeted NGS in Moroccan families clinically diagnosed with a neuromuscular disorder

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A B S T R A C T

Background and aims: The identification of underlying genes of genetic conditions has expanded greatly in the past decades, which has broadened the field of genes responsible for inherited neuromuscular diseases. We aimed to investigate mutations associated with neuromuscular disorders phenotypes in 2 Moroccan families.

Material and methods: Next-generation sequencing combined with Sanger sequencing could assist with understanding the hereditary variety and underlying disease mechanisms in these disorders.

Results: Two novel homozygous mutations were described in this study. The *SILI* mutation is the first identified in the Moroccan population, the mutation was identified as the main cause of Marinesco-Sjogren syndrome in one patient. While the second mutation identified in the fatty acid 2-hydroxylase gene (*FA2H*) was associated with the Spastic paraplegia 35 in another patient, both transmitted in an autosomal recessive pattern.

Discussion and conclusions: These conditions are extremely rare in the North African population and may be underdiagnosed due to overlapping clinical characteristics and heterogeneity of these diseases. We have reported in this study mutations associated with the diseases found in the patients. In addition, we have narrowed the phenotypic spectrum, as well as the diagnostic orientation of patients with neuromuscular disorders, who might have very similar symptoms to other disease groups.

1. Introduction

Neuromuscular disorders (NMD) as a group, encompass a wide range of diseases that affect peripheral nerves or muscle [1], they are linked by anatomy, with overlapping but distinct phenotypes, as well as different pathogenic mechanisms, clinical expression, and disease progression [2]. Symptoms vary depending on the speed of onset of the respiratory muscle weakness [3]. The position of damage can be in the cell bodies, neuromuscular junction, axons, Schwann cells, muscle, or any combination of these sections [1]. NMD are difficult to diagnose due to the

abundance of possible sites of injury. Clinical presentation of NMD differs largely; many diseases of the peripheral nervous system (PNS) will manifest with muscle atrophy, distal sensory loss, and/or reduced tendon reflexes. Most NMD are hereditary [4], and only a few mutations have been identified in this context, especially in northern African countries, where inbreeding is at a high rate and access to diagnosis and health care is limited [5,6]. In the following sections we will detail the two hereditary diseases found in our patients previously diagnosed with muscle damage suggestive of a neuromuscular disease.

1.1. Marinesco-Sjögren syndrome (MSS) (MIM# 248800)

It's a rare autosomal recessive multisystem disorder. Cerebellar ataxia, bilateral cataracts, hypergonadotropic hypogonadism, mild to moderate mental retardation, chronic myopathy, short stature, and skeletal abnormalities comprise the cardinal features of the disease. Mutations in the *SIL1* gene on chromosome 5q31, which encodes an endoplasmic reticulum resident co-chaperone, were identified as the principal cause of Marinesco-Sjögren syndrome [7,8]. *SIL1* (also called BAP) encodes a nucleotide exchange factor for the protein chaperone HSPA5, which is a key regulator of the main functions of the endoplasmic reticulum. The main endoplasmic reticulum (ER) chaperone and the primary sensor of ER stress is *SIL1* a nucleotide exchange factor for BiP. In the endoplasmic reticulum cell membrane and secretory proteins are synthesized and a network of molecular chaperones and folding factors assure correct protein folding and export to post-ER compartments. In case of rupture of this process, the accumulation of unfolded/misfolded proteins, ER stress, and activation of the unfolded protein response (UPR) will take place, a complex signaling pathway aimed at restoring ER homeostasis, whose failure eventually leads to cell death [9,10].

1.2. Autosomal recessive hereditary Spastic Paraplegia (HSP) (MIM# 611026)

The disease describes a heterogeneous group of 27 autosomal recessive neurodegenerative disorders, in which the predominant clinical features are a lower extremity weakness and spasticity [11,12]. The genetics of HSPs is complex and has been reported with all modes of inheritance pattern, the autosomal recessive form of diseases are often common in consanguineous populations such as Middle East and North African population, with a prevalence of 0.6/100,000 identified in the Norway population. Thus, a remarkable prevalence was found in the Tunisian population is 5.75/100,000 the overall prevalence of all HSPs ranges between 4,3 and 9.8/100,000 [12,13]. The most common autosomal recessive HSP identified is the SPG11 subtype associated with mutation in the SPG11, followed by SPG15 associated to mutations in the *ZFYVE26*. One more kind of connection among autosomal-recessive HSPs is exemplified by studies recognizing changes in the *FA2H* gene at 16q21-q23 chromosome, across a disorder range including SPG35, leukodystrophy, and neurodegeneration with brain iron aggregation (NBIA) [14]. SPG35 is defined by childhood-onset (6–11 years) and variable neurologic features in which the cardinal pathological feature is upper motor neuron degeneration leading to progressive spasticity and weakness of the lower limbs, with extrapyramidal features. Other variable neurologic features are dystonia, optic atrophy, and seizures may also occur [11,15,16]. 2-Hydroxy fatty acids (hFA) have been found present in additional tissues and cell types such as tumors. hFA are necessary components of a subset of mammalian sphingolipids. Their presence in sphingolipids is described in the nervous system, epidermis, and kidney. However, Biosynthesis of hFA-sphingolipids requires fatty acid 2-hydroxylase, This protein is an NADPH-dependent mono-oxygenase that catalyzes the synthesis of free fatty acids to 2-hydroxy fatty acids. These are then incorporated into myelin galactolipids containing hydroxy fatty acid as the N-acyl chain, which helps maintain the myelin sheath [14,17].

In the present study, Next-Generation sequencing allowed us to identify variants associated with rare neuromuscular disorders in a 2 Moroccan family. To this date 24 pathogenic and likely pathogenic *SIL1* variants have been reported in LOVD and Clinvar. While, only 22 pathogenic and likely pathogenic *FA2H* variants were reported in the same specific locus databases. The rarity of the identified cases and the diversity of the clinical characteristics of these diseases constitutes a barrier in terms of identification and harmonization of the phenotypic spectrum, from where the need for further exploration, essentially in the North African and Middle Eastern populations having a rather important

consanguinity rate.

2. Material and methods

2.1. Ethics approval

The study was approved by the ethics committee for biomedical research in Rabat and written informed consent was collected from all volunteers.

2.2. Molecular genetic analysis

Genomic DNA was extracted from peripheral leukocytes with Qiagen Kit, QIAamp DNA Blood Mini Kits. The enriched libraries utilizing the HaloPlex Target (Agilent, CA, USA) Enrichment System for Illumina sequencing later sequenced using Next Generation Sequencing (NGS). The Panel gene approach was conducted using the NextSeq 550 (Illumina, CA, USA).

2.3. Data analysis

Therefore, targeted NGS sequencing was performed for 306 neuromuscular genes [18]. The list must essentially cover the initial "Limb-Girdle Muscular Dystrophies" clinical entry-diagnosis group defined by the FILNEMUS network guidelines except for a few newly involved genes added very recently to the list of the corresponding genes [19]. Clean reads were aligned to UCSC hg19 human reference genome. Annotated VCF files including all variants were analyzed to prioritize mutations using VarAFT software. VarAFT Interactive filtration features provide the progressive reduction of the list of candidate mutations by combining the various annotations [20]. The identification of the disease-causing mutations from the 2 index patients VCF corresponding to diseases transmitted in an autosomal recessive pattern. It was performed in many steps using data from the variant annotations and the phenotype layers, excluding all variants types except splicing and exonic, removing variant with a Minor Allele Frequency (MAF) > 0.01 in the GnomAD database [21], polymorphism, and probable polymorphism were also excluded. The remaining variants were classified according to the ACMG classification system [22]. Following gene assessment and variant classification according to the American College of Medical Genetics and Genomics (ACMG) guidance for variant classification, integration of results with patients' phenotype was performed to provide a clinically relevant interpretation of the findings [22].

2.4. Sanger verification

Exon 6 and 4 of the *SIL1* and *FA2H* genes respectively, were amplified by a polymerase chain reaction from the DNAs of affected and unaffected individuals of families, of each index patient. The amplicons were subsequently sequenced by the Sanger method using ABI Big-Dye terminator v3.1 sequencing + reaction Kit (Applied Biosystems) and analyzed on an ABI 3130 Genetic analyzer (Applied Biosystems). All the sequences were analyzed with the ChromasPro software.

3. Results

The sequence analysis of the *SIL1* gene revealed the novel mutation NM_001037633: c.453 + 5G > A (hg19) chromosome5: 138378304, associated with Marinesco-Sjogren Syndrome for the first patient. All the mutations reported in the literature and responsible for the same disease are gathered in Table 1. Thus, the homozygous status of the mutation that we have identified is consistent with the parental consanguinity. This variant may most probably affect splicing, as predicted by Human Splice Finder (HSF) [23]. Also, the patient phenotype observed sticks with the clinical features described in OMIM (# 248800).

Moreover, the second patient harbored the variant NM_024306:

Table 1*SIL1* mutations in patients with Marinesco–Sjögren syndrome autosomal recessive, identified in this study and earlier reports.

Nucleotide change	Effect on coding sequence	Number of patients	Geographic origin	Reference
c.178G > T	p.E60X	1	Vietnam	[28]
c.212dupA	p.H71fs	1	France	[29]
c.244 + 1G > A	splice error predicted		Turkey	[7]
c.274C > T	p.R92W	1	Pakistan	[30]
c.302_303delAG	p.E101fs	1	Pakistan	[7]
c.331C > T	p.R111X		Iran, Turkey, Italy	[28,29,31,32]
c.347delG	p.G116fs		Vietnam	[28]
chr5:g.138376244_138378903del	p.L119fs		India	[7]
c.424delG	p.A142fs		Germany	[7]
chr5:g.138311133_138369401delinsTGCA	p.A152fs	1	Japan	[33]
c.460C > T	p.Q154X		France	[7]
c.506_509dupAAGA	p.D170fs	3	Finland, Sweden, Norway	[29]
c.603_607delGAAGA	p.E201fs	1	Japan	[33]
c.645 + 1G > A	p.A152_Q215del	2	Turkey, Argentina	[7,28]
c.645 + 2 T > C	p.A152_Q215del	1	Sweden	[29]
c.691_696delGTGATC	p.V231_1232del		Turkey	[7,34]
c.768-1G > A	p.S256fs		Italy	[7]
chr5:g.(138271912_138277581) _(138345106_138349134)del	p.S256_R461del		Russia	[7]
c.811C > T	p.Q271X		Turkey	[7]
c.866dupT	p.L290fs		France	[7]
c.934G > A	p.G312R		USA	[7,34]
c.936dupG	p.L313fs	4	Japan, Argentina	[7,35]
c.947dupT	p.R317fs	2	Germany, Russia	[7,28]
c.1029 + 1G > A	p.V289_K343del	1	Bosnia	[28]
c.1030-9G > A	p.F345fs	4	Norway, USA, Pakistan	[7,29]
c.1030-18G > A	p.M344fs		Germany	[28]
c.1035delC	p.F345fs		Germany	[7]
c.1126C > T	p.Q376X		Pakistan	[7]
c.1137C > A	p.C379X		Iraq	[7]
c.1240C > T	p.Q414X		Pakistan	[30]
c.1249C > T	p.Q417X		Mali	[28]
c.1276_1282delCAGGCTG	p.Q426fs		Germany	[7]
c.1312C > T	p.Q438X	4	Egypt	[36]
c.1367delT	p.L456fs		Russia	[28]
c.1367 T > A	p.L456X	1	Turkey	[29]
c.1370 T > C	p.L457P	1	Japan	[29]
chr5: 138,282,808 A > G	stop codon (TGA) to an arginine codon (CGA)	1	Chinese	[37]
c.740C > T	p.A247V	2	Canada	[38]
c.783G > C	p. Q261H			
c.-197_90delinsCTGTACTTTCTCAGTTCACT	affected the 5' UTR, translation initiation site and the endoplasmic reticulum-targeting signal sequence	1	Emirates	[39]
c.1060C > T	p.Gln354X	2	Dutch	[40]
c.935G > A	p.Gly312Glu	2	Dutch	[40]
c.1207_1209delinsT	p.Val403Serfs*25	1	Italy	[41]
c.244 + 1G > A	splice error predicted	1	Turkey	
c.453 + 5G > A		1	Moroccan	This study

c.581delG (G194Afs*47) (hg19) chromosome16: 74,760,155 associated with a novel homozygous *FA2H* mutation leading to a frameshift of the reading frame. In the literature, only few cases have been reported with HSP type 35 (Table 2). The two variants reported in this study were novel and never have been reported in any locus databases, and several prediction programs were used to assess the pathogenicity (SIFT[24], REVEL[25], MetaLR[26]), the novel missense variants were found to have severe predicted pathogenic effects (Table 3), regarding the Human Splice Finder (HSF[23]) predictions, the first variant in the *SIL1* was considered to have an effect of alteration of the WT donor site which is most probably affecting splicing, the same program predicted an alteration of the exonic splicing enhancer site and a potential alteration of splicing for the second *FA2H* variant. In addition, according to the ACMG[22] classification, the *SIL1* variant has been predicted as uncertain significance, studies have shown that this type of mutation is consistent with splicing errors, and often leads to open reading frame alterations due mainly to incorrect deletion of introns[27]. A previous study found this type of intronic splicing mutations in individuals with

MSS, which result from a disruption of donor splice sites, the same study proved that *SIL1* mutations are the main cause of MSS[28]. As well, the *FA2H* variant reported in this study has a pathogenic verdict according to the ACMG[22] classification, a study also reported the involvement of *FA2H* loss-of-function mutations in three Israeli families[16]. All of the above evidence supported the involvement of these variants in the diseases diagnosed in both patients, including predictions of pathogenicity, the absence of these variants from generic databases (1000G and GnomAD), the similarity of the phenotype in the patient to phenotypes described in the literature, and the potential impact on splice sites. All variants identified by the Illumina NextSeq 550 sequencer were confirmed by Sanger sequencing (Fig. 1). After family segregation analyses, the first patient harboring mutation in the *SIL1* gene, the exon 6 mutation was found in a heterozygous state in a non-affected brother. Moreover, the mutation was found in the parents as a heterozygous state (Fig. 1.A). The second patient carried the novel homozygous frameshift deletion in the *FA2H* gene, the family segregation allowed the identification of the variant in a heterozygous state in the parents (Fig. 1.B).

Table 2
FA2H mutations in patients with Hereditary spastic paraplegias (HSPs) type 35 (SPG35), identified in this study and earlier reports.

Nucleotide change	Effect on coding sequence	Number of patients	Geographic origin	Reference
c.130C > T	p. Pro44Ser	1	Turkey	[42]
c.388C > T / c.506 + 6C > G	p.L130F / affect the splicing of the FA2H mRNA	1	Chinese	[43]
c.230 T > G	p.Leu77Arg	1	Chinese	[43]
C.688G > A; c.968C > A; c.976G > A	p.E230K; p.P323Q; p.G326S	2	Chinese	[44]
c.786 + 1G > A	Skipping of exons 5 and 6	2	Arab-Muslim	[45]
c.103G > T	p.D35Y	1	Arab-Muslim	[45]
c.703C > T	p.R235C	1	Omani	[16,46]
c.157_174 del	p.R53_158del	1	Pakistani	[16,46]
c.460C > T	p.R154C	2	Italian/ NR	[47,48]
c.509_510delAC	p.Y170X	1	Albanian	[47]
c.270 + 3A > T	Loss of exons 2–7	–	NR	[49]
c.707 T > C	p. F236S	–	NR	[50]
c.209C > T; c.968C > T	p.S70L; p.P323L	–	Filipino/ Vietnamese	[51]
c.509A > G	Y170C	–	NR	[52]
c.527G > A	p.Trp176*	2	Serbian / NR	[5348]
c.131C > A	p.Pro44Gln	2	German / NR	[5348]
c.133G > T	p.Gly45Trp	2	German/ NR	[5348]
c.785A > C	p.Lys262Thr	2	Spanish / NR	[5348]
C.265C > T	p.Q89*	1	Egypt	[54]
chr16:74760208C > G	p.Trp176Cys	1	Sudan	[54]
chr16:74750478C > A	p.Arg269Leu	1	Yemen	[54]
c.169_170insGCGGCCAGG	p.Asp57Glyfs*66	1	NR	[55]
c.117C > A	p.Phe39Leu	–	–	–
c.620C > T; c.460C > T	p.T207M; p.R154C	1	Italy/UK	[56]
c.1051A > C; c.486G > C	p.S351R; p.E162D	1	UK	[56]
c.798C > G; c.782dupA	p.D266E; p.H261Qfs*	1	Ireland/UK	[56]
c.157_174del	p.Arg53_158del	1	Pakistan	[56]
c.509_510delAC	p.Y170X	4	Montenegro/ Albanian/NR	[47,48,57]
c.160_169dup	p.D57Gfs*66	1	Turkey	[58]
c.21delC	p.A8Pfs*91	1	NR	[48]
c.160_169del GCGGCCAGG	p.A54Tfs*42	2	NR	[48]
c.205C > T	p.H69Y	1	NR	[48]
c.232G > A	p.E78K	2	NR	[48]
c.262G > T	p.E88*	1	NR	[48]
c.443C > T	p.P148L	1	NR	[48]
c.503_506del TCTG	p.V168Gfs*72	1	NR	[48]
c.704G > A	p.R235H	2	NR	[48]
c.859 T > C	p.C287R	1	NR	[48]
c.908G > T	p.G303V	1	NR	[48]
c.956A > G	p.H319R	1	NR	[48]
c.968C > T	p.P323L	2	NR	[48]
c.805C > T; c.1501A > G	p.R269C; p.S351G	1	Italian	[15]
c.193C > T	p.P65S	1	NR	[15]
c.1055C > T; [c.340_363del24][c.363 + 1_8del8]	p.T352I	1	Brazilian	[15]
c.445G > T; c.780C > A	p.Val149Leu; p.His260Gln	1	Japanese	[59]
c.806G > A	p.R269H	2	Moroccan	[60]
c.1006C > A	p.H336N	3	Saudi Arabia	[60]
c.130C > T	p.Pro44Ser	1	Czech	[61]
c.137G > A	p.Gly46Asp	1	Moroccan	[62]
c.101A > G; c.620C > T	p.Tyr34Cys; p.Thr207Met	1	NR	[62]
c.677G > A	p.Trp226*	1	NR	[62]
c.581delG	G194Afs*47	1	Moroccan	This study

Table 3

Mutational data of the two patients.

Patient	RefSeq Transcript	HGVS coding	HGVS Protein	Variant type [63]	Location	Coding impact [63]	ACMG classification [64]	SIFT [24]	REVEL [25]	DANN [65]	HSF[26]	GERP [66]
IC1	NM_001037633.1	c.453 + 5G > A	-	SNV	intron 5 of 9 position 5 of 15,627 (splicing, intronic)	-	Uncertain Significance	NA	NA	0.7779	Alteration of the WT donor site, Most probably affecting splicing.	4.11
IC2	NM_024306.4	c.581delG	G194Afs*47 (p. Gly194AlafsTer47)	Deletion	exon 4 of 7 position 75 of 107 (coding, NMD)	Frameshift	Pathogenic	NA	NA	NA	Alteration of an exonic ESE site. Potential alteration of splicing.	4.2933

The table provides information about the three Index Cases (ICs) and prediction by several tools.

SNV: Single nucleotide Variant.

NA: Not available (SIFT, REVEL, DANN do not provide prediction for variants creating a stop or a frameshift).

ACMG [64]: American College of Medical Genetics SIFT [24]: Sort Intolerant From Tolerant; REVEL [25]: Rare Exome Variant Ensemble Learner; DANN [65]: Deleterious Annotation of genetic variants using Neural Networks, HSF [26]: Human Splicing Finder; GERP [66]: Genomic Evolutionary Rate Profiling.

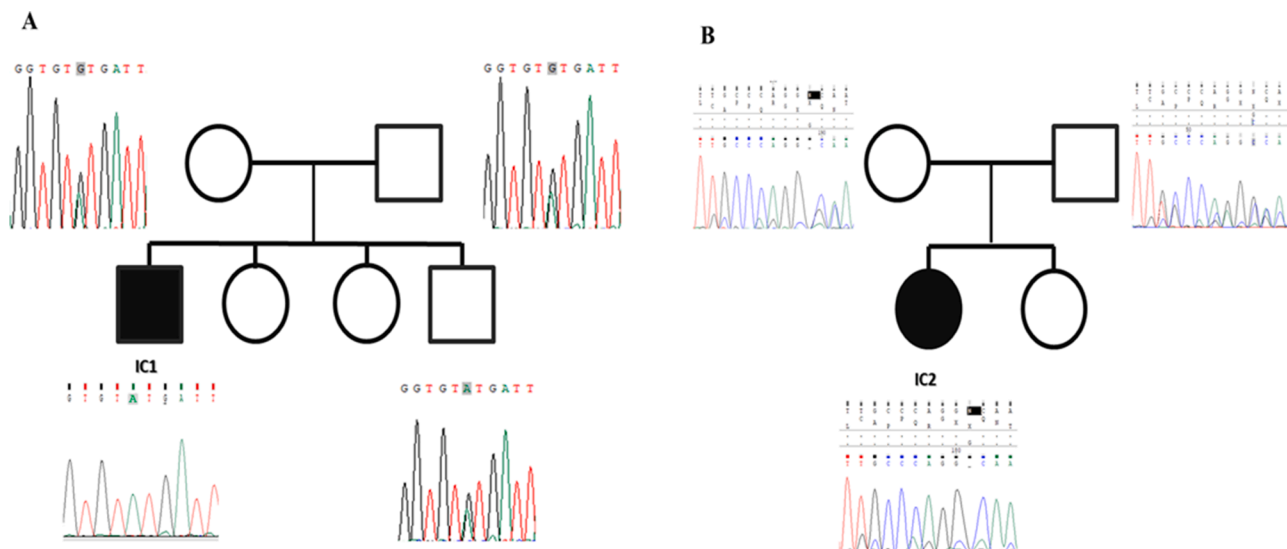


Fig. 1. Pedigree and Electropherogram corresponding to the Sanger sequencing of the Index Cases (IC), and members of the families. (A), corresponds to pedigree the electropherogram of the IC1 family. The parents, brother, and the patient were analyzed in this case. (B), corresponds to the pedigree and Electropherogram of the IC2 family. The patient and his parents were analyzed.

4. Discussion

In this study, we identified another example of an instance where NGS using a panel approach established the genetic cause of disease in two recessive families (Table 4). Using a panel approach, we identified two novel mutations. The first mutation was responsible for Marinesco-Sjogren syndrome, the second for Autosomal Recessive Spastic Paraplegia 35 (SPG35), these variants have never been reported neither in the gnomAD database, or scientific literature. The clinical phenotype helped to lead the diagnosis, the differential diagnosis for NMD broad. Hence, we opted to use the massive parallel sequencing using the panel approach of 306 neuromuscular genes to identify the specific genetic defects leading to the diseases. The VarAFT software was used to prioritize causative variants, using filters with different annotation and prediction tools, as well as the patient's phenotype, we only kept the exonic and splicing variants and the variants with an allelic frequency lower than 1% in the gnomAD database. In addition, we excluded

variants suspected to be polymorphisms or likely polymorphisms. Bio-computational analyses of these mutations did not spare the pathogenic status of the variants, using the different prediction tools, as well as the ACMG classification and the HSF program prediction tools allowed the support of the involvement of these variants in the diseases found in the patients. After using these different levels of filtering, we kept the variants that correlate with the patient's phenotypes for further molecular analysis.

The clinical diagnosis of the first patient presented neonatal hypotonia, the 8-year-old patient had first-degree consanguineous parents, as well as walking deficit. The patient is described with clinical and pathological features of a neuromuscular disorder, accompanied by cerebellar dysfunction, possibly the result of cerebellar atrophy, a myogenic involvement has also been mentioned in this context. The patient does not fulfill the clinical diagnostic criteria for MSS, as myopathy and cataracts are absent in him. In addition, the disease was present earlier in the child. As well, *SIL1* mutations were reported to be

Table 4

Disorders data of the two Index Cases.

Patient	Disorder[67]	Protein name	Locus/ Gene	Cellular functions [68]	Disease clinical features [67]	Inheritance	Prevalence [69]	Treatment[69]
IC1	Marinesco-Sjogren syndrome # 248,800	SIL1 Nucleotide Exchange Factor OMIM: *608005 UniProtKB: Q9H173 Ensembl: ENSG00000120725	5q31.2	SIL1 is a resident endoplasmic reticulum (ER) glycoprotein that interacts with the ATPase domain of BIP (HSPA5; 138120) and enhances nucleotide exchange.	Characterized primarily by congenital cataracts, cerebellar ataxia, progressive muscle weakness due to myopathy, and delayed psychomotor development. Other features include short stature, hypergonadotropic hypogonadism, and skeletal deformities due to muscle weakness.	AR	Prevalence: <1/1000000 (Worldwide)	Treatment is symptomatic. Cataracts often require surgical removal to preserve vision. Hormonal replacement therapy may be needed if hypogonadism is present. Physical and occupational therapy are crucial.
IC2	- Spastic Paraplegia 35, Autosomal Recessive (SPG35) - Fatty Acid Hydroxylase-Associated Neurodegeneration (FAHN) # 612,319	FA2H Fatty acid 2-hydroxylase OMIM : *611026 UniProtKB: Q7L5A8 Ensembl: ENSG00000103089	16q23.1	Myelin lipid hydroxylation	seizures, ataxia, urgency of micturition	AR	Prevalence: <1/1000000 (Worldwide)	At the present time, therapy is predominantly symptomatic. Physical therapy, stretching, and activity are mainstays of treatment, seeking to maintain mobility and avoid complications from inactivity or falls

The table provides several information about the disorder, Protein affected, phenotype and prevalence for each Index Case (IC). AR: Autosomal recessive.

rare (2.7 %) in MSS patients[7]. The patient harbored a novel mutation in the *SIL1* gene (Nucleotide Exchange Factor protein; *608005), associated with the Marinesco-Sjogren syndrome. Thus, the parent's consanguinity demonstrates the homozygous status of this variant. Moreover, the Sanger verification demonstrated the heterozygous status of this variant in the parent's analysis. In addition, family segregation presented a heterozygous status of the variant in the patient's brother. Therefore, we suggest that screening of *SIL1* mutations should be considered in patients with neonatal hypotonia, cerebellar atrophy, and myogenic damage, even without other associated features.

This second novel mutation harbored by the second patient in the *FA2H* (Fatty acid 2-hydroxylase *611026) gene, the frameshift mutation seems to be associated with autosomal recessive Spastic Paraplegia 35 (SPG35). The 4-years-old patient diagnosis presented normal brain CT, besides early welding of inters temporoparietal and metopic sites, epileptic seizures were also present. The available phenotype correlated with the clinical features already reported in the databases, in addition to the bioinformatics prioritization of causative variants, lead us to conclude that the variant was linked with the Spastic Paraplegia 35 (SPG35) in an autosomal recessive mode of inheritance. Molecular analysis of the various relatives made it possible to confirm the homozygous status of the mutation found in the patient, explained by the consanguinity of the parents, as well as the heterozygous status of this mutation in the latter. Generally these diseases represent involuntary cramps (dystonia), in association or not with problems in coordination and balance (ataxia). The evolution of regression of movements is progressive and can eventually require in some cases the wheelchair assistance. These muscular impairments, as well as the age of onset of the disease, suggest at first a neuromuscular disease diagnosis. The new generation sequencing and with the use of the gene panel approach, has allowed to reveal an impairment of the nervous system (neurodegeneration), the condition belongs rather to the class of diseases called neurodegeneration with brain iron accumulation (NBIA).

5. Conclusion

These results should be taken into consideration in order to expand scientific research to properly illustrate these diseases and improve the phenotypic barriers of such conditions that may have symptoms or phenotypes that intersect. Finally, these rare conditions could lead to a false diagnosis when they are underdiagnosed or underrated, which causes a misidentification of the causative disease. One of the major problems with this type of rare diseases is that the phenotypic spectrum as well as the age of onset, which vary considerably from a case to another. Increasing awareness of this clinically and genetically diverse group of diseases is particularly important for patients and researchers, as progress in this field is rapid with the advent of next-generation sequencing. Thus further improvement and exploration in this area will open up promising prospects for treatment and harmonization of diagnosis, especially in populations with a high rate of inbreeding that leads to the diversification of inherited genetic diseases.

CRedit authorship contribution statement

Khaoula Rochdi: Methodology, Software, Writing – original draft. **Mathieu Cerino:** Methodology, Software, Validation, Writing – review & editing. **Nathalie Da Silva:** Methodology. **Valerie Delague:** Conceptualization, Validation, Resources. **Ayman Bouzidi:** Methodology. **Halima Nahili:** Investigation. **Ghizlane Zouiri:** Investigation. **Yamna Kriouile:** Investigation. **Svetlana Gorokhova:** Validation, Writing – review & editing. **Marc Bartoli:** Conceptualization, Validation, Resources, Writing – review & editing, Supervision. **Rachid Saïle:** Conceptualization, Validation, Writing – review & editing, Supervision. **Abdelhamid Barakat:** Conceptualization, Validation, Writing – review & editing, Supervision. **Martin Krahn:** Conceptualization, Validation, Resources, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] Morrison. Neuromuscular diseases. (2016).
- [2] J.M. Bhatt, The Epidemiology of Neuromuscular Diseases, *Neurol. Clin.* 34 (4) (2016) 999–1021.
- [3] D. Hutchinson, K. Whyte, Neuromuscular disease and respiratory failure, *Pract. Neurol.* 8 (2008) 229–237.
- [4] N.G. Laing, Genetics of neuromuscular disorders, *Crit. Rev. Clin. Lab. Sci.* 49 (2) (2012) 33–48.
- [5] R. Khaoula, M. Cerino, N. Da Silva, V. Delague, H. Nahili, Y. Kriouile, S. Gorokhova, M. Bartoli, R. Saïle, A. Barakat, M. Krahn, First characterization of congenital myasthenic syndrome type 5 in North Africa, *Mol. Biol. Rep.* 48 (10) (2021) 6999–7006, <https://doi.org/10.1007/s11033-021-06530-7>.
- [6] L. Al-Gazali, H. Hamamy, S. Al-Arrayad, Genetic disorders in the Arab world, *BMJ* 333 (7573) (2006) 831–834.
- [7] Krieger, M. *et al.* SIL1 mutations and clinical spectrum in patients with Marinesco-Sjögren syndrome. *Brain* 136, 3634–3644 (2013).
- [8] U. Yiş, S. Cirak, S. Hiz, H. Cakmakçı, E. Dirik, Heterogeneity of Marinesco-Sjögren syndrome: report of two cases, *Pediatr. Neurol.* 45 (2011) 409–411.
- [9] R. Chiesa, M. Sallèse, Protein misfolding diseases - The rare case of Marinesco-Sjögren syndrome, *Neuropathol. Appl. Neurobiol.* (2019), <https://doi.org/10.1111/nan.12588>.
- [10] G. De Michele, A. Filla, Other autosomal recessive and childhood ataxias, *Handb. Clin. Neurol.* 103 (2012) 343–357.
- [11] J.K. Fink, Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms, *Acta Neuropathol. (Berl.)* 126 (2013) 307–328.
- [12] F.J. Aguirre-Rodríguez, *et al.*, Novel FA2H mutation in a girl with familial spastic paraplegia, *J. Neurol. Sci.* 357 (2015) 332–334.
- [13] J. Finsterer, *et al.*, Hereditary spastic paraplegias with autosomal dominant, recessive, X-linked, or maternal trait of inheritance, *J. Neurol. Sci.* 318 (2012) 1–18.
- [14] Blackstone, C. Hereditary spastic paraplegia. in *Handbook of Clinical Neurology* vol. 148 633–652 (Elsevier, 2018).
- [15] F. Mari, B. Berti, A. Romano, J. Baldacci, R. Rizzi, M. Grazia Alessandri, A. Tessa, E. Procopio, A. Rubegni, C.M. Lourenço, A. Simonati, R. Guerrini, F.M. Santorelli, Clinical and neuroimaging features of autosomal recessive spastic paraplegia 35 (SPG35): case reports, new mutations, and brief literature review, *Neurogenetics* 19 (2) (2018) 123–130.
- [16] K.J. Dick, M. Eckhardt, C. Paísán-Ruiz, A.A. Alshehhi, C. Proukakis, N.A. Sibtain, H. Maier, R. Sharifi, M.A. Patton, W. Bashir, R. Koul, S. Raeburn, V. Gieselmann, H. Houlden, A.H. Crosby, Mutation of FA2H underlies a complicated form of hereditary spastic paraplegia (SPG35), *Hum. Mutat.* 31 (4) (2010) E1251–E1260.
- [17] H. Hama, Fatty Acid 2-Hydroxylation in Mammalian Sphingolipid Biology, *Biochim. Biophys. Acta* 1801 (2010) 405–414.
- [18] S. Gorokhova, *et al.*, Comparing targeted exome and whole exome approaches for genetic diagnosis of neuromuscular disorders, *Appl. Transl. Genomics* 7 (2015) 26–31.
- [19] M. Krahn, *et al.*, A National French consensus on gene lists for the diagnosis of myopathies using next-generation sequencing, *Eur. J. Hum. Genet.* 27 (2019) 349–352.
- [20] Desvignes, J.-P. *et al.* VarAFT: a variant annotation and filtration system for human next generation sequencing data. *Nucleic Acids Res.* 46, W545–W553 (2018).
- [21] Karczewski, K. J. *et al.* The mutational constraint spectrum quantified from variation in 141,456 humans. <http://biorxiv.org/lookup/doi/10.1101/531210> (2019) doi: 10.1101/531210.
- [22] S. Richards, *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. Off. J. Am. Coll. Med. Genet.* 17 (2015) 405–424.
- [23] F.-O. Desmet, *et al.*, Human Splicing Finder: an online bioinformatics tool to predict splicing signals, *Nucleic Acids Res.* 37 (2009), e67.
- [24] P.C. Ng, S. Henikoff, SIFT: predicting amino acid changes that affect protein function, *Nucleic Acids Res.* 31 (2003) 3812–3814.
- [25] N.M. Ioannidis, *et al.*, REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants, *Am. J. Hum. Genet.* 99 (2016) 877–885.
- [26] C. Dong, *et al.*, Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies, *Hum. Mol. Genet.* 24 (2015) 2125–2137.
- [27] A. Anna, G. Monika, Splicing mutations in human genetic disorders: examples, detection, and confirmation, *J. Appl. Genet.* 59 (2018) 253–268.
- [28] J. Senderek, *et al.*, Mutations in SIL1 cause Marinesco-Sjögren syndrome, a cerebellar ataxia with cataract and myopathy, *Nat. Genet.* 37 (2005) 1312–1314.
- [29] A.-K. Anttonen, *et al.*, The gene disrupted in Marinesco-Sjögren syndrome encodes SIL1, an HSPA5 cochaperone, *Nat. Genet.* 37 (2005) 1309–1311.
- [30] S.A. Riazuddin, *et al.*, Novel SIL1 mutations in consanguineous Pakistani families mapping to chromosomes 5q31, *Mol. Vis.* 15 (2009) 1050–1056.
- [31] G. Annesi, *et al.*, SIL1 and SARA2 mutations in Marinesco-Sjögren and chylomicron retention diseases, *Clin. Genet.* 71 (2007) 288–289.
- [32] A. Terracciano, F. Renaldo, G. Zanni, A. D’Amico, A. Pastore, S. Barresi, E. M. Valente, F. Piemonte, G. Tozzi, R. Carozzo, M. Valeriani, R. Boldrini, E. Mercuri, F.M. Santorelli, E. Bertini, The use of muscle biopsy in the diagnosis of undefined ataxia with cerebellar atrophy in children, *Eur. J. Paediatr. Neurol.* 16 (3) (2012) 248–256.
- [33] T. Takahata, *et al.*, Novel mutations in the SIL1 gene in a Japanese pedigree with the Marinesco-Sjögren syndrome, *J. Hum. Genet.* 55 (2010) 142–146.
- [34] L. Kollipara, S. Buchkremer, J.A.G. Coraspe, D. Hathazi, J. Senderek, J. Weis, R. P. Zahedi, A. Roos, In-depth phenotyping of lymphoblastoid cells suggests selective cellular vulnerability in Marinesco-Sjögren syndrome, *Oncotarget* 8 (40) (2017) 68493–68516.
- [35] M. Eriguchi, H. Mizuta, K. Kurohara, J. Fujitake, Y. Kuroda, Identification of a new homozygous frameshift insertion mutation in the SIL1 gene in 3 Japanese patients with Marinesco-Sjögren syndrome, *J. Neurol. Sci.* 270 (2008) 197–200.
- [36] M.A. Karim, *et al.*, A novel mutation in BAP/SIL1 gene causes Marinesco-Sjögren syndrome in an extended pedigree, *Clin. Genet.* 70 (2006) 420–423.
- [37] N. Gai, *et al.*, Novel SIL1 nonstop mutation in a Chinese consanguineous family with Marinesco-Sjögren syndrome and Dandy-Walker syndrome, *Clin. Chim. Acta Int. J. Clin. Chem.* 458 (2016) 1–4.
- [38] A. Noreau, R. La Piana, C. Marcoux, P.A. Dion, B. Brais, G. Bernard, G.A. Rouleau, Novel SIL1 mutations cause cerebellar ataxia and atrophy in a French-Canadian family, *Neurogenetics* 16 (4) (2015) 315–318.
- [39] P. Nair, *et al.*, Marinesco-Sjögren Syndrome in an Emirati Child with a Novel Mutation in SIL1 Affecting the 5' Untranslated Region, *Med. Princ. Pract. Int. J. Kuwait Univ. Health Sci. Cent.* 25 (2016) 580–582.
- [40] M. Horvers, *et al.*, Marinesco-Sjögren syndrome due to SIL1 mutations with a comment on the clinical phenotype, *Eur. J. Paediatr. Neurol. EJPJN Off. J. Eur. Paediatr. Neurol. Soc.* 17 (2013) 199–203.
- [41] C. Cerami, *et al.*, Marinesco-Sjögren syndrome caused by a new SIL1 frameshift mutation, *J. Neurol. Sci.* 354 (2015) 112–113.
- [42] F. Incecik, S. Besen, S.T. Bozdogan, Hereditary Spastic Paraplegia Type 35 with a Novel Mutation in Fatty Acid 2-Hydroxylase Gene and Literature Review of the Clinical Features, *Ann. Indian Acad. Neurol.* 21 (2018) 335–339.
- [43] X. Liao, *et al.*, SPG35 contributes to the second common subtype of AR-HSP in China: frequency analysis and functional characterization of FA2H gene mutations, *Clin. Genet.* 87 (2015) 85–89.
- [44] L. Cao, X.-J. Huang, C.-J. Chen, S.-D. Chen, A rare family with Hereditary Spastic Paraplegia Type 35 due to novel FA2H mutations: a case report with literature review, *J. Neurol. Sci.* 329 (2013) 1–5.
- [45] S. Edvardson, *et al.*, Mutations in the Fatty Acid 2-Hydroxylase Gene Are Associated with Leukodystrophy with Spastic Paraparesis and Dystonia, *Am. J. Hum. Genet.* 83 (2008) 643–648.
- [46] K.J. Dick, R. Al-Mjeni, W. Baskir, R. Koul, M.A. Simpson, M.A. Patton, S. Raeburn, A.H. Crosby, A novel locus for an autosomal recessive hereditary spastic paraplegia (SPG35) maps to 16q21-q23, *Neurology* 71 (4) (2008) 248–252.
- [47] M.C. Krueger, *et al.*, Defective FA2H leads to a novel form of neurodegeneration with brain iron accumulation (NBIA), *Ann. Neurol.* 68 (2010) 611–618.
- [48] T.W. Rattay, *et al.*, FAHN/SPG35: a narrow phenotypic spectrum across disease classifications, *Brain J. Neurol.* 142 (2019) 1561–1572.
- [49] C. Garone, *et al.*, FA2H-related disorders: a novel c.270+3A>T splice-site mutation leads to a complex neurodegenerative phenotype, *Dev. Med. Child Neurol.* 53 (2011) 958–961.
- [50] T.M. Pierson, D.R. Simeonov, M. Sincan, D.A. Adams, T. Markello, G. Golas, K. Fuentes-Fajardo, N.F. Hansen, P.F. Cherukuri, P. Cruz, C. Blackstone, C. Tiff, C. F. Boerkoel, W.A. Gahl, Exome sequencing and SNP analysis detect novel compound heterozygosity in fatty acid hydroxylase-associated neurodegeneration, *Eur. J. Hum. Genet. EJHG* 20 (4) (2012) 476–479.
- [51] R. Ruppas, *et al.*, Novel Mutations in FA2H-Associated Neurodegeneration: An Underrecognized Condition? *J. Child Neurol.* 28 (2013) 1500–1504.
- [52] A. Tonelli, *et al.*, Atypical adult onset complicated spastic paraparesis with thin corpus callosum in two patients carrying a novel FA2H mutation, *Eur. J. Neurol.* 19 (2012) e127–e129.
- [53] A.S. Soehn, T.W. Rattay, S. Beck-Wödl, K. Schäferhoff, D. Monk, M. Döbler-Neumann, K. Hörtnagel, A. Schlüter, M. Ruiz, A. Pujol, S. Züchner, O. Riess, R. Schüle, P. Bauer, L. Schöls, Uniparental disomy of chromosome 16 unmasks recessive mutations of FA2H/SPG35 in 4 families, *Neurology* 87 (2) (2016) 186–191.
- [54] M.S. Zaki, *et al.*, Mutations in FA2H in three Arab families with a clinical spectrum of neurodegeneration and hereditary spastic paraparesis, *Clin. Genet.* 88 (2015) 95–97.
- [55] J.L. Pedroso, B.W. Handfas, A. Abrahao, F. Kok, O.G.P. Barsottini, A.S.B. Oliveira, Fatty acid 2-hydroxylase deficiency: clinical features and brain iron accumulation, *Neurology* 84 (9) (2015) 960–961.
- [56] E. Kara, *et al.*, Genetic and phenotypic characterization of complex hereditary spastic paraplegia, *Brain J. Neurol.* 139 (2016) 1904–1918.

- [57] S. Donkervoort, et al., Phenotypic variability of a likely FA2H founder mutation in a family with complicated hereditary spastic paraplegia, *Clin. Genet.* 85 (2014) 393–395.
- [58] G. Bektaş, et al., Hereditary spastic paraplegia type 35 caused by a novel FA2H mutation, *Turk. J. Pediatr.* 59 (2017) 329–334.
- [59] M. Kawaguchi, et al., Novel biallelic FA2H mutations in a Japanese boy with fatty acid hydroxylase-associated neurodegeneration, *Brain Dev.* 42 (2020) 217–221.
- [60] C. Marelli, et al., Cerebral Iron Accumulation Is Not a Major Feature of FA2H/SPG35, *Mov. Disord. Clin. Pract.* 2 (2015) 56–60.
- [61] A.U. Meszarosova, et al., Autosomal recessive hereditary spastic paraplegia type SPG35 due to a novel variant in the FA2H gene in a Czech patient, *J. Clin. Neurosci.* 59 (2019) 337–339.
- [62] Pensato, V. et al. Overlapping phenotypes in complex spastic paraplegias SPG11, SPG15, SPG35 and SPG48. *Brain* 137, 1907–1920 (2014).
- [63] Kopanos, C. et al. VarSome: the human genomic variant search engine. *Bioinformatics* 35, 1978–1980 (2019).
- [64] 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. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4544753/>.
- [65] D. Quang, Y. Chen, X. Xie, DANN: a deep learning approach for annotating the pathogenicity of genetic variants, *Bioinformatics* 31 (2015) 761–763.
- [66] G.M. Cooper, et al., Single-nucleotide evolutionary constraint scores highlight disease-causing mutations, *Nat. Methods* 7 (2010) 250–251.
- [67] Searching Online Mendelian Inheritance in Man (OMIM): A Knowledgebase of Human Genes and Genetic Phenotypes. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5662200/>.
- [68] Rappaport, N. et al. MalaCards: an amalgamated human disease compendium with diverse clinical and genetic annotation and structured search. *Nucleic Acids Res.* 45, D877–D887 (2017).
- [69] A. Rath, et al., Representation of rare diseases in health information systems: The orphanet approach to serve a wide range of end users, *Hum. Mutat.* 33 (2012) 803–808.