Exome Sequencing Identifies DYNC1H1 Variant Associated with Vertebral Abnormality and SMA-LED

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Abstract

Background—Molecular diagnosis of the distal spinal muscular atrophies or distal hereditary motor neuropathies remains challenging due to clinical and genetic heterogeneity. Next generation sequencing offers potential for identifying *de novo* mutations of causative genes in isolated cases.

Patient—We describe a 3.6 year old girl with congenital scoliosis, equinovarus and L5/S1 left hemivertebra who showed delayed walking and lower extremities atrophy. She was negative for SMN1 deletion testing and parents show no sign of disease.

Results—Whole exome sequencing of the affected girl showed a novel *de novo* heterozygous missense mutation c.1792C>T; (p.Arg598Cys) in the tail domain of the *DYNC1H1* gene encoding for cytoplasmic dynein heavy chain 1. The mutation changed a highly conserved amino acid, and was absent from both parents.

Conclusion—*De novo* mutations of *DYNC1H1* have been found in cases of autosomal dominant mental retardation with neuronal migration defects. Dominantly inherited mutations of *DYNC1H1* have been reported to cause spinal muscular atrophy with predominance of lower extremity involvement (SMA-LED) and Charcot-Marie-Tooth type 2O (CMT2O). This is the first report of a *de novo DYNC1H1* mutation in the SMA-LED phenotype with a spinal deformity (lumbar hemi vertebrae). This case also demonstrates the power of next generation sequencing to discover *de novo* mutations at a genome-wide scale.

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Keywords

spinal muscular atrophy with lower extremity predominance; SMALED; SMA-LED; distal spinal muscular atrophy; dSMA; whole exome sequencing; *DYNC1H1*; Charcot-Marie-Tooth

Introduction

The hereditary motor neuropathies (HMN), also known as distal spinal muscular atrophy (dSMA) or spinal Charcot-Marie-Tooth (CMT), are characterized by length-dependent neuropathies of primarily motor axons, although sensory axons could be minimally involved as well.[1] Other associated features include pyramidal signs or myelopathy (neuronopathies).[2] The current classification is based on phenotype and mode of inheritance (dominant, recessive). However, there is extensive genetic heterogeneity, with more than 18 disease-causing genes and varying phenotypes (Table 1). Molecular diagnosis by a candidate gene approach using traditional Sanger single gene sequencing methods remains problematic. The recent ability to carry out highly parallel DNA sequencing (nextgen) enables rapid scans of the patient's entire genome for potential causative mutations. This approach has been particularly informative in isolated cases, where *de novo* mutations may be seen in the proband that are not seen in either parent. Here, we report the clinical and molecular work up of a young girl with a Spinal muscular atrophy with lower extremity predominance SMA-LED) phenotype using next generation (nextgen) methods.

Case report

This female patient, with non-consanguineous healthy parents, was born after 40 weeks of gestation and presented with scoliosis and pes equinovarus deformity at birth. Pregnancy was complicated with gestational diabetes and hypothyroidism, which was treated, delivery was vaginal and uncomplicated. Growth parameters were within range of norms for gestational age at birth. In terms of motor development, the parents did not recall initial time for acquisition of early motor milestones. They reported that she was able to turn over at 10 months, and soon after she started crawling. She was able to walk with support at 15 months of age and independently at 2 years of age. Medical history was only significant for two typical febrile seizures. Physical examination at time of initial neurological evaluation at age 2 showed inability to rise from the floor, waddling gait, distal lower extremities atrophy and absent deep tendon reflexes on lower extremities only. Plantar responses were normal. She had increased scoliosis, calf atrophy (Figure 1A) and showed selective atrophy of lower extremities (Figure 1B).

Clinical laboratory work up included normal levels of full blood count, creatine kinase, thyroid stimulating hormone, lactic acid and ammonia levels. Genetic analysis ruled out the deletion of SMN1, a common cause of SMA. Non-contrast brain MRI was normal. Spine MRI showed L5/S1 left hemivertebra (Figure 1C, 1D). Nerve conduction studies displayed normal motor and sensory conductions. Concentric needle electromyography of proximal and distal leg muscles showed no evidence of abnormal spontaneous activity along with decreased recruitment of high amplitude (5 to 7mV) motor units action potentials, consistent with a chronic neurogenic pattern On the most recent neurological examination at 3.6 years

of age, the patient could walk unassisted but showed waddling gait. She has not had further deterioration of motor function. While standing, the patient demonstrated hyperlordosis of the spine. No sensory disturbance of ataxia has been recognized as of yet. Cognitive development was normal for age.

Results

Exome sequencing of unaffected parents and affected proband was performed. Informed consent was obtained under protocol 2405 from the Office for the Protection of Human Subjects and Institutional Review Board at Children's National Medical Center, Washington DC, USA. Exome enrichment was carried out using TruSeq exome enrichment kits (Illumina, San Diego, CA) with sequencing performed on the Illumina HiScan SQ sequencer (2x100cycles, paired end sequencing). Exome sequencing data was analyzed using NextGENe software (SoftGenetics, State College, PA) for alignment, variant calling and mutation detection. Filtering of variants was carried out by limiting to coding sequences, splice junctions (+/− 5 bases from exons), removing synonymous SNPs, and removing polymorphisms already reported in dbSNP and 1000Genomes. A candidate gene filter with 18 known dSMA genes (Table 1) was added to facilitate identification of known disease-causing variants associated with dSMA. A novel heterozygous variant c.1792C>T; (p.Arg598Cys) in *DYNC1H1* (NM_001376.4) gene was found in the proband. We confirmed that the change occurred *de novo* in the patient including confirmation of maternity and paternity (data not shown); this variant was also confirmed by Sanger sequencing (Figure 2A). All *de novo* variants for proband were further filtered against our in-house sequencing database; 12 *de novo* variants were studied to confirm *DYNC1H1* as the best candidate gene for our proband. The *DYNC1H1* c.1792C>T variant was absent from the NHLBI GO Exome Sequencing Project (ESP) database which further confirms it is not a common variation. Overall coverage of exomes was ~ 35x. Coverage of candidate dSMA genes in our exome data was ~92% with coverage of 10x and ~96% with coverage of ~5x. Most dSMA exons with lower or no coverage showed high GC content which probably resulted in low hybridization efficiency.

Discussion

Mutations in *DYNC1H1* (dynein, cytoplasmic 1, heavy chain 1) have been known to cause autosomal dominant forms of axonal Charcot-Marie-Tooth disease type 2O (CMT2O; OMIM#614228), spinal muscular atrophy with lower extremity predominance (SMA-LED; OMIM#158600) and mental retardation with neuronal migration defects (OMIM#614563). *DYNC1H1* mutations were first reported by Weedon *et al.* using whole exome sequencing in a large family with axonal CMT.[3] Subsequently, other authors have reported missense mutations in the tail domain of *DYNC1H1* in families with dominant spinal muscular atrophy with predominance of lower extremity involvement (SMA-LED or SMA-LED).[4,5] More recently, *de novo* missense mutations have been reported in patients with severe cognitive disability, microcephaly and cortical development defects.[6,7,8] The role of *DYNC1H1* has thus seen an expansion in its phenotypic spectrum encompassing development of both the central and peripheral nervous systems (Table 2). This wide spectrum likely reflects the numerous functions of the dynein complex. Consistent with this,

mutations in the cytoplasmic dynein *DNCHC1* mouse ortholog of *DYNC1H1* cause widespread neuronal deficits (Loa mouse; legs at odd angles).[9] Loa mice show deficits in both proprioceptive sensory neurons and motor neurons.[10,11] Altered dendritic morphology and reduced number of trigeminal motor neurons was also described in Loa mice.[9]

Our patient showed a *de novo* heterozygous variant c.1792C>T; (p.Arg598Cys) in exon 8 of *DYNC1H1* which is present in the mutation hotspot tail domain (exon 5–15) of *DYNC1H1*, very close to the original p.Ile584Leu mutation as described by Harms *et al.*[4] Other indications of pathogenicity include high conservation of Arginine at position 598 in 9 species (Figure 2B) and bioinformatics predictions of a deleterious effect on the protein by all disease prediction software algorithms (dbNSFP database)[12] (Figure 2C).

The main clinical features of autosomal dominant SMA with *DYNC1H1* mutations include congenital or very early onset patterns of weakness that are most severe in the proximal legs, and a static or minimally progressive course (Table 4). Our patient showed these symptoms along with an additional lumbar hemivertebra phenotype. A different missense mutation (H306R) in the tail domain of DYNC1H1 was described in a single family with Charcot-Marie-Tooth disease (CMT 2O, OMIM 614228).[3] Although the CMT2O family shares early childhood onset of symptoms with the families in the present study, their phenotype was that of length-dependent weakness and sensory loss. These studies support the concept that both motor and tail domain mutations differentially impair *DYNC1H1* function, leading to variable involvement of the peripheral or central nervous systems. In contrast, the patient reported here uniformly showed predominant weakness in lower extremities without sensory abnormalities on examination or electrophysiology, a combination that led to classification as SMA-LED rather than CMT. Interestingly, 1 of 13 patients with CMT 2O was noted to have predominant proximal weakness similar to our SMA subject.[3] This range of phenotypes is reminiscent of those found in patients with *TRPV4* mutations, which causes congenital HMN, scapuloperoneal SMA, and CMT 2C (*TRPV4*, OMIM#605427).

Several forms of skeletal abnormalities have been previously reported in association with some forms of CMT, but not SMA-LED. Chen *et al.*[11] reported a 3-generation family of CMT where individual affected individuals showed proportional short stature, vocal cord paresis, reduced height of the vertebral bodies, and dolichocephaly.[13] Other cases of *TRPV4* mutations have since been described showing association of skeletal dysplasia and peripheral neuropathy.[14]

From a clinical standpoint, this is the first reported case of a de novo *DYNC1H1* mutation leading to co-occurrence of skeletal dysplasia and a SMA-LED phenotype. Next generation sequencing for detection of *de novo* mutations is an effective way to provide a molecular diagnosis, although it should be noted that both parents must also be subjected to exome sequencing (trio sequencing) in order to define such *de novo* mutations in the proband.

Acknowledgments

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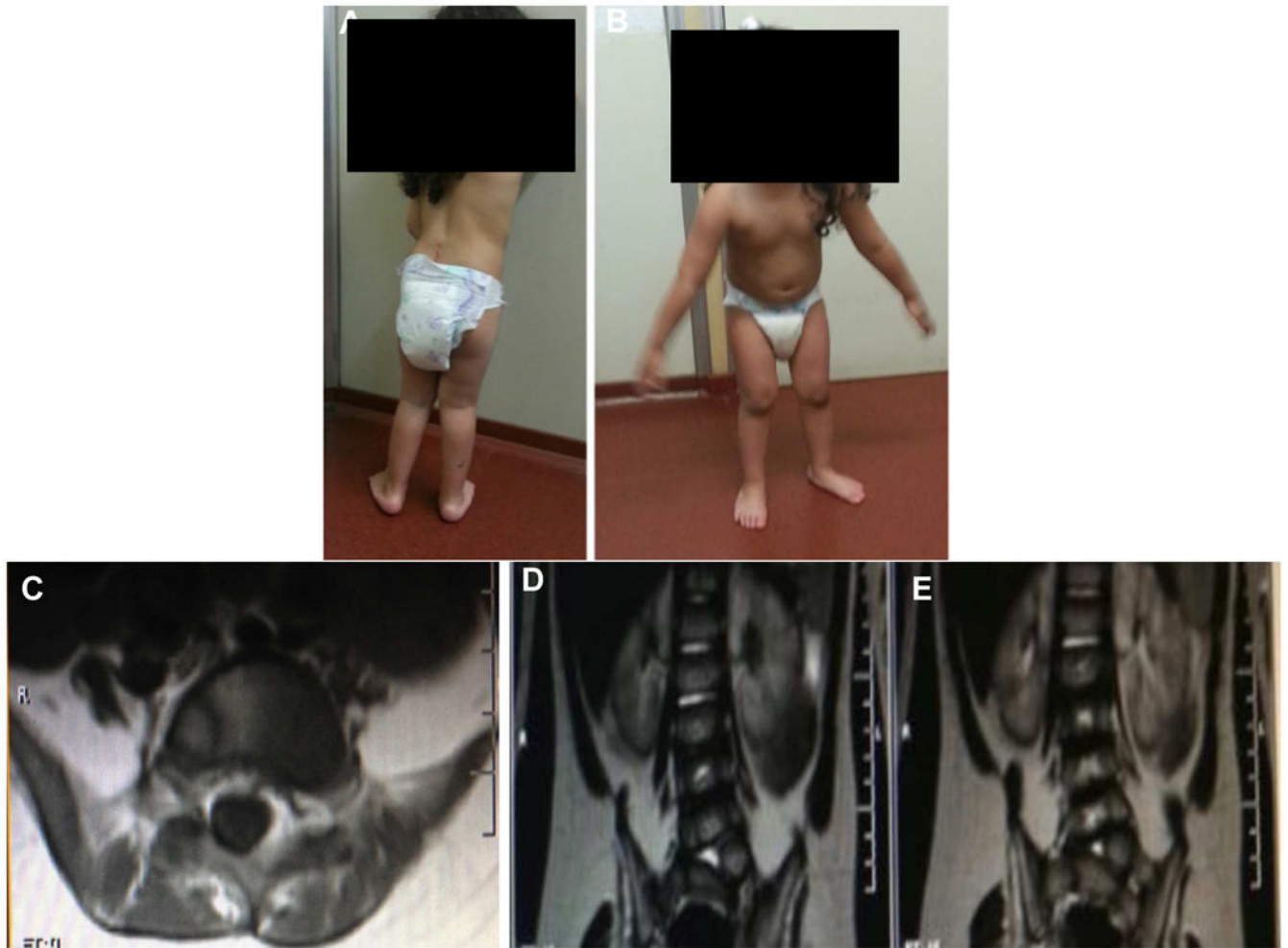
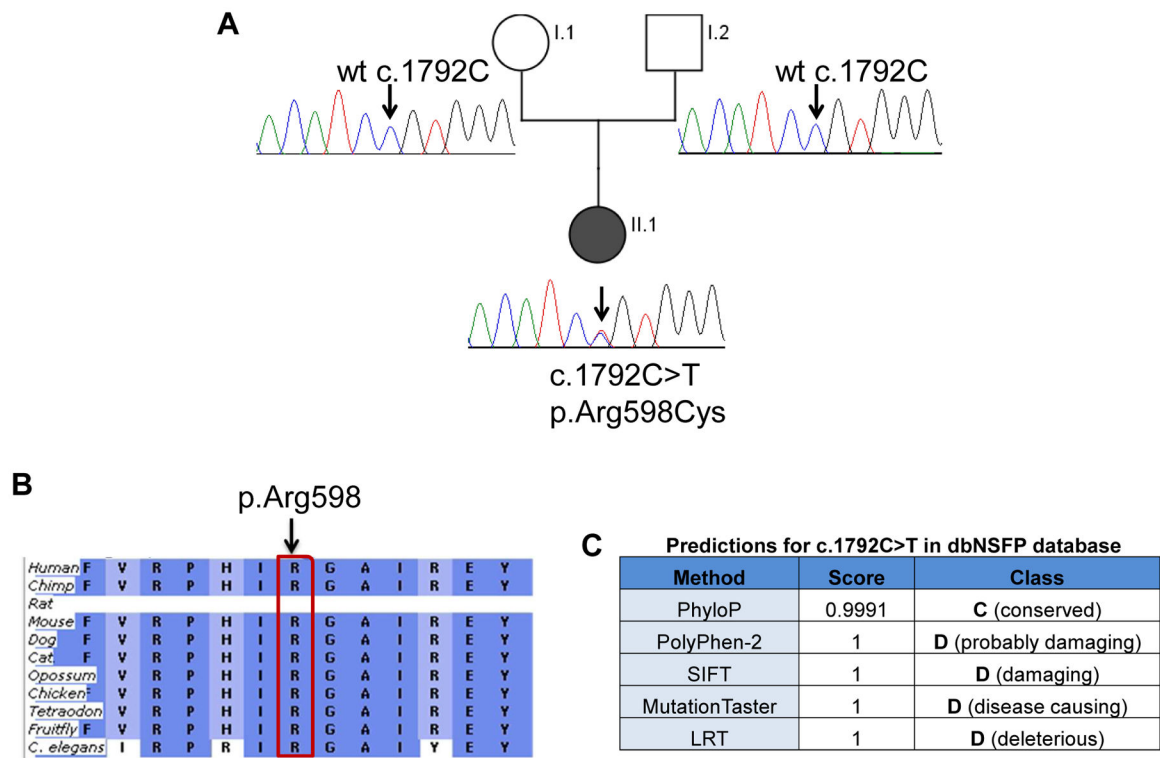**Figure 1.**
Patient at 3.6 years of age. **Panel A**: Patient in standing position showed left scoliosis and calf atrophy. **Panel B**: Patient in standing position showed selective atrophy of lower extremities. **Panel C**: Coronal and **Panel D**: Axial lumbarsacral spine MRI of patient (long and short arrow respectively) showed left vertebral anomaly (hemivertebra) corresponding to level L5/S1.

Figure 2.
Detection of mutation in *DYNC1H1*. **Panel A**: Sanger sequencing confirmation of novel *DYNC1H1* variant (NM_001376.4:c.1792C>T) in proband and parents. Sanger sequencing chromatograms confirm that both unaffected parents have normal alleles at position c.1792 (arrow) and proband is heterozygous at position c.1792C>T causing Arg at position 598 to be changed to Cys. Wt=wildtype. **Panel B:** Arginine (R) at position 598 in *DYNC1H1* is a highly conserved amino acid (generated using Alamut and the Ensembl Compara dataset). **Panel C**: Pathogenicity prediction for c.1792C>T in dbNSFP (database for nonsynonymous SNPs' functional predictions) with probability scores ranging from 0 to 1 (0 being benign and 1 being disease causing).

Table 1

Candidate gene list for distal SMA.

#	Gene	Description	Disease (SMA)	Inheritance	OMIM	Other diseases
1	HSPB1	heat shock 27kDa protein 1	distal hereditary motor neuropathy, type IIB	AD, AR	602195	Charcot-Marie-Tooth disease, axonal, type 2F
2	HSPB3	heat shock 27kDa protein 3	Neuronopathy, distal hereditary motor, type IIC	AD	604624	
3	HSPB8	heat shock 22kDa protein 8	distal hereditary motor neuropathy type IIA	AD	608014	Charcot-Marie-Tooth disease, axonal, type 2L
4	BSCL2	Berardinelli-Seip congenital lipodystrophy 2 (seipin)	distal hereditary motor neuropathy, type V	AD	606158	Lipodystrophy, congenital generalized, type 2; Silver spastic paraplegia syndrome
5	GARS	glycyl-tRNA synthetase	distal hereditary motor neuropathy, type V	AD	600287	Charcot-Marie-Tooth disease, type 2D
7	AARS	alanyl-tRNA synthetase	distal hereditary motor neuropathy	AD	601065	Charcot-Marie-Tooth disease, axonal, type 2N
8	DYNC1H1	dynein, cytoplasmic 1, heavy chain 1	Spinal muscular atrophy, lower extremity-predominant	AD	600112	Charcot-Marie-Tooth disease, axonal, type 20; Mental retardation, autosomal dominant 13
9	REEP1	receptor accessory protein 1	Neuronopathy, distal hereditary motor, type VB	AD	609139	Spastic paraplegia 31, autosomal dominant
10	IGHMBP2	immunoglobulin mu binding protein 2	Neuronopathy, distal hereditary motor, type VI (also DSMA1)	AR	600502	
11	PLEKHG5	pleckstrin homology domain containing, family G, member 5	Spinal muscular atrophy, distal, 4 (DSMA4)	AR	611101	
12	DCTN1	dynactin 1	Neuronopathy, distal hereditary motor, type VIIB	AD	601143	Perry syndrome; susceptibility to amyotrophic lateral sclerosis (ALS)
13	TRPV4	transient receptor potential cation channel, subfamily V, member 4	Scapuloperoneal spinal muscular atrophy; Spinal muscular atrophy, distal, congenital nonprogressive	AD	605427	Brachyolmia type 3; Digital arthropathy-brachydactyly, familial; Hereditary motor and sensory neuropathy, type IIC; Metatropic dysplasia; Parastremmatic dwarfism; Spondyloepiphyseal dysplasia, Maroteaux type; Spondylometaphyseal dysplasia, Kozlowski type
14	ATP7A	ATPase, Cu++ transporting, alpha polypeptide	Spinal muscular atrophy, distal, 3 (DSMA4)	X-linked	300011	Menkes disease; Occipital horn syndrome
15	DNAJB2/ HSJ1	DnaJ (Hsp40) homolog, subfamily B, member 2	Spinal muscular atrophy, distal 5 (DSMA5)	AR	604139	
16	UBA1/UBE1	ubiquitin-like modifier activating enzyme 1	X-linked infantile spinal muscular atrophy (XLSMA)	X-linked	314370	
17	VAPB	VAMP (vesicle-associated membrane protein)-associated protein B and C	Spinal muscular atrophy, late-onset, Finkel type	AD	605704	Amyotrophic lateral sclerosis 8
18	SLC5A7	solute carrier family 5 (choline transporter), member 7	Neuronopathy, distal hereditary motor, type VIIA	AD	608761	
19	?	linked to chromosome 9p21.1-p12	Neuropathy, distal hereditary motor, Jerash type (DSMA2)	AR	605726	

#	Gene	Description	Disease (SMA)	Inheritance	OMIM	Other diseases
20	?	linked to chromosome 11q13.3	Spinal muscular atrophy, chronic distal (DSMA3)	AR	607088	

Table 2

Reported mutations in DYNC1H1 gene: main clinical and laboratory features.

Published reference	Inheritance/ Clinical phenotype	Clinical features	Other features	EDX/Biopsy/MRI
Weedon et al., 2011[3] (N.=23)	Dominant Axonal CMT disease	Delayed motor milestones, abnormal gait and difficulty running Distal LE weakness and wasting. Pes cavus deformity Ambulation maintain through adulthood	Variable learning disabilities in some Variable sensory impairment in some	Nerve conductions normal Sural nerve biopsy: axonal degeneration
Harms et al., 2012[4] (N=34)	Dominant (Three generation family) Dominant SMALED	Waddling gait Difficulty running (Significant quadriceps weakness) Variable heel cord contractures	1 patient with fasciculation of calves 1 patient with developmental delay and polymicrogyria	NCS: Low amplitude CMAP and normal sensory responses EMG chronic denervation
Willemsen et al., 2012[5] (N=2)	Severe Mental retardation, cortical dysplasia and dimorphism	Severe mental retardation Inability to walk or speak Club feet Kyphoscoliosis Spastic quadriplegia Swallowing difficulties	Short stature Microcephaly Small hands and feet with short toes Seizures	CT Head: Enlarged ventricles, cortical malformation with wide opercular regions and abnormal flat cortex
Tsurusaki et al., 2012[6] (n=3)	Dominant, incomplete penetrance	Prominent weakness and atrophy of proximal muscles of lower extremities Waddling gate. Ambulant	None reported	Muscle biopsy: severe grouping atrophy of type 2 fibers and sparse enlarged type 1 fiber
Poirier et al., 2013[7] (N=9)	Dominant	Early onset seizures Variable microcephaly 3 patients spastic quadriparesis 3 patients with foot deformity (axonal neuropathy)	Severe Mental retardation, cortical dysplasia and dimorphism	MRI: posterior pachygyria Some frontal polymicrogyria or nodular heterotopia
Fiorillo et al., 2014[8] (N=2)	Dominant	Global developmental delay, cognitive delay, foot deformities at birth. Ambulation maintain through adulthood.	Hyperlordosis, right curve scoliosis	Brain MRI: Perisylvian abnormal gyration and polymicrogyria.

Abbreviations: SMALED (Spinal muscular atrophy with lower extremity predominance), EMG (Electromyogram), CMT (Charcot-Marie-Tooth), NCS (Nerve conduction studies), CMAP (Compound muscle action potential), CT (Computed tomography), MRI (Magnetic resonance imaging).