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Exome Sequencing Identifies Five Mutations in the *DYNC1H1* Gene Associated with Severe Neurological Phenotypes

BACKGROUND

- Mutations in the highly conserved *DYNC1H1* gene, encoding for cytoplasmic dynein heavy chain 1, have been associated with variable neurological and neurodegenerative phenotypes including autosomal dominant spinal muscular atrophy with lower extremity predominance, intellectual disability, and Charcot-Marie-Tooth (OMIM:600112).
- This gene is located on chromosome 14q32 and is part of a group of microtubule-activated ATPases which act as molecular motors involved in retrograde transport along microtubules.
- *DYNC1H1* mutations likely interfere with dynein-dependent mitochondrial trafficking, resulting in abnormal mitochondrial function that leads to neurodegeneration. To date only 14 mutations have been reported, of which the majority are *de novo* missense.

METHODS

- **Patients/study population:** Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from probands and relatives referred to Ambry Genetics (Aliso Viejo, CA) for diagnostic exome sequencing (DES). Informed consent was obtained from all family members involved in the testing process, and has met criteria for IRB approval exemption under Federal regulations.
- **Whole exome sequencing:** Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA) or or SeqCap EZ VCRome 2.0 (Roche NimbleGen, Madison, WI). The enriched exome libraries were sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA).
- **Characterized and Disease-causing (ChaD) and Novel gene databases**: The Characterized and Disease-causing (ChAD) gene database was curated on a weekly basis to include genes currently known to be responsible for causing human disease. The ChAD database included genes which are associated with syndromes listed in the Human Gene Mutation Database (HGMD) (Stenson, 2009) and the Online Mendelian Inheritance in Man (OMIM) database. Novel genes were defined as those not known to underlie a Mendelian condition at the time of data analysis. Any RefSeq gene not included in the ChAD database was included in the novel gene database.
- **Bioinformatics annotation, filtering of variants, and Family history Inheritance-based Detection (FIND):** HGMD, OMIM, the Single Nucleotide Polymorphism database (dbSNP) (Sherry, 2001), 1000 genomes, HapMap data (International HapMap, 2003) and online search engines (e.g., PubMed) were used to search for previously described gene mutations and polymorphisms. Stepwise filtering included the removal of common SNPs, intergenic and 3′/5′ UTR variants, non-splice-related intronic variants, and lastly synonymous variants. Variants were then filtered further based family history and possible inheritance models using the informatics program "FIND" (Family history Inheritance-based Detection).
- **Personalized Medical Review with Enhanced and Comprehensive Assessment (PRECISE) of potentially causal variants:** Each candidate mutation was assessed by a molecular geneticist to identify the most likely causative mutation(s) using the "PRECISE" (Personalized Medical Review with Enhanced and Comprehensive Assessment) analysis method. In brief, interpretive filtering was based on the deleterious nature of the candidate alterations, literature search, and analysis of the relevance of the candidate genes' function in relation to the patient's phenotype. Most candidate alterations undergo Sanger sequencing confirmation and familial co-segregation analysis.
- Statistical analyses were computed by chi^2 goodness of fit tests and Fisher's Exact Probability.

DYNC1H1 Gene Domains and Clinical Presentations

DYNC1H1 Variant	Proband's Phenotype	Reported By	Gene Domain Location
K129I	Malformations of cortical development	Poirier et al., 2013	Tail domain
H306R	Charcot-Marie-Tooth, axonal	Weedon et al., 2011	Tail domain
I584L	Spinal muscular atrophy	Harms et al., 2012	Tail domain
K671E	Spinal muscular atrophy	Harms et al., 2012	Tail domain
Y970C	Spinal muscular atrophy	Harms et al., 2012	Tail domain
E1518K	ID w/ neuronal migration defects	Willemsen et al., 2012	Tail domain
R1567Q	Malformations of cortical development	Poirier et al., 2013	Tail domain
R1962C	Malformations of cortical development	Poirier et al., 2013	Motor Domain (linker region)
K3241T	Malformations of cortical development	Poirier et al., 2013	Stalk Domain/MTBD
K3336N	Malformations of cortical development	Poirier et al., 2013	Stalk Domain/MTBD
R3344Q	Malformations of cortical development	Poirier et al., 2013	Stalk Domain/MTBD
R3384Q	Malformations of cortical development	Poirier et al., 2013	Stalk Domain/MTBD
H3822P	Severe intellectual disability	Vissers et al., 2010	Motor Domain

Tail Domain= (amino acid 53-1867)

Motor Domain= (amino acid 1686-4646)

Stalk Domain/MTBD= (Pro3285-Pro3409)

Proband	Phenotype	Gene Domain Location
Patient #1 (R1567Q)	absence-like episodes; SMA	Tail Domain
Patient #2 (E3345K)	Encephalopathy; Neuronal migration defects	Stalk Domain/MTBD
Patient #3 (I4071V)	Infantile spasms, epilepey, abnormal MRI	Motor Domain
Patient #4 (9754_9756del)	ID; motor neuroapthy (axonal)	Stalk Domain/MTBD
Patient #5 (R1943Q)	early onset epileptic spasms	Motor Domain

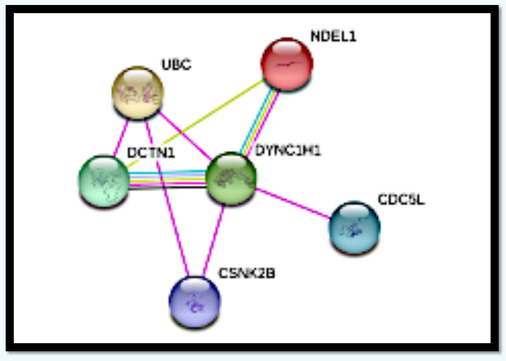
Proband	Neurological Phenotype	Sequencing Info	*DYNC1H1* Alteration	Previously Reported?
Patient #1 (POSITIVE):	Short absence-like episodes and stops activity; microcephaly, eye-rolling; unsteady gait; significant developmental and verbal disabilities; normal EEG; EMG=chronic denervation consistent with mild spinal muscular atrophy; polymicrogyria (esp. frontal regions) on MRI; head CT normal	mutation *de novo*	Heterozygous c.4700G>A; p.R1567Q	Porier *et al*., 2013 paper
Patient #2 (POSITIVE):	bilateral frontal polymicrogyria with mild ventriculomegaly; cerebral palsy, encephalopathy, cortical visual impairments, hyperactivity, unsual facial features, mild central hypotonia, wide gait	mutation *de novo*	Heterozygous c.10033G>A; p.E3345K	novel alteration; not prev. described
Patient #3 (UNCERTAIN):	infantile spasms, early infantile seizures, global developmental delay, growth delay, facial dysmorphism, microcephaly, FTT, hypotonia, EMG normal, mild microcephaly, mildly prominent ventricles and reduced brain volume, significantly delayed myelination, prominent interhemispheric fissure and sylvian fissures.	parents consanguineous; alteration inherited	Homozygous c.12211A>G; p.I407IV	novel alteration; not prev. described
Patient #4 (POSITIVE):	moderate cognitive delay, developmental delay, wide-based gait, language disabilities, low muscle tone/hypotonia, frontal bossing, unusual facial features, microcephaly, EMG showed motor neuropathy of axonal and chronic nature, small amounts of frontal extra axial fluid on MRI, normal EEG, staring spells, ASD-spectrum	mutation *de novo*	Heterozygous c.9754_9756del	novel alteration; not prev. described
Patient #5 (POSITIVE):	epileptic spasms (onset 5.5 months), abnormal MRI with slight enlargement of pericerebral spaces (both supra and infratentorial)	mutation *de novo*	Heterozygous c.5828G>A; p. R1943Q	novel alteration; not prev. described

RESULTS

- We describe four *de novo* and one inherited *DYNC1H1* alterations identified through exome sequencing associated with severe neurological phenotypes.
- Of the five mutations in this cohort, four are novel (c.10033G>A, c. 12211A>G, c.9754_9756del, and c.5828G>A) and one (c.4700G>A) was recently reported by Poirier *et al.* (2013).
- All of our probands showed *de novo* alterations except for one proband from consanguineous (first-cousin) parents who carried a homozygous familial alteration (c.12211A>G).
- Four of the five mutations identified in our cohort were located in the motor domain (amino acid 1686-4646) of the *DYNC1H1* gene and the stalk domain/MTBD region , while the fifth mutation was located in the tail domain (amino acid 53-1867).
- To the best of our knowledge, most reported mutations in *DYNC1H1* in the literature to date are located in the tail domain of the gene except for two which were reported in the motor domain by Vissers *et al.* (2010) and Poirier *et al*., (2013).
- Patient #1 in our cohort also presented with mild SMA and a tail domain mutation.
- Patients #2-5 presented with more severe neurological findings and had mutations in the motor domain and the stalk domain/MTBD.
- Mutations in the microtubule binding domain (MTBD) of *DYNC1H1* are expected to result in weakened microtubule binding and a more severe clinical phenotype.

Literature & Reported Variant Overview

Phenotype Associated	# of Mutations in HGMD	Reference
Malformation(s) of cortical development	15 mutations described	Poirier et al., 2013
Spinal muscular atrophy	6 mutations described	Harms et al., 2012
Charcot-Marie-Tooth disease, axonal	1 mutation described	Weedon et al., 2011
Intellectual disability with neuronal migration defects	1 mutation described	Willemsen et al., 2012
Mental retardation	1 mutation described	Vissers et al., 2010



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TAKE-HOME POINTS

- The *DYNC1H1* gene is highly conserved and is known to be associated with neurological phenotypes in the literature. Our cohort builds upon the previously reported cases and all cases confirm neurological involvement.
- Most previously reported mutations in the *DYNC1H1* gene are located in the tail domains of the gene, which are reportedly associated with SMA/CMT phenotypes.
- Based on the location of reported pathogenic variants in this gene, mutations in the motor domain and MTBD appear to be associated with more severe neurological phenotypes.
- Continued reporting of mutations in the *DYNC1H1* gene is recommended in order to better delineated genotype-phenotype correlations.

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