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A novel *de novo* mutation in *DYNC1H1* gene underlying malformation of cortical development and cataract

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ABSTRACT

Mutations in *DYNC1H1*, the gene encoding the largest cytoplasmic dynein, have been associated with a wide spectrum of neurodegenerative disorders. In this study, we describe a child in whom a novel *de novo* likely pathogenic variant in the motor domain of DYCN1H1 was identified through whole exome sequencing. The affected child presented with severe neurological symptoms and more extensive cortical malformations compared to previously reported cases with mutations in this gene, including diffuse pachygyria-lissencephaly and bilateral symmetric subcortical gray matter heterotopia. A more distinct aspect of the phenotype in this child is the presence of cataract in infancy. So far, only acquired bilateral cataract in adulthood has been described in this disorder in a patient with a much milder neurological phenotype. These findings could extend the phenotype associated with defective DYNC1H1 and suggest a possible important role in human ocular development.

1. Introduction

Identification of the genetic mutations associated with disease is an important step for understanding disease pathogenesis and often provide clues for the development of novel diagnostic and therapeutic modalities (Majewski et al., 2011). Over the past few years, whole-exome sequencing (WES) has been successfully used for disease gene identification and diagnosis of many complex neurological disorders (Foo et al., 2012; Srivastava et al., 2014).

Dyneins are large multi-subunit protein complexes that play crucial roles within the cell of many organisms. Based on function, dyneins can be divided into two classes; axonal and cytoplasmic dyneins. Axonemal dyneins are involved in the movement of the cilia and flagella. Two types of cytoplasmic dynein have been identified. The most abundant type is cytoplasmic dynein 1 which is an essential protein in higher eukaryotes with housekeeping functions in the cell and is responsible for spindle-pole organization and nuclear migration during mitosis (Raaijmakers et al., 2013). Moreover, it is important for the cellular positioning of several organelles including the endoplasmic reticulum, Golgi apparatus and the nucleus and the retrograde axonal transport in neurons. Cytoplasmic Dynein 1 heavy chain 1 (DYNC1H1) is the largest cytoplasmic dynein with 4600 residues and a molecular weight of about 530 kDa (heavy chains) (Pfister et al., 2006) and was first identified in rat spinal cord and brain. *DYNC1H1* is a highly conserved 78-exon gene encoding an isoform of DYNC1H1 (Schiavo et al., 2013). Mutations in this gene in humans have been associated with a number of neurological manifestations including developmental delay, intellectual disability, neuronal migration defects and malformation of the cortical development, spinal muscular atrophy, Charcot–Marie–Tooth type 20 and hereditary spastic paraplasia associated with thin corpus callosum (Fiorillo et al., 2014; Harms et al., 2012; Strickland et al., 2015; Tsurusaki et al., 2012; Willemsen et al., 2012). Functional studies previously demonstrated that DYNC1H1 mutations cause weakness in the binding of the dynein complexes to microtubules which leads to abnormal brain morphology with motor and sensory neuron defects (Schiavo et al., 2013).

A variety of *in silico* web based analysis tools have been developed to assist in the interpretation of the effect of variants at the nucleotide and amino acid level (Richards et al., 2015). The most common computational approaches in assessing the effect of a missense variant used in clinical laboratories are Mutation Taster, PolyPhen2 and SIFT (Richards et al., 2015). Mutation Taster rapidly evaluates the disease causing potential of a variant with an accuracy of 91.1 ± 0.1% by integrating information from different databases such as GeneDistiller, BioMart, dbSNP, SwissProt and UniProt (Schwarz et al., 2010). Polymorphism phenotyping v2 (PolyPhen2) is designed to predict the impact of amino acid alteration on protein structure and function (Adzhubei et al., 2013). The output result is a qualitative outcome based on several

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evolutionary conservation scores from multiple sequence alignment (Adzhubei et al., 2013). The Sorting Intolerant From Tolerant (SIFT) predicts the effect of a coding variant on protein function bases on sequence homology (Sim et al., 2012).

In this study we report a *de novo* likely pathogenic variant in *DYNC1H1* gene in a patient with a malformation of cortical development associated with cataract in infancy.

2. Patients and methods

2.1. Patient and ethic statement

We ascertained a non-consanguineous Syrian family residing in UAE with a single affected child who presented with severe neurological symptoms including motor and speech delay, intellectual disability, seizures, generalized hypotonia, microcephaly and cataract. This study has been approved by the Al-Ain Medical Human Research Ethics Committee according to national regulations (protocol number 10/09).

2.2. Whole-exome sequencing, in silico and segregation analysis

Peripheral blood samples were collected in EDTA tubes from the affected child who was one year old at that time. After obtaining informed written consent, blood samples were collected from parents for segregation analysis. Genomic DNA was extracted using the Flexigene DNA kit (Qiagen Gmbh, Germany) following the manufacturers' protocol. Whole exome sequencing for the affected child was performed as a service at the Medical Genetics Laboratories, Mayo Medical Genetics Laboratories, Houston, USA (www.bcmgeneticlabs.org). The annotated SNPs were mapped to dbSNP v131, v132 and 1000 genome databases. Moreover, selected variants were cross compared with the Ensembl database (www.ensembl.org) as well as the Exome variant server (http://gvs.gs.washington.edu/gvs/). Prediction analysis of the consequence of the identified variants was carried out using Mutation Taster (http://www.mutationtaster.org/), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT/Provean (http://sift.jcvi.org/). DNA Sanger sequencing was performed using an ABI 330xl Genetic Analyzer on samples of the affected child, his parents and non-affected siblings (Komara et al., 2015). Sanger sequencing results were analyzed based on NCBI reference sequence number NM_001367.

3. Results

3.1. Clinical features

The affected male child was born to non-consanguineous Syrian parents who also have five healthy older sisters (Fig. 1A). The family history was unremarkable except for congenital deafness in a paternal aunt and uncle as well as in two daughters of a maternal cousin. The pregnancy was complicated by maternal pyelonephritis in the 4th month of gestation. Fetal ultrasound in the 8th month showed agenesis of the corpus callosum but no other abnormalities were identified. Delivery was

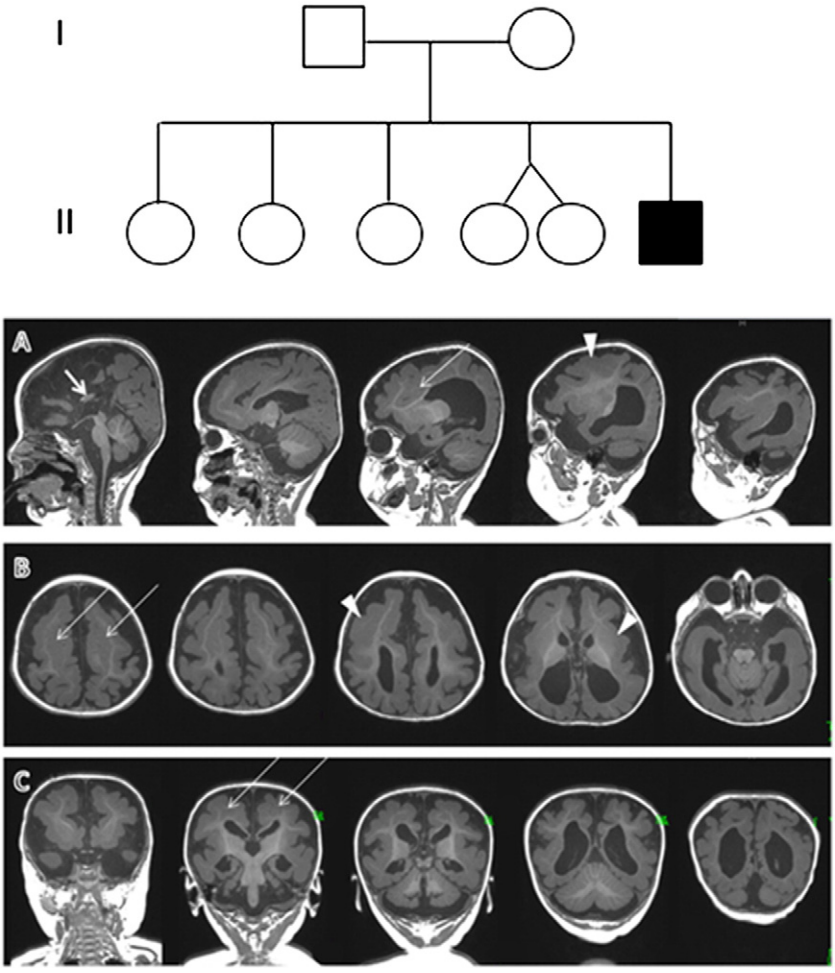

Fig. 1. a. Family pedigree. Black filled symbol represents the affected child. b. Brain MRI done at age 12 months: A sagittal, B axial and C coronal thin slice T1 weighted images. Flat and thickened cortical sulci are seen (arrow heads) bilaterally predominantly anteriorly. In addition bilateral symmetrical subcortical islands of gray matter heterotopia are seen in the frontoparietal region (arrows). The MRI also shows dysgenesis of the posterior part of the corpus callosum, with only the anterior part visible (thick arrow); as well as reduced volume of white matter, large CSF spaces and dilated ventricles indicating brain atrophy.

normal with a birth weight of 2.75 kg. However, at birth bilateral talipes equinovarus was noticed and this was corrected surgically at the age of 6 months with good results. At the age of 4 months the child was still unable to focus or follow. Bilateral cataracts were found and were operated on. After the operation the child continued to have very poor vision. However, a visual evoked potential (VEP) test was reported as normal. The child was also noted to have global developmental delay with poor head control, delay of social smile and cooing. The first documented head circumference at age of 12 days was 37 cm (P50–75 — CDC growth charts 2000). Subsequent head measurements were consistent with acquired microcephaly (41 cm at four months — P10–25, 43.5 cm at nine months — P5–10, and 44 cm at 12 months — below 3rd percentile). At the age of 6 months he had an episode of generalized tonic seizure lasting for about 10 min. On retrospect the child had episodes suggestive of seizures since the age of three months. They were characterized by rhythmical tongue movements and head turning from side to side lasting a few seconds. No treatment was started until he had further seizures at the age of 8 months. The seizures were consistent with flexor spasms. The EEG done at another facility showed an attenuated background of mixed theta and delta frequencies and semiperiodic spike and slow wave activity in the right temporal occipital region. Prednisolone was commenced as per UKISS protocol and resulted in partial improvement of spasms. Vigabatrin was added with further improvement of seizures. Progress EEG showed persistence of the background attenuation, but less frequent right posterior epileptic activity. The child was evaluated at our clinic at the age of 16 months. He continued to have seizures as well as global developmental delay. He had poor head control and was not rolling. He was smiling and cooing but not babbling. He was not fixing or following, had hypotonia and head lag, as well as reduced deep tendon reflexes.

Brain MRI at the age of 12 months showed poorly formed, flat and thickened cortical sulci compatible with lissenencephaly-pachygyria, in association with bilateral symmetric extensive gray matter heterotopia in the frontoparietal regions. In addition, it showed severe hypoplasia of the corpus callosum, widened CSF spaces and reduced volume of white matter (Fig. 1B). The following investigations were normal or negative: blood count, glucose, urea and electrolytes, magnesium, calcium, creatinine kinase, liver transaminases, plasma and urine amino acids and urine organic acids.

3.2. Whole exome sequencing revealed de novo missense variant in a conserved ATP-binding dynein motor region of DYNC1H1

Since the parents were phenotypically normal and come from a highly consanguineous population, we initially assumed a recessive mode of inheritance for the phenotype (Al-Gazali and Ali, 2010). However, whole exome sequencing failed to reveal any suspected variant for this mode of inheritance but it revealed a novel heterozygous missense variant (c.10973G>A) in exon 58 of *DYNC1H1* gene. This variant was confirmed by Sanger sequencing as heterozygous in the affected child but absent from both parents and the tested siblings. The variant causes a substitution of guanine by adenine at nucleotide position 10973 leading to amino acid change from glycine to glutamic acid (p.G3658E) at a highly conserved residue (Fig. 2) which indicates that Gly3658 is likely to be essential for DYNC1H1 structure and/or function.

3.3. In silico analysis of (c.10973G>A, p.G3658E) variant

In silico analysis of the identified variant using Mutation Taster indicated the variant to be disease causing. This result is based on the fact that the variant causes an amino acid change from a highly conserved glycine residue to glutamic acid which is physiochemically different from the wild type residue. Moreover, the variant was neither found in ExAC nor in the 1000 Genome database. PolyPhen2 predicted the variant to be damaging with a score if 1.00. Polyphen2 scores range from 0.00 to 1.00 based on the effect of amino acid substitution on stability and function of proteins. A score of 1.00 is the highest score indicating that a change at this position cannot be tolerated. Similarly, Provean and SIFT evaluation of the variant was deleterious (scores −7.36 and 0.00 respectively). Collectively, it can be suggested that c.10973G>A is likely pathogenic.

4. Discussion

Cytoplasmic dynein 1 is the primary ATP-hydrolyzing motor responsible for retrograde axonal transport in eukaryotic cells (Levy and Holzbaur, 2006; Schiavo et al., 2013). Mutations in the largest cytoplasmic dynein DYNC1H1 have been associated with a wide spectrum of neurodegenerative diseases in humans, due to neuronal migration defects, motor neuropathies, neuron degeneration, and locomotor abnormalities (Eschbach and Dupuis, 2011; Schiavo et al., 2013). The tail domain of DYNC1H1 is located in the N-terminus and is required for heavy chain dimerization whereas the motor domain is responsible for the ATP-binding activity (Scoto et al., 2015) (Fig. 2). In this study, we identified a novel mutation in the motor domain of DYCN1H1 in a child presenting with a severe neurological disorder in association with extensive malformations of cortical development and cataract. This variant is absent from both parents suggesting a *de novo* origin. Moreover, computational approach analysis suggested a deleterious effect on the substitution from glycine to glutamic acid at the highly conserved 3658 amino acid residue. Glycine is a unique amino acid in terms of structure and function. It contains hydrogen as a side chain unlike all other amino acids and could exert unique functional roles in protein conformational structure. All together a change at a highly

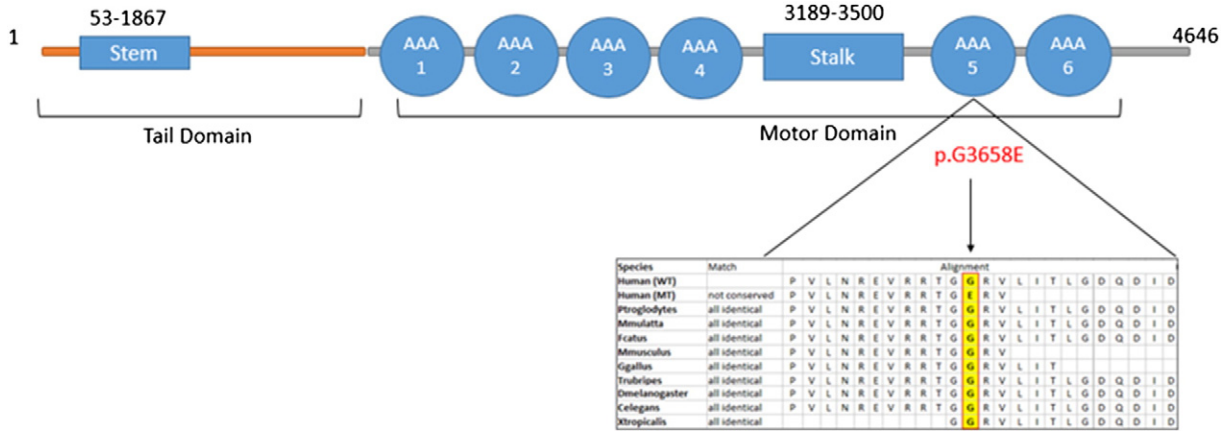

Fig. 2. Model representing the domains of the large DYNC1H1 protein. The N-terminal is known as the stem. The C terminal comprises 6 hepatometric rings of ATPase domains (AAA) and a microtubule-interacting stalk region (Pfister et al., 2006).The identified variant is located at the highly conserved 5th ATP-binding region of *DYNC1H1*. Scale indicates amino acid sequence.

conserved glycine residue in a protein will most likely cause functional/structural changes (Betts and Russell, 2007).

Previous reports of mutations in the motor domain of DYCN1H1 described malformation of the cortical development, including polymicrogyria (Fiorillo et al., 2014; Poirier et al., 2013; Scoto et al., 2015), focal cortical dysplasia (Willemsen et al., 2012) and focal pachygyria (Poirier et al., 2013). The cortical malformations seen in our patient are more extensive and diffuse than what have been previously reported, including diffuse pachygyria-lissencephaly and bilateral symmetric subcortical gray matter heterotopia. It is interesting that our case had cataract in infancy in addition to the severe malformations of cortical development which has not been reported in other documented cases in children with mutations in *DYNC1H1* (Cohen et al., 2013; Fiorillo et al., 2014; Harms et al., 2012; Tsurusaki et al., 2012; Weedon et al., 2011; Willemsen et al., 2012). However, recently Strickland et al. (2015) reported a mutation in the *DYNC1H1* gene in a family with hereditary spastic paraplegia associated with a thin corpus callosum which was complicated by mild ataxia of the extremities and bilateral cataract in adulthood which is different to our patient who has severe malformations of cortical development associated with cataract manifested within the first few months of life with the absence of spastic paraplegia.

In this study, whole exome sequencing identified a *de novo* mutation in a well-conserved residue in DYNC1H1 in a patient with severe malformation of cortical development associated with cataract. The exact mechanism by which the substitution affects protein structure or function is yet to be determined, however, the highly conserved protein region across species is predicted to be untolerated. We therefore concluded that the c.10973G>A variant is likely the cause of malformation of cortical development associated with cataract in our patient.

Conflict of interest

All authors declare no conflict of interest.

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