DIHYDROPYRIDINE RECEPTOR CONGENITAL MYOPATHY IN A CONSANGINEOUS TURKISH FAMILY

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Abstract

Dihydropyridine receptor congenital myopathy is a recently described congenital myopathy. To date, only 11 patients from 7 families were described. Here, we describe a consanguineous family with three affected children, presenting congenital hypotonia, contractures, ophtalmoplegia and respiratory insufficiency, with a novel homozygous mutation in CACNA1S gene in whole exome sequencing. Cognitive delay, pes equinovarus deformity and neurogenic changes has not been associated with this myopathy in previous report. This report expends the phenotypic spectrum of dihydropyridine receptor congenital myopathy and underscores the importance of whole exome sequencing in early onset neuromuscular disorders.

Introduction

The congenital myopathies are a group of genetic muscle disorders, which are characterized on the basis of morphological features seen on muscle biopsy. There are early and late onset forms. The clinical course is static or slowly progressive. Many of the congenital myopathies can be caused mutations in more than one gene or mutations in the same gene can cause diverse muscle pathologies. On the other hand, same mutation in a family can lead to different pathologic features.[1] Increasing use of exome and whole genome sequencing has led to identification of new congenital myopathy genes, but there are still many to be discovered.

Excitation-contraction coupling occurs at the triad. There is a specialized membrane structure, T-tubule and two sarcoplasmic reticulum saccules containing the ryanodine receptors. Dihydropyridine receptors are located on T-tubules. Activation of dihydropyridine receptors induce the opening of ryanodine receptors and release of Ca^{+2} from sarcoplasmic reticulum stores, which leads to muscle contraction.[2] *CACNA1S* gene encodes the pore-forming subunit of dihydropyridine receptor in the skeletal muscle. Heterozygous dominantly acting *CACNA1S* mutations have been associated with malignant hyperthermia susceptibility, hypokalemic periodic paralysis and thyrotoxic periodic paralysis.[3-5] Recently, both recessive and dominant *CACNA1S* mutations as a cause of congenital myopathy has been identified.[6]

Here, we describe a consanguineous Turkish family whose three children presented with hypotonia, muscle weakness, respiratory distress, swallowing dysfunction, ophtalmoplegia and pes equinus deformity. Two of the siblings died at three months of age due to respiratory insufficiency. Whole exome sequencing showed a homozygous variant in *CACNA1S* in the surviving patient. There is only one previous report describing a cohort of 11 patients from 7 families. The cases described in our report expands the spectrum of *CACNA1S* related congenital myopathy and show the importance whole exome sequencing in diseases associated with consanguineous marriages.

Clinical Report

The probands are Turkish descent and were offsprings of consanguineous parents (third cousin). In addition to the three probands, the couple had one healthy 10-year-old girl and one spontaneous pregnancy loss.

Proband 1 was a male infant born at 38 weeks gestation via cesarean section. Anthropometric measurements were not available. He was taken to neonatal intensive care unit because of

respiratory insufficiency on the first day of life. He had severe hypotonia, absent suck, muscle weakness, ophtalmoplegia and pes equinus deformity. In histopathological evaluation of the muscle biopsy of the proband 1, mild dystrophic changes like contraction, regeneration, degeneration, nuclear internalization, and fibrosis were visible. In addition, many pathological immature myofibers were visualized using the neonatal myosin staining. Based on immunostaining, dystrophin, merosin and sarcoglycans were present at normal levels. Interestingly there were several huge type 1 fibers which are specific for infantile denervation. He died at three months of age due to respiratory comprise.

Proband 2 was a female infant born at 38 weeks gestation via cesarean section. The mother noted decreased fetal movements during pregnancy. Prenatal ultrasonography showed ventricular enlargement and pes equinus deformity. She was not breathing spontaneously and was taken to neonatal intensive care unit. She had severe hypotonia, absent deep tendon reflexes, absent suck and Moro reflex and pes equinus deformity. She stayed in neonatal intensive care unit for five months and was discharged with tracheostomy and percutaneous gastric feeding tube. Metabolic investigations, serum creatine kinase, chromosome analysis, SMN and IGHMBP2 gene analyses were normal. Electromyography showed myopathic changes in all examined muscles and nerve conduction velocities were normal. Cardiac examination was normal. Brain magnetic resonance imaging showed mild ventricular enlargement with thin corpus callosum. In histopathological evaluation of the muscle biopsy of Proband 2, there was a marked variation in fiber size and shape. There was also increasing of nuclear internalization. There were no myofibrillary irregularity with modified trichrome or NADH-TR enzyme staining. Based on immunostaining, dystrophin, merosin and sarcoglycans were present at normal levels. There were also grouping fascicules of large and small myofiber which are specific for neuropathies (Figure 1). Last examination at four years of

age showed severe motor delay with generalized and axial weakness. She was bedridden. She had mild facial involvement with high arched palate and total ophtalmoplegia. She also had scoliosis and pes equinus deformity. She was fed by percutaneous gastrostomy. She was mechanical ventilator dependent all day (Figure 2). She also had moderate cognitive delay.

Proband 3 was also a female infant born at 38 weeks gestation via cesarean section. She was also taken to neonatal intensive care unit because of respiratory insufficiency on the first day of life. She had severe hypotonia, absent suck, muscle weakness, ophtalmoplegia and pes equinus deformity. She died at three months of age due to respiratory comprise.

Material and Methods

Clinical studies

Probands 2 and 3, and parents were enrolled in research protocol for diseases related to consanguineous marriages that was approved by Dokuz Eylül University, School of Medicine Institutional Review Board. Informed consent was obtained from both parents prior to participation including for whole exome sequencing and the publication of medical information.

Whole exome sequencing and Sanger Sequencing

Whole exome sequencing (WES) was performed on DNA obtained from proband 2 and 3, and both parents, at the Broad Institute of MIT and Harvard, using Illumina Exome Capture Kit (38 Mb target). Sequencing data was processed at the Centro Nacional de Análisis Genómico (CNAG), Barcelona, and data analysis carried out on the RD-Connect Genome-Phenome Analysis Platform (https://platform.rd-connect.eu/genomics) using standard filtering criteria for rare diseases, including Minor Allele Frequency (MAF) <0.01, Variant Effect Predictor (VEP)=mod/high and Combined Annotation Dependent Depletion (CADD) >20.

Results

Whole exome sequencing (WES) was performed using DNA from proband 2 and 3 and revealed a novel homozygous missense variant in the CACNA1S gene (c.2366G>A) that causes substitution of a highly conserved (GERP=3.91) Arginine by Histidine (p.Arg789His). Both parents were heterozygous for this variant. WES results and segregation of the CACNA1S variant with disease were confirmed by Sanger sequencing (Figure 3a). All bioinformatic prediction tools tested (Poylphen, SIFT, MutationTaster) classified the variant as pathogenic/damaging and the CADD score is 35. The variant was not observed in any of the 150,524 gnomAD exomes and was observed in heterozygous state in only one out of 30,934 gnomAD exomes (frequency=0.00003233). Moreover, none of the 1182 Turkish exomes (TUBITAK-BILGEM) contained the variant. No DNA was available for other affected siblings with similar clinical findings.

Discussion

Advances in genetic technologies led to the identification of new congenital myopathy genes, most of which are associated with sarcomere structure and its stability. Despite advances in genetic area, about half of the patients with congenital myopathy remain without a genetic diagnosis. Very recently, 11 congenital myopathy patients from 7 families have been described with *CACNA1S* gene. Mutations were recessive, dominant and de novo. All patients in this cohort presented with early onset hypotonia, progressive muscle weakness with prominent axial involvement. All of them had mild facial involvement and four had ophtalmoplegia. There were mild to severe respiratory and swallowing problems in all patients. There was no cardiac involvement and only two patients had elevated serum creatine kinase values. Six patients had scoliosis. All of the patients were surviving and age ranged from 8 to 60 years.[6] The clinical course in our patients were severe and only proband 2 was alive. They had severe respiratory and swallowing problems and two of them

deceased in the third month of life because of respiratory insufficiency. The surviving patient was dependent on mechanical ventilator all day and fed by percutaneous gastric tube. They all had ophtalmoplegia. The surviving patient also had scoliosis and lack of neck control showing the involvement of axial muscles. One striking finding was congenital pes equinus deformity that was not reported in other patients. The surviving patient also had moderate cognitive delay and ventricular enlargement with thin corpus callosum on brain magnetic resonance imaging. The cognitive status of other reported patients were not described. Cognitive delay and brain magnetic resonance imaging findings may be a feature of severe early onset cases but also may be due to prolonged neonatal intensive care stay, high oxygen exposure and episodes of respiratory comprise.

In a recent case series, histopathologic analyses of muscle biopsies showed an alveolar aspect of the intermyofibrillar network on NADH-TR staining, centralized nuclei, fiber size variability, core-like features, uniformity of type 1 and a dystrophic process. Ultrastructure of muscles on electron microscopy showed dilated T-tubules and sarcoplasmic reticulum and focal zones of myofibrillar disorganization. We could only evaluate the muscle biopsies of two siblings. Histopathological evaluation of the muscle biopsies revealed mild dystrophic and/ or myopathic changes, fiber size variability, nuclear internalization and fibrosis. In addition, many pathological immature myofibres were seen using the neonatal myosin staining. Immunohistochemically, common structural proteins of muscle cell showed normal expression patterns and levels. All these changes except the myofibrillary disorganization and vacuolization were similar to those previously reported.[6] Interestingly, there was also grouping of large and small size fibers, which is hallmark for denervation with reinnervation seen in neuropathies and spinal muscular atrophy.

Arginine residues in the S4 transmembrane helix of voltage-gated ion channels play critical roles in voltage sensing .[7] Indeed, most of the mutations identified so far in hypokalemic periodic

paralysis 1 (HOKPP1) affect arginine residues within S4 voltage-sensing regions of $Ca_v1.1$, encoded by the *CACNA1S* gene, and most of them are arginine-to-histidine substitutions, just like the R789H variant that we identified in this study.[8,9] However, localization of Arg-789 to the cytoplasmic loop II-III of $Ca_v1.1$ points to a different role for this residue than in voltage-sensing. The II-III loop of $Ca_v1.1$ is critical for transmitting the excitation-contraction coupling to Ca^{2+} release by the gating of Ryanodine receptor (RyR1). This process is mediated by interaction of the loop II-III of $Ca_v1.1$ with the SH3-domain(s) of STAC3, STAC2 and STAC1 .[10-12] Recent studies identified residues 720-765, particularly 750-756, of $Ca_v1.1$ to be critical for binding to STAC3.[10,11] Localization of $Ca_v1.1$ to the cell membrane in muscle and neuronal cells depends on its interaction with Stac proteins, particularly with Stac3, and loss of these interactions lead to strongly reduced channel activity and perturbation of skeletal muscle excitation-contraction coupling .[13] Interaction of SH3-domain proteins with their ligands is mediated by proline-rich sequences flanked by an arginine on the ligand .[14] Yuen et al. identified Arg-757 of $Ca_v1.1$ as a critical residue that makes multiple interactions via hydrogen bonding with the first SH3 domain of Stac2 .[11] Although the pathologic variant R789H is not located exactly within the critical Stac3 interacting region, it sits in a highly conserved region (Figure 3b) that is 25-30 amino acids apart from the critical region.[15] Moreover, this conserved region might possibly modulate the $Ca_v1.1$-Stac3 interaction .[10]

Severity of the phenotype observed in the 3 siblings presented here points to a critical role for the Arg-789 residue, and possibly the flanking region, in $Ca_v1.1$ functioning. Therefore, we propose that a possible molecular etiopathology for the cases presented here might be perturbation of $Ca_v1.1$-Stac3 interaction, resulting in reduced channel activity and disruption of the excitation-contraction coupling. Interestingly, in two different families reported by Schartner et al., the same residue Proline-742, within the critical binding region to Stac3, was mutated to either glutamine or

serine, with significant differences in the onset, severity and the range of tissues affected .[6] Further studies are required to provide mechanistic explanations to the observed discrepancies and to understand why R789H mutation causes such a severe phenotype.

In summary, the clinical course of *CACNA1S* congenital myopathy may be so severe and patients may be lost in the first months of life because of respiratory comprise. Patients may also have cognitive delay and congenital pes equinus deformity. There may be neurogenic changes in muscle biopsy in addition to myopathic findings. Since clinical and histologic features of *CACNA1S* myopathy are diverse, this gene should be included in early NGS panels of onset early onset neuromuscular disorders.

References

1. North KN, Wang CH, Clarke N, Jungbluth H, Vainzof M, Dowling JJ, et al. Approach to the diagnosis of congenital myopathies. Neuromuscul Disord. 2014;24(2):97-116.

2. Rebbeck RT, Karunasekara Y, Board PG, Beard NA, Casarotto MG, Dulhunty AF (2014) Skeletal muscle excitation–contraction coupling: who are the dancing partners? Int J Biochem Cell Biol 48:28–38.

3. Monnier N, Procaccio V, Stieglitz P, Lunardi J (1997) Malignant-hyperthermia susceptibility is associated with a mutation of the alpha 1-subunit of the human dihydropyridine-sensitive L-type voltage-dependent calcium-channel receptor in skeletal muscle. Am J Hum Genet 60:1316–1325

4. Jurkat-Rott K, Lehmann-Horn F, Elbaz A, Heine R, Gregg RG, Hogan K, Powers PA, Lapie P, Vale-Santos JE, Weissenbach J et al (1994) A calcium channel mutation causing hypokalemic periodic paralysis. Hum Mol Genet 3:1415–1419

5. Kung AW, Lau KS, Fong GC, Chan V (2004) Association of novel single nucleotide polymorphisms in the calcium channel alpha 1 subunit gene (Ca(v)1.1) and thyrotoxic periodic paralysis. J Clin Endocrinol Metab 89:1340–1345.

6. Schartner V, Romero NB, Donkervoort S, Treves S, Munot P, Pierson TM, et al. Dihydropyridine receptor (DHPR, CACNA1S) congenital myopathy. Acta Neuropathol. 2017;133(4):517-533.

7. Catterall WA. Ion channel voltage sensors: structure, function, and pathophysiology. Neuron. 2010 Sep 23;67(6):915-28.

8. Matthews E, Labrum R, Sweeney MG, Sud R, Haworth A, Chinnery PF, Meola G, Schorge S, Kullmann DM, Davis MB, Hanna MG. Voltage sensor charge loss accounts for most cases of hypokalemic periodic paralysis. Neurology 2009 72:1544-1547
9. Beam TA, Loudermilk EF, Kisor DF. Pharmacogenetics and pathophysiology of CACNA1S mutations in malignant hyperthermia.Physiol Genomics. 2017 Feb 1;49(2):81-87.
10. Polster A, Nelson BR, Papadopoulos S, Olson EN, Beam KG. Stac proteins associate with the critical domain for excitation-contraction coupling in the II-III loop of CaV1.1. J Gen Physiol. 2018 Apr 2;150(4):613-62.
11. Wong King Yuen SM, Campiglio M, Tung CC, Flucher BE, Van Petegem F. Structural insights into binding of STAC proteins to voltage-gated calcium channels. Proc Natl Acad Sci U S A. 2017 Nov 7;114(45):E9520-E9528.
12. Campiglio M, Flucher BE. STAC3 stably interacts through its C1 domain with CaV1.1 in skeletal muscle triads. Sci Rep. 2017 Jan 23;7:41003.
13. Polster A., Perni S., Bichraoui H., Beam K. G. Stac adaptor proteins regulate tra cking and function of muscle and neuronal L-type Ca2+ channels. Proc. Natl. Acad. Sci. USA 2015 112, 602–606
14. Feng S, Kasahara C, Rickles RJ, Schreiber SL. Specific interactions outside the proline-rich core of two classes of Src homology 3 ligands. Proc Natl Acad Sci U S A. 1995 Dec 19;92(26):12408-15.
15. Bannister RA, Beam KG. Ca(V)1.1: The atypical prototypical voltage-gated Ca^{2+} channel. Biochim Biophys Acta. 2013 Jul;1828(7):1587-97.

Figure Legends

Figure 1: Note the marked variation in fiber size and shape (HE x 200), B. There are no marked myofibrillary irregularity (NADH-TR x 200), C. Normal sarcolemmal expression of merosin as well as grouping of large and small myofiber fascicles (DAB x 200), D. Note the presence of huge type 1 fibers with fast myosin antibody (DAB x 100).

Figure 2: A-B: proband 2 with tracheostomy and nasogastric feeding. She is bedridden and no anti-gravity movements are avaliable. She also has congenital onset pes equinus deformity. C: proband 3 has severe respiratory comprise. She also has congenital onset pes equinus deformity.

Figure 3: a) Pedigree and Sanger sequencing validation of WES results for CACNA1S c.2366G>A mutation. Segregation of the mutation is indicated by the number of red dots: 1 dot indicates carriers, 2 dots indicates homozygotes for this mutation. b) ClustalOmega multiple protein sequence alignment of the regions flanking the "critical region", which is required for binding to Stac3, of human CACNA1S, human CACNA1C, human CACNA1D, mouse CACNA1S and zebrafish CACNA1Sb. Note that the region C-terminal to the *critical region*, that contains Arg-789, is highly conserved. Darker colors indicate higher level of consensus between sequences. Lower panel indicates the level of consensus as a bar-graph.