A Deletion of SGCE Gene in a Chinese Family with Myoclonus-Dystonia Syndrome

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Abstract

Background: Myoclonus-dystonia syndrome is a rare neurological disorder characterized by a combination of myoclonic jerks and dystonia. To investigate the causative factor in a big family with myoclonus-dystonia syndrome, we performed whole-exome sequencing and Sanger sequencing for affected family members.

Methods and Results: The proband and her affected family members manifested typical neurological symptoms of myoclonus alone or with dystonia. The proband did not respond to antiepileptic and other symptomatic medications. We identified a heterozygous single-nucleotide deletion of SGCE gene, c.360delT (p.E121Kfs*11), in the proband. Affected members in this family are heterozygous carriers of this variant. The patient’s father and his two brothers are carriers but asymptomatic. It is consistent with the autosomal dominant pattern of inheritance with maternal imprinting which caused incomplete penetrance of SGCE-associated myoclonus-dystonia syndrome.

Conclusion: This study reports a 4-generation family with myoclonus-dystonia syndrome and expands the phenotypic spectrum of variant c.360delT associated disease.

Abbreviations

ACMG: American College of Medical Genetics and Genomics guidelines; EEG: Electroencephalography; EMG: Electromyogram; HGMD: Human Gene Mutation Database; MDS: Myoclonus-Dystonia Syndrome; MRI: Magnetic Resonance Imaging; PMA: Progressive Myoclonus Ataxia; SGCE: ε-Sarcoglycan; WES: Whole-Exome Sequencing

Introduction

Myoclonus-Dystonia Syndrome (MDS), also called Dystonia-11 (DYT11) and SGCE myoclonus-dystonia (SGCE-M-D) is a rare neurological disorder characterized by a combination of myoclonic jerks, typically affecting neck, trunk and upper limbs, and twisting and repetitive movements resulting in abnormal postures (dystonia). Approximately 50% patients present with writer’s cramp and cervical dystonia [1]. This disease is a genetically heterogeneous movement disorder with an autosomal dominant pattern of inheritance [2]. The ε-Sarcoglycan (SGCE) gene (Dystonia-11, myoclonic; OMIM 604149), located in the chromosome region 7q21.3, is associated with familial MDS [3]. SGCE gene encodes epsilon-sarcoglycan, a single pass transmembrane proteins that are part of the dystrophin-glycoprotein complex, which contribute to mediating the stability of the plasma membrane. According to Human Gene Mutation Database (HGMD), there are 133 mutations in SGCE gene reported to be associated with myoclonus dystonia or similar symptoms, including 131 confirmed disease-causing mutations [4]. However, no genotype-phenotype correlations have been identified [1]. Herein we report clinical information and genetic data on a 4-generation Chinese family of MDS caused by a deletion, c.360delT (p.E121Kfs*11), in SGCE gene.

Materials and Methods

Clinical data collection

Clinical information of the proband were collected from medical records and of her family members were recorded through personal follow-up visits. The written consent forms were obtained with the informed consent of all participants.
Further examinations

Physical examination was to check the patient’s motor function. Laboratorial assessments were also performed, including complete blood count, urinalysis, stool test, basic metabolic panel, blood lactate and ammonia. Electromyogram (EMG), brain Magnetic Resonance Imaging (MRI) and Electroencephalography (EEG) were also applied.

Whole-exome sequencing

Whole-exome sequencing was performed by Running Gene Inc. (Beijing, China) following the manufacturer’s protocol. DNA was isolated from peripheral blood using a blood DNA Isolation Kit (#CW2553, Cwbio, Taizhou, China). Genomic DNA sample was shared by sonication. The sheared genomic DNA fragments were processed by end-repairing, A-tailing, adaptor ligation and a 4-cycle pre-capture PCR amplification. The amplified products were then captured by IDT and xGen Lockdown Probes (Integrated DNA Technologies, Coralville, IA) to enrich the exonic DNA. The libraries were first tested for enrichment by qPCR and for size distribution and concentration using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA). Two parallel reactions were performed for each sample. Exon-enriched DNA was sequenced by the Illumina Novaseq platform following the manufacturer’s instructions. Raw image files were processed by the Illumina Sequence Control Software (Illumina) for base calling and generating the raw data. The low-quality variations were filtered out based on an in-house algorithm. The high-quality sequencing reads were aligned to the human reference genome sequence (GRCh37/hg19) using Burrows-Wheeler Alignment tool [5]. The Single-Nucleotide Polymorphisms (SNPs) and insertions and deletions (indels) were called using the GATK software [6]. SNPs and indels with low-quality were filtered out. For candidate variants, all were annotated using public databases (1k Genomes Project [7], ExAC [8], gnomAD [9], ESP6500 [10], Ensembl [11], HGMD etc.). The pathogenicity of variants will be generated based on American College of Medical Genetics and Genomics (ACMG) guidelines [12]. Sanger sequencing was then used to verify the variant and analyze their genetic origin (SGCE-forward: TAGGCGAGATTAGTAATGATCCCA; SGCE-reverse: CTITTATAAACAGAGAAGATGG-CAC). PCR products were sequenced by ABI 3730 (Applied Biosystems, Carlsbad, CA) and analyzed by Chromas Lite v2.01 (Technelysium Pty Ltd., QLD, Australia).

Results

Clinical presentation

The proband (IV-31) was a 12-year-old girl and her parents (III-42 and III-43) are non-consanguineous. The pregnancy was unremarkable and the proband was delivered by caesarean section at gestational 39th week. She developed normally in infancy. Since the age of 2, she began to exhibit exaggerated startle response with or without acoustic and/or tactile stimuli during awake, and her motor development dramatically delayed compared with peers. She could not walk independently because an unexpected startle during walk could make her fall down. During a startle attack, her arms became fully extended and her whole body involuntarily tightened. Each episode lasted for a few seconds, during which she was fully conscious. The frequency of attacks is more than a hundred per day and also depended on the environment around her. All symptoms

Figure 1: Pedigree analysis and sequencing of the 4-generation family. (A) The proband (IV-31), her father (III-42), brother (IV-30), grandmother (II-11), two uncles (III-38 and III-40) and two cousins (IV-27 and IV-28) carry a heterozygous variant of SGCE gene, c.360delT (p.E121Kfs*11). Her mother (III-43) and grandfather (II-10) are wild-type at this site. MDS-affected members are labelled in black. (B) Binary alignment map of whole-exome sequencing shows heterozygous c.360delT. (C) Chromatograms from Sanger sequencing show wild-type and c.360delT (p.E121Kfs*11) of SGCE gene in family members.
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4th generation, torticollis became not obvious. The proband's mother Symptoms in generation III manifested as slight torticollis. In the in generation II presented with both myoclonus and torticollis. or dystonic symptoms to varying degrees. Affected family members in generation II presented with both myoclonus and torticollis. Symptoms in generation III manifested as slight torticollis. In the 4th generation, torticollis became not obvious. The proband's mother (III-43), two uncles (III-38 and III-40) and grandfather (II-10) were asymptomatic. The proband's brother (IV-30) reported a reduction in myoclonus in response to alcohol ingestion. Therefore, this family is fully compliant with the diagnosis of MDS.

Physical and biochemical examinations

The proband (IV-31) was admitted to our hospital for further examinations. On admission, she was conscious with normal facial features and without horizontal nystagmus. The muscle strength of his upper and lower extremities and muscle tone were all normal. Tendon reflexes were active on all extremities and bilateral pathological reflexes were negative. Pain sensation was present symmetrically. Romberg's sign was negative. No abnormal signs were present in his heart, lungs and abdomen. The results of laboratorial assessments were within normal limits and unremarkable. The result of Wechsler Intelligence Scale for Children [13] was normal. No abnormal findings were observed in the results of EMG, brain MRI and EEG.

Genetic analysis

Whole-exome sequencing identified a heterozygous deletion, c.360delT (p.E121Kfs*11), in exon 3 of SGCE gene (NM_003919, chr7:94257544) in the proband (Figure 1B & Table 1). This deletion will lead to mRNA degradation by nonsense-mediated mRNA decay or frame shift causing premature termination of the peptide chain from 462 amino acids to 130 (PVS1). This variant is absent from public databases (PM2). The deletion also co-segregates with disease in multiple affected family members (PP1). Multiple in silico algorithms predicted it deleterious (Mutation Taster 2 [14], 1, disease causing; SIFT_indels [15], damaging, confidence score =0.858; FATHMM_indel [16], score =0.988; cutoff =0.5, pathogenic; CADD [17], 26.8-15, deleterious; CAPICE [18], 0.986<0.02, pathogenic) (PP3). The proband's phenotypes and family histories are highly specific for MDS (PP4). According to HGMD database, this deletion has been reported as a disease-causing mutation in a family with Progressive Myoclonus Ataxia (PMA) but without laboratorial evaluation (PP5) [19]. Thus, this variant is classified as 'pathogenic', based on ACMG guidelines. Sanger sequencing verified this pathogenic variant was also carried by her father (III-42), brother (IV-30), grandmother (II-11), two uncles (III-38 and III-40) and two cousins (IV-27 and IV-28) (Figure 1C). The proband's mother (III-43) and grandfather (II-10) are wild-type at this site. The disease in this family was inherited as an autosomal dominant pattern with maternal imprinting.

Table 1: Variant table.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>HGVS (NM_003919, NP_003910)</th>
<th>Variant type</th>
<th>Predicted effect</th>
<th>dbSNP/ClinVar ID</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGCE</td>
<td>7:94257544</td>
<td>c.360delT, p.E121Kfs*11</td>
<td>Deletion</td>
<td>Frame shift</td>
<td>NA</td>
<td>Heterozygous</td>
</tr>
</tbody>
</table>

Discussion

MDS is a rare autosomal dominant neurological disorder. The onset age is usually less than 20 years old and with an estimated prevalence of 1-9/1000000 in European population [20]. It manifests as myoclonus and dystonia. Among them, myoclonic jerks mainly occur in the upper limbs and trunk, with a few involving the lower limbs. The myoclonus is not under conscious control and often occurs when stimulated by sound. Other factors eliciting the movement include stress, sudden noise, tactile stimuli and caffeine. Focal or segmental dystonia manifests as writing spasm and/or torticollis and dystonia in the lower limbs is rare. Due to early onset and frequent myoclonic seizures, the normal life of patients is severely disrupted and they are often accompanied by many mental disorders, such as anxiety, depression and compulsive behaviors [21]. In addition, patients are often addicted to alcohol since most patients reported alcohol could alleviate myoclonic symptom dramatically [22-24]. However, clinical manifestations of different patients were various. Myoclonic symptoms are prominent and only some patients present dystonia. Some of manifestations are too mild to be noticed. Usually, patient's intelligence is not affected and the results of neurological examinations are normal. No specific abnormality shows in cranial MRI, and EEG findings are not consistent with corticogenic myoclonus. Thus, MDS is often misdiagnosed as Tourette syndrome, epilepsy, or psychogenic non-epileptic seizure. The diagnosis of MDS is usually based on both clinical manifestations and genetic background of patients. Mutations SGCE gene is the causative factor of MDS [25]. Pedigree analysis showed that different origin of the pathogenic allele would lead to a remarkable difference in penetrance, indicating the maternal imprinting mechanism of MDS. Although pathogenic alleles with either maternal or paternal origin can be inherited to offspring, only paternal alleles can be expressed and most of maternal alleles will be silenced by DNA methylation [2,25]. Approximately 5% of patients inherited disease alleles with maternal origin but presented milder symptoms. The reason for loss of suppression is unknown. A heterozygous deletion, c.360delT (p.E121Kfs*11), of SGCE gene was first identified in the proband (IV-31) and verified in other family members. Variant c.360delT was considered as a causative variant of MDS. Although 126 disease-causing mutations of SGCE gene have been reported to be associated with myoclonus dystonia, variant c.360delT has been reported in a family with Progressive Myoclonus Ataxia (PMA), also referred to as dyssynergia cerebellaris myoclonica rather than MDS [19]. In the current family, our patient (IV-31) presented involuntary body tremors in upper limbs and trunk and frequent falls, which result in inability to walk. Other family members presented with mild myoclonus and/or torticollis and symptoms could be alleviated after alcohol ingestion. In the PMA family, the proband suffered from dysmetria and ataxia gait, which did not present in MDS family, and frequent falls and limb myoclonus, which also showed in the present family. Similarly, both patients had normal intelligence and showed normal results in neurological examinations, MRI and EEG. They both did not respond to symptomatic treatments but PMA patient experienced progressive worsening of symptoms. Both families showed an autosomal dominant pattern of inheritance with maternal imprinting. It is intriguing to note that the identical variant may cause two different, but somewhat similar, diseases. Personal
genetic background, lifestyle or environmental factor may cause heterogeneity of phenotypes. We also suppose that the inconsistent symptoms, dysmetria and ataxia, were probably caused by other gene(s), which they missed due to lack of whole-exome sequencing (only a SGCE gene fragment (from intron 5 to intron 7) was sequenced), irrelevant to c.360delT in SGCE. This hypothesis could also explain why the phenotypes of this PMA family are unique (in 133) among SGCE mutations in HGMD. However, the correlation between phenotypes and variants should be explored and analyzed with more cases. Currently, no drugs are specifically designed for MDS. Antiepileptic drugs have been reported in individual cases and clinical series with varying responses. A randomized, double-blind, placebo-controlled crossover study showed that zonisamide was the only drug with level 1 evidence of effectiveness in both myoclonus and dystonia [26]. Other antiepileptic including valproate, levetiracetam, clonazepam, gabapentin and topiramate have also been trialed in patients with MDS [27-29]. Only valproate has been reported to be effective in reducing the occurrence of myoclonus in some patients [30]. However, levetiracetam has limited efficacy and gabapentin caused a severe worsening the conditions [31,32]. Other than antiepileptic medications, benzodiazepines, anticholinergic agents, dopaminergic agents, serotoninergic agents have been administrated to treat myoclonus and/or dystonia but also with various responses [33-38]. Except for medications, surgical treatment, such as deep brain stimulation, has also been reported as an effective and safe treatment for MDS when medications failed [37,39]. In this case, the patient failed in all medication attempts, bilateral implantation of deep brain stimulation could be an opportunity to improve myoclonus and dystonia. To summarize, MDS is a neurological movement disorder which mainly manifested as myoclonus and dystonia. It is genetically characterized by an autosomal dominant pattern of inheritance with maternal imprinting. In this study, we reported a 4-generation MDS family who carried c.360delT deletion in SGCE gene. Affected members presented with myoclonic jerks, involuntary body tremors, frequent falls and torticollis with varying degrees, and alcohol ingestion could help to alleviate symptoms in some patients. However, patients responded to antiepileptic and other symptomatic drugs in varying outcomes. We hope that medications specific for MDS will be developed sooner rather than later.

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