Short Report

Myoclonus-dystonia and Silver–Russell syndrome resulting from maternal uniparental disomy of chromosome 7

Myoclonus-dystonia (M-D; OMIM #159900) is a movement disorder characterized by bilateral, alcohol-sensitive myoclonic jerks and dystonia typically diagnosed in the first 2 decades of life (1). The age of onset can range from 4 months to 75 years (2, 3). The M-D phenotype may include non-motor symptoms such as depression, obsessive compulsive disorder and panic attacks (4). It is associated with mutations in the epsilon-sarcoglycan gene (SGCE), located at 7q21.3 (5). SGCE is widely expressed with the highest levels in lung, heart, brain, and skeletal muscle (6). It is a component of the sarcoglycan complex in muscle, but a specific role for SGCE in the brain has not yet been described (7). SGCE is maternally imprinted and preferentially expressed from the paternal allele (8, 9). The inheritance pattern of M-D is autosomal dominant with variable penetrance dependent on the parent of origin of the mutation. Penetrance is nearly complete in paternal transmission and very rare in maternal transmission (10). Previously reported M-D-associated
mutations are predicted to cause loss of SGCE function (11). Mutations affecting the methylation status of SGCE have not been reported (1).

Silver–Russell syndrome (SRS; OMIM #180860) is characterized by intrauterine growth restriction and postnatal growth deficiency with proportionate short stature and normal head circumference (12). SRS is genetically heterogeneous with 30–60% of cases associated with hypomethylation of the paternal allele of imprinting center region (ICR1) at 11p15.5 (13), whereas approximately 5–10% of cases are due to maternal uniparental disomy of chromosome 7 (mUPD7) (12). Patients with mUPD7 should not express SGCE as they lack the paternal unmethylated SGCE allele. This genotype is consistent with an M-D phenotype, but abnormal movements are not part of the classically described SRS phenotype. There are, however, three previous reports of M-D-like movement disorders in five patients with SRS due to mUPD7 (14–16). We report the sixth patient with SRS and M-D due to mUPD7.

Methods

SNP array

Blood samples were obtained from the proband, his brother, and parents with informed consent following the guidelines of the Institutional Review Board at the Johns Hopkins School of Medicine. Genomic DNA was extracted using the QIAamp kit (Qiagen, Valencia, CA) and single nucleotide polymorphism (SNP) array analysis was performed using the Illumina Human Omni1-Quad (1,000,000 markers) and the Illumina CytoSNP12 (300,000 markers) (Illumina, San Diego, CA). Allele ratios and signal intensity were analyzed with the CNV Partition 2.4.4.0 algorithm in KARYSO STUDIO (v.1.4.3.0) and GENOME STUDIO (v.2010.3) (Illumina). Genotypes of SNPs on chromosome 7 from the proband and his parents were manually compared to determine the parent of origin of chromosome 7. Genomic positions are based on the February 2009 Human Genome Build (hg19).

Karyotype and fluorescence in situ hybridization

A high-resolution karyotyping was performed using phytohemagglutinin-stimulated lymphocytes following standard G-banding protocols. Fluorescence in situ hybridization (FISH) was performed on interphase nuclei using two commercially available probes on chromosome 7 (LSI D7S522.7q31 SpectrumOrange/CEP 7 SpectrumGreen Probe; Abbott Molecular, Des Plaines, IL).

SGCE methylation

DNA samples were treated with sodium bisulfite using the QiagenEpiTect Bisulfite Kit (Qiagen). A 197-nucleotide region in the SGCE promoter was amplified from 2 ng of bisulfite-converted DNA using the

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PyroMark PCR Kit (Qiagen) and a commercially available biotinylated SGCE PCR primer pair (PyroMarkCpG Assay PM00030205; Qiagen) according to the manufacturer’s specifications. The resulting non-biotinylated strand was sequenced on a Pyromark Q24 system (Qiagen).

Case report

The proband is a 24-year-old man with short stature evaluated for jerking of the arms and shoulders with unknown etiology. He was born at 42 weeks gestation and was small for gestational age at 2.3 kg (<3rd percentile). His adult height (151.9 cm) and weight (52.7 kg) are both below the fifth percentile and he is significantly shorter than his parents (midparental percentile: 25–50th percentile). The proband was macrocephalic (56 cm, >90th percentile). Dysmorphic features included mild frontal bossing, triangular face, deep set eyes, mild fifth finger clinodactyly, arm length discrepancy and three café au lait spots. There was no history of developmental delay or school difficulties. His brother also has short stature (160.02 cm, <5th percentile) without abnormal movements.

The patient’s abnormal movements began at about 7 years of age as difficulty in writing and throwing. He currently has intermittent, action-induced myoclonic jerking of both his hands and arms and transient action-induced dystonic posturing of his hands and arms. The frequency of his abnormal movements decreases with alcohol intake. He has a history of anxiety attacks. His symptoms were substantially reduced by clonazepam.

Results

SNP array showed a male genotype with no significant copy number changes. However, there were three long continuous stretches of homozygosity on chromosome 7 (Fig. 1a,b). Two segments were on the short arm between bands 7p22.3–7p21.3 and 7p21.3–7p13 from nucleotide 10,704 (the first SNP on this platform) to 8,249,663 and 9,173,694 to 44,142,792, respectively. The third stretch of homozygosity was on the long arm of chromosome 7 between bands 7q21.3 and 7q31.33 from nucleotide 97,616,154 to 125,604,657. No other large regions of homozygosity were seen. Large homozygous region(s) confined to one chromosome are suggestive of chromosomal uniparental disomy with regions of isodisomy and heterodisomy.

To test this hypothesis, the proband and both of his parents were genotyped using an Illumina 300K array that contained 15,344 SNPs on chromosome 7. The genotypes of the 7065 SNPs present in the regions of loss of heterozygosity (LOH) were consistent with maternal isodisomy. The 8279 SNPs outside of the LOH regions were identical between the proband and his mother at all but one SNP. This one discordant SNP was determined to be a genotyping error in the patient’s mother. Collectively, these data are consistent with mUPD7 with segments of heterodisomy and
isodisomy due to five meiotic crossover events on chromosome 7. A high-resolution chromosome analysis showed a normal male karyotype. FISH showed no evidence of chromosome 7 trisomy in 508 interphases examined. The patient’s brother with short stature was also genotyped using SNP array and showed biparental inheritance of chromosome 7.

Because of the patient’s M-D-like phenotype, we assessed the methylation status of SGCE. We predicted that his SGCE promoter would be completely methylated because he carries two maternal SGCE alleles. DNA samples from both of the proband’s parents as well as his brother were used as controls. We used sodium bisulfite treatment followed by pyrosequencing to determine the methylation status of four CpG residues within the SGCE promoter in these four individuals (Fig. 2a). These residues are part of a differentially methylated CpG island that extends 1117 nucleotides upstream and 552 nucleotides downstream of SGCE (8). In individuals with biparental inheritance of chromosome 7, approximately 50% of the CpG residues at these four loci should be methylated. This was the pattern observed in the controls (Fig. 2b). Conversely, these four residues were 91–100% methylated in the proband. These data confirm that the SGCE promoter is fully methylated in the proband, consistent with M-D.

Discussion

Utilizing SNP array, we identified extended regions of homozygosity on chromosome 7 in a patient with short stature and M-D. Parental analysis of SNP array genotyping showed that the patient had mUPD7 with segments of isodisomy and heterodisomy. This scenario can be explained by the occurrence of five crossover events followed by a segregation error leading to an aneuploid oocyte. Because there are pericentromeric heterozygous SNPs, this event probably occurred during maternal meiosis I (17). The high number of crossovers and relative proximity of one to the centromere may have led to entanglement of the maternal meiotic bivalent resulting in either non-disjunction or precocious separation of sister chromatids (18). This aneuploid gamete, when fertilized, resulted in a zygote with trisomy 7 and subsequent loss of the paternal chromosome 7 homolog by trisomy rescue. No cells with trisomy 7 were identified in the patient’s lymphocytes, suggesting that this trisomy rescue event occurred very early in development.

Although the diagnoses of SRS or M-D had not been previously considered in this patient, the identification of mUPD7 provides a unifying diagnosis for his physical features and movement disorder. His short stature, relative macrocephaly, facial features, mild fifth finger clinodactyly, and arm length discrepancy are all consistent with SRS. Similarly, his myoclonus and dystonia that began at age 7 is typical for M-D due to loss of SGCE expression. He was initially prescribed clonazepam to control his abnormal movements, but recently began having more frequent symptoms and was switched to carbamazepine. Following the diagnosis of M-D, his medication was switched back to a higher dose of clonazepam, as this has been reported to be an effective treatment for myoclonic jerks as seen in M-D (19, 20). Decreased severity of symptoms with alcohol consumption, as observed in our patient, is a hallmark feature of M-D (5). Additionally, M-D is consistent
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Fig. 2. Loss of the unmethylated SGCE allele in the proband. (a) A diagram of the genomic location of the CpG island in the SGCE promoter. The methylation status of the four underlined CpG dinucleotides was assessed in the patient and his family members using sodium bisulfite treatment followed by pyrosequencing. This graphic was constructed using the UCSC Genome Browser (GRCh37/hg19). (b) Summary of SGCE promoter CpG methylation studies.

with the patient’s panic attacks as individuals with M-D have been described to have an increased frequency of psychiatric features including anxiety disorders (4).

A comprehensive search of the literature identified three additional reports of abnormal movements in SRS. The phenotypes of these five patients are described in Table 1. All patients with mUPD7 should lack SGCE expression due to loss of the paternal SGCE allele, yet our patient is only the sixth to have been described with M-D-like features. The fact that mUPD7 accounts for only 5–10% of SRS explains why abnormal movements are not a well-established feature of the SRS phenotype. Additionally, the majority of subjects with mUPD7 who have been reported in the literature are young. M-D typically develops in the first or second decade of life (1), so it is possible that abnormal movements develop after the initial mUPD7 diagnosis and evaluation. For example, myoclonic jerks and dystonia were only noted at 17 years in the mUPD7 patient reported by Guettard et al. (14). Long-term follow-up may be necessary to determine the actual frequency of M-D in this population. There is also the potential for additional genetic or

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>mUPD7</th>
<th>Age at presentation of movement disorder (years)</th>
<th>Movement disorder phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>+</td>
<td>17</td>
<td>Shock-like myoclonic jerks of the upper limbs, trunk, and face; mild dystonia&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Guettard et al. (14)</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>&lt;6</td>
<td>Generalized myoclonus with ‘shock-like’ jerks of all four extremities; no dystonia</td>
<td>Stark et al. (15)</td>
</tr>
<tr>
<td>14.9</td>
<td>+</td>
<td>–</td>
<td>Intermittent episodes of head shaking</td>
<td>Wakeling et al. (16)</td>
</tr>
<tr>
<td>14.2</td>
<td>+</td>
<td>–</td>
<td>Slight tremor affecting left arm</td>
<td>Wakeling et al. (16)</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Myoclonic jerks in infancy (3 weeks to 1 year) which resolved</td>
<td>Wakeling et al. (16)</td>
</tr>
<tr>
<td>24</td>
<td>+</td>
<td>7</td>
<td>Intermittent, action-induced myoclonic jerking of both hands and arms; transient action-induced dystonic posturing of hands and arms</td>
<td>Current report</td>
</tr>
</tbody>
</table>

<sup>a</sup> This patient had mUPD7 and an abnormal mosaic karyotype (47,XY+mar[22]/46,XY[11]) with a small marker chromosome derived from chromosome 7. <sup>b</sup> mRNA studies confirmed that this patient lacked SGCE expression.
environmental factors that could relax SGCE imprinting and lead to sufficient expression to allow for some mUPD7 patients to escape the M-D phenotype.

Despite only being reported in six patients, our findings illustrate that the presence of M-D differentiates SRS due to mUPD7 vs other causes. Because of the variability of the age of onset of M-D this distinction may not be possible in young SRS patients. Nevertheless, this is important, as currently the genetic etiology of SRS cannot be predicted based on clinical features alone (16, 21). Moreover, similar to our patient, it is also possible that some individuals with M-D who have short stature could have undiagnosed SRS. Thus, testing for mUPD7 in any patient with short stature and M-D should be considered.

Acknowledgements

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References