Myoclonus-Dystonia Due to Maternal Uniparental Disomy

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Background: Myoclonus-dystonia is a movement disorder often associated with mutations in the maternally imprinted ε-sarcoglycan (SGCE) gene located on chromosome 7q21. Silver-Russell syndrome is a heterogeneous disorder characterized by prenatal and postnatal growth restriction and a characteristic facies, caused in some cases by maternal uniparental disomy of chromosome 7.

Objectives: To describe and investigate the combination of a typical myoclonus-dystonia syndrome and Silver-Russell syndrome.

Design: Clinical and neurophysiological examination as well as cytogenetic and molecular analyses.

Setting: Movement disorder clinic.

Patient: A 36-year-old man with typical myoclonus-dystonia and Silver-Russell syndrome.

Main Outcome Measures: Clinical description of the disease and its genetic cause.

Results: Cytogenetic analysis revealed mosaicism for a small chromosome 7 marker chromosome. Microsatellite analysis indicated loss of the paternal allele and maternal uniparental disomy of chromosome 7. In keeping with the maternal imprinting mechanism, no unmethylated allele of SGCE was detected after bisulfite treatment of the patient’s DNA, and reverse transcription–polymerase chain reaction demonstrated loss of SGCE expression. Molecular analysis ruled out mutations in the SGCE gene.

Conclusions: We identified a new genetic alteration—maternal chromosome 7 disomy—that can cause myoclonus-dystonia. This alteration results in repression of both alleles of the maternally imprinted SGCE gene and suggests SGCE loss of function as the disease mechanism.

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Myoclonus-dystonia (M-D) is a movement disorder characterized by a combination of myoclonic jerks and dystonia. Myoclonus, the principal feature, predominates in the arms and axial muscles and is often responsive to alcohol. Dystonia is usually mild and often manifests as cervical dystonia or writer’s cramp.1 Myoclonus-dystonia usually emerges in the first or second decade of life. A major M-D gene, the ε-sarcoglycan gene (SGCE) (GenBank NT_007933), is located on chromosome 7q21.2 Myoclonus-dystonia is inherited in an autosomal dominant fashion with reduced penetrance. The SGCE gene is maternally imprinted with methylation of the maternal allele. This results in expression of the paternal allele only and therefore reduces the penetrance of maternally transmitted mutations.3 Mutation analysis in SGCE reveals mutations in 30% to 80% of patients with familial M-D,4,5 suggesting that the syndrome is genetically heterogeneous.

Silver-Russell syndrome (SRS) is mainly characterized by intrauterine and postnatal growth retardation and by characteristic craniofacial dysmorphism. It is clinically and genetically heterogeneous and has been linked to various modes of inheritance and abnormalities involving chromosomes 7, 8, 11, 15, 17, and 18.6,7 Maternal uniparental disomy of chromosome 7 (mUPD7) is reported in about 10% of sporadic cases.8,9 To our knowledge, SRS and M-D have never previously been reported together in the same patient. Here we describe clinical and molecular findings in such a patient and discuss the link...
between the two disorders, including a novel genetic abnormality causing M-D.

### METHODS

#### CYTOGENETIC ANALYSIS AND FLUORESCENCE IN SITU HYBRIDIZATION

Blood samples were collected with written informed consent for cytogenetic analysis and extraction of genomic DNA. Conventional cytogenetic analysis of peripheral blood lymphocytes from the proband and his parents was performed with standard techniques and processed by RHG and GTG-banding chromosomes. We also applied fluorescence in situ hybridization (FISH) analysis to metaphase and interphase lymphocyte preparations by using mixtures of chromosome-specific centromere probes (Qbiogen, Ilkirch, France) according to the manufacturer’s recommendations. Further FISH analysis with a whole chromosome 7 paint probe (Qbiogen) was used to screen the supernumerary marker chromosome (SMC) for euchromatin. To assess the size of the marker chromosome and to define the break points, we used FISH analysis with a set of bacterial artificial chromosome (BAC) clones selected from the Genome Database (http://www.ensembl.org/index.html); Wellcome Trust Sanger Institute, Cambridge, England, and M. Rocchi, University of Bari, Bari, Italy). Clones were plated and propagated and glycerol stocks were prepared. The DNA was isolated and purified with the Qiagen Plasmid Maxi kit (Qiagen SA, Courtaboeuf, France) according to the manufacturer’s instructions. The FISH probes were created using purified BAC DNA labeled by nick translation with digoxigenin (Roche Diagnostics, Meylan, France). The digoxigenin-labeled DNA probes were applied to the patient’s interphase and metaphase cells.

#### MOLECULAR GENETIC ANALYSES

To screen for mutations in SGCE, we sequenced all of the exons as described and used multiplex ligation-dependent probe amplification to detect deletions of whole exons. To investigate the parental origin, we used 28 microsatellite markers in the proband and his parents covering chromosome 7 (Figure 1). To test for SGCE expression in the patient, RNA was extracted using the Pax gene blood kit (Qiagen). After reverse transcription (SuperScript; Invitrogen, Carlsbad, California), we performed nested polymerase chain reaction with primers in exons 3 and 7 for the first round and in exons 4 and 6 for the second round. We amplified a part of the B-globin gene to ensure equal loading of reverse-transcribed RNA.

#### BISULFITE SEQUENCING

Bisulfite treatment of genomic DNA converts all unmethylated cytosine residues to uracil but leaves methylated residues unaffected. Subsequent polymerase chain reaction and sequencing yields sequences in which all unmethylated cytosine residues appear as thymines.

Bisulfite treatment of the proband’s genomic DNA was implemented with the EZ DNA Methylation Gold kit (Zymo Research Corp, Orange, California). To investigate the methylation pattern of the patient’s SGCE gene, we used polymerase chain reaction to amplify the PromM region located 773 to 1148 base pairs upstream of the SGCE translation start site compris-
ing 25 CpG dinucleotides that are usually methylated on the maternal allele only. To control for differential methylation in the patient, a fragment of the SNRPN gene (GenBank NT_026446) promoter on chromosome 15 was amplified. Both polymerase chain reaction products were visualized on ethidium bromide–stained agarose gels, extracted with Perfectprep Gel Cleanup (Eppendorf, Hamburg, Germany), and directly sequenced.

RESULTS

CASE REPORT

The patient, a 36-year-old man, developed a movement disorder at age 17 years. At the time of this study, his condition had recently started to deteriorate. He had healthy unrelated parents and no noteworthy family history. The pregnancy and delivery (at 40 weeks) were normal except for unexplained intrauterine growth retardation. At birth he measured 1800 g (−3.6 SDs) and 44 cm (−3.8 SDs) and had an occipitofrontal circumference of 32 cm (−2.9 SDs). He had difficulty feeding in infancy as well as growth retardation and premature puberty. He measured 152 cm when referred to us at age 36 years. On examination he had shocklike myoclonic jerks of the upper limbs, trunk, and face that responded very well to alcohol. He also had mild dystonia with retrocollis, blepharospasm, and writer’s cramp. He had no mental retardation. His dysmorphic features were clearly recognizable on photographs from infancy (Figure 2). He had no fifth-finger clinodactyly or hemihypertrophy. Together, his history and clinical features suggested both a typical M-D syndrome and SRS. Brain magnetic resonance imaging results were normal. A neurophysiological study showed subcortical myoclonus.

CYTOGENETIC FINDINGS

Conventional analysis of the patient’s chromosomes revealed mosaicism (46,XY/47,XY,+mar) for an SMC of unknown origin in 22 of 30 cells (73%) analyzed. The SMC was a small ring chromosome (Figure 3A). Both parental karyotypes were normal.

Analysis by FISH with the D7Z1 probe (specific to the centromere of chromosome 7) showed a signal on the SMC, indicating that it was derived from chromosome 7 (Figure 3B). Further FISH analysis with a whole chromosome 7 paint probe and BAC clones indicated that the marker chromosome had a small amount of pericentric euchromatin. The distal break points mapped to BAC RP5-1091E12 on 7p and to BAC RP11-34016 on 7q (Figure 3C and D). The pericentric regions of both 7p and 7q were approximately 1.8 megabases, with break points at 7p11.2 and 7q11.21.

MOLECULAR FINDINGS AND IMPRINTING

No mutations were found in SGCE by sequence and multiplex ligation-dependent probe amplification analyses.

Genotyping of 28 microsatellite markers on chromosome 7 revealed identical alleles in the mother and the patient; they were heterozygous at 22 markers and homozygous in both mother and son at 1 marker; the son was homozygous for 1 of the maternal alleles at 5 markers (Figure 1). The father shared no alleles with his son at 13 markers, indicating complete mUPD7 in the patient’s blood cells. The maternal disomy took the form of heterodisomy, with the exception of the centromeric region and the 7q telomeric region (Figure 1). No markers revealed the presence of 3 alleles that might have arisen from the SMC.

Both alleles of the SGCE promoter region were methylated as shown by the presence of only unconverted cytosines in CpG dinucleotides (Figure 4A). In contrast, a pattern of differential methylation was found in the promoter region of the SNRPN gene on chromosome 15, indicating a specific effect of mUPD7 on the patient’s chromosome 7. These results supported the sole presence of 2 maternal alleles in the patient as the maternal allele was the methylated allele.

No SGCE transcripts were detected in the patient owing to methylation (and thus silencing) of the 2 maternal alleles (Figure 4B).

COMMENT

We describe the case of a 36-year-old man with both M-D and SRS associated with mUPD7 and mosaicism for a supernumerary ring chromosome 7. Maternal imprinting leading to the absence of SGCE gene expression is likely to account for the M-D, whereas altered expression of a different imprinted gene might be responsible for the SRS.

The combination of M-D and SRS in this patient might have arisen in 3 different ways. First, mUPD might have led to abnormal expression of imprinted genes located on chromosome 7. Silver-Russell syndrome is a genetically heterogeneous syndrome, but about 10% of cases are related to mUPD7. Disruption of genomic imprinting is the most likely cause of SRS in mUPD7 owing either to the absence of the active paternal allele involved in growth promotion or to an excess of the maternal allele involved in growth inhibition. The SGCE gene associated with M-D is located on chromosome 7 and is subject to maternal imprinting, usually expressed only from the paternal allele. The second possible mechanism is an increased dosage of 2 or more genes located at the SMC due to trisomy 7 mosaicism, resulting in genetic imbal-
ance. Increased dosage of the SGCE gene can be excluded as a cause of the M-D phenotype in our patient because SGCE was not located at the SMC. Moreover, the SMC is small, containing only a centromere and the proximal part of either the short arm (to 7p11.2) or the long arm (to 7q11.21) of chromosome 7. According to the location of the BAC clones, the supernumerary small ring chromosome of our patient included only 1 gene, \( \text{EGFR} \) (GenBank NT_033968), spanned by BAC RP5-1091E12 on 7p11.2, whereas 7q11.21 was found to be gene-free. A maternal duplication of 7p13-p11.2 that did not encompass the \( \text{EGFR} \) gene was reported in a patient with SRS.8 This finding excluded a possible role of the \( \text{EGFR} \) gene in the SRS phenotype. We thus consider that SMC7 contributes little if anything to the patient’s phenotype. In addition, the association with SMC7 is exceptional, meaning that this mechanism could not explain most other cases of SRS with isolated mUPD7. The third possible explanation is fortuitous coexistence of SRS and M-D, but this is very unlikely given the rarity of the two disorders.

Our patient’s karyotype could be related to the fusion of a disomic gamete with a normal monosomic gamete, with subsequent loss of 1 of the extra-homologues from the trisomic conceptus, a phenomenon called \textit{functional trisomic rescue}.9 A trisomic zygote probably originated by maternal nondisjunction at the first meiotic cell division. Uniparental heterodisomy—inheritance of 2 different homologues from 1 parent—arises through this mechanism. This can also be accompanied by trisomy mosaicism in the placenta. Mosaicism for SMC7 probably reflected the instability of supernumerary partial chromosomes during cell replication, which can lead to disappearance of the SMC in some cells. The presence of small homozygous segments of chromosomes (isodisomic segments), as in the centromeric part of chromosome 7 in our patient, is a common finding in patients with uniparental heterodisomy due to meiotic nondisjunction and subsequent trisomic rescue. It reflects the occurrence of meiotic recombination in meiosis I and does not correspond to a deletion or duplication.10

Given the paternal expression of SGCE, patients with SRS and mUPD7 would be expected to develop M-D, but this neurological disorder has not previously been reported in patients with SRS due to mUPD. This may be because most patients described so far are younger than 5 years and may not have developed the M-D phenotype yet. In addition, M-D features can be difficult to identify. Maternal imprinting of the SGCE gene has been demonstrated in humans but the maternal allele might escape imprinting under certain conditions, providing a further possible explanation for why this association has not yet been reported in patients with SRS.

Figure 3. Cytogenetic status of the patient. A, The GTG-banded chromosomes showing the supernumerary marker chromosome (arrow). B, Fluorescence in situ hybridization analysis with a centromere 7 probe (D7Z1) showing a signal on the normal chromosome 7 and on the supernumerary marker chromosome (arrow). C, Fluorescence in situ hybridization with bacterial artificial chromosome RP5-1091E12 (red) and D7Z1 (green) probes showing hybridization on the supernumerary marker chromosome 7 (arrow). D, Fluorescence in situ hybridization with bacterial artificial chromosome RP11-3N2 (red) and D7Z1 (green) probes (arrow) showing hybridization only on the normal chromosome 7. Bacterial artificial chromosome RP11-3N2 was the first bacterial artificial chromosome to be negative in the 7q11.21 region; RP11-340I6 was the last to be positive. These findings defined the 7q break point between these 2 bacterial artificial chromosome clones.
We have identified a new genetic alteration—mUPD7 with subsequent repression of the 2 alleles of the SGCE gene—that can result in M-D. This provides further evidence of maternal imprinting of this gene. Patients with dystonic or myoclonic symptoms as well as growth retardation or dysmorphic features (which may be difficult to recognize in adulthood) should have cytogenetic investigations and be tested for mUPD7. Because patients with SRS associated with mUPD7 are prone to developing M-D, they should be carefully screened for the presence of this movement disorder.

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