Genetic heterogeneity in ten families with myoclonus-dystonia


Background: Myoclonus-dystonia (M-D) is a movement disorder with autosomal dominant inheritance and reduced penetrance but may also occur sporadically. Recently, mutations in the epsilon-sarcoglycan gene (SGCE) were shown to cause M-D. Furthermore, single variants in the dopamine D2 receptor (DRD2) and DYT1 genes were found in combination with SGCE mutations in two M-D families, and another M-D locus was recently mapped to chromosome 18p11 in one family.

Methods: The authors clinically and genetically characterised ten consecutive cases with myoclonus-dystonia; seven familial and three sporadic. Twenty-nine M-D patients and 40 unaffected family members underwent a standardised clinical examination by a movement disorder specialist. Index cases were screened for mutations in the SGCE, DYT1, and DRD2 genes and for deletions of the SGCE gene. Suitable mutation negative families were tested for linkage to the SGCE region and to chromosome 18p11.

Results: Two SGCE mutations were detected among the seven familial but no mutation in the sporadic cases. Haplotype analysis at the new M-D locus was compatible with linkage in two families and excluded in another family, suggesting at least one additional M-D gene. There were no obvious clinical differences between M-D families with and without detected mutations.

Conclusion: M-D is genetically heterogeneous with SGCE mutations accounting for the disease in only part of the clinically typical cases.

Myoclonus-dystonia (M-D, DYT11) occurs as an autosomal dominant or sporadic movement disorder, characterised by myoclonic jerks affecting mostly proximal muscles. Dystonia, usually torticollis or writer’s cramp, is observed in most but not all patients and can occasionally be the only symptom of the disease. Symptoms often respond to alcohol and patients can show psychiatric abnormalities.1

The epsilon-sarcoglycan gene (SGCE) located on human chromosome 7 was recently identified as the major M-D gene.2 Molecular evidence shows that it is a maternally imprinted gene, resulting in paternal expression and thus reduced penetrance upon maternal transmission.3 To date, SGCE mutations have been reported mostly in pedigrees previously linked to chromosome 7 or in single families.2-7 However, the frequency of SGCE mutations in larger, clinically ascertained M-D patient cohorts is currently unknown.

Different reported SGCE mutation types include missense and nonsense mutations, small deletions, and a heterozygous deletion of the complete gene.8-11 Additionally, single variants that may represent functional mutations were reported in both the dopamine D2 receptor (DRD2) and the DYT1 gene in combination with SGCE mutations in single families with an M-D phenotype.5 Further, a new gene locus was recently identified on chromosome 18p11.10 We present the detailed clinical and genetic analysis of 10 consecutive M-D families.

MATERIALS AND METHODS

Subjects
We included 10 consecutive index patients of Serbian origin presenting with early onset (<20 years) familial or sporadic myoclonus and dystonia, with a relatively benign course and alleviation of symptoms by alcohol in 15 of 17 cases (88%) tested. Exclusion criteria were other neurological deficits, pathological findings on electroencephalogram (EEG), somatosensory evoked potentials, or neuroimaging. All patients and unaffected family members underwent a standardised neurological examination by a movement disorder specialist (VK). Clinical information on two affected deceased individuals was available by history only. After obtaining informed consent, a blood sample was collected from all available family members. A core branch of family 1 underwent imprinting studies and is presented elsewhere as family V.3 Several additional members of family 1 have recently been collected and are described in this article (fig 1).

Mutational analysis
All 12 exons and flanking intron regions of the SGCE gene were tested for mutations using SSCP and DHPLC (Wave system, Transgenomics, Crewe, UK) analysis, followed by cycle sequencing of polymerase chain reaction (PCR) products in cases of suspected sequence alterations. In addition, gene dosage studies of exon 6 were performed on the LightCycler (Roche Diagnostics, Mannheim, Germany) by a quantitative duplex PCR assay to study for large genomic deletions. The method was adapted from an assay for the Parkin gene.11 Furthermore, all seven exons of the DRD2 gene were tested by SSCP analysis, and all samples were tested for the GAG deletion and the newly detected 18-bp deletion in exon 5 of the DYT1 gene.12 Fifty Centre d’Etude du Polymorphisme Humaine (CEPH) controls were screened for the detected missense mutation by DHPLC. Primers and PCR conditions are available upon request.

Haplotype analysis
Genotyping of the SGCE region on 7q21-31 and the new locus on 18p11 was carried out with microsatellite markers in mutation negative families suitable for linkage analysis (families 3–5; fig 2). PCR products were analysed on an

Abbreviations: DHPLC, denaturing high performance liquid chromatography; DRD2, dopamine D2; PCR, polymerase chain reaction; SGCE, epsilon-sarcoglycan.
shift (321FS333X). In family 2, all three affected individuals carried a mutation in the SGCE gene (966delT), causing frame shift. In family 1, two affected females and their unaffected father responded to various antiepileptic drugs including clonazepam, and gabapentin.

RESULTS
Clinical examination
We identified 26 familial M-D cases in seven M-D families (families 1–7), along with 40 unaffected family members. DNA samples were available for 14 affected and 12 unaffected individuals in these families (figs 1 and 2). In addition, we identified three isolated M-D cases. Mode of inheritance appeared autosomal dominant in families 1–6 and showed evidence for reduced penetrance in five of these families, consistent with paternal expression of the disease gene in families 2 and 3. In family 7, two siblings were the only affected family members, suggesting autosomal dominant inheritance with reduced penetrance or recessive inheritance. Mean (standard deviation, SD) age of onset was at 11.9 (SD 5.5) years (range 3–24 years) for all examined patients, 12.0 (SD 5.5) years (range 3–24 years) in the familial, and 11.0 (SD 7.2) years (range 5–19 years) in sporadic cases.

In all patients clinical findings were fully compatible with a diagnosis of M-D (table 1). Eighteen of 29 (62.1%) patients had a combination of myoclonus and dystonia, eight patients (27.6%) showed only myoclonus, and two (6.9%) only dystonia. One patient had a history of myoclonus; information on dystonic signs was unavailable. No psychiatric abnormalities were reported; however, no formal testing was performed. In families 1 and 2, prominent leg involvement was noted as unusual clinical feature in two family members. M-D was ameliorated by intake of alcohol in at least one affected of eight of the families, negative in sporadic cases. M-D was ameliorated by intake of alcohol in at least one affected of eight of the families, negative in sporadic cases.

Haplotype analysis
In families 3 and 4, both affected and unaffected family members shared an allele at markers flanking the SGCE gene, whereas in family 5 only affected members carried a common haplotype in that region (fig 2). Screening of the DRD2 gene revealed the NcoI RFLP polymorphism (His313His) in exon 6 in four families but no mutations. No mutations in any of the three genes were found in families 3–7 or in the sporadic cases of families 8–10.

DISCUSSION
Analysis of the three known genes associated with M-D revealed mutations only in the SGCE gene and only in two families, resulting in an overall low mutation rate in our M-D cohort (2/10). Separating out the familial cases, the mutation frequency (2/7) was in accordance with a very recent similar study that identified SGCE mutations in three of six familial M-D cases. By contrast, no mutations were found in our three sporadic patients. Similarly, a recent study failed to identify mutations in at least 10 sporadic patients. However, given the occurrence of pseudosporadic cases due to reduced penetrance and the possibility of de novo mutations, the SGCE mutation rate in apparently sporadic cases needs to be evaluated in larger series.

The present study also tested for mutations in the DRD2 and DYT1 gene; however, no support was found in this
relatively small sample for the theory that mutations in these genes cause M-D.

The phenotype in the mutation negative M-D patients was fully compatible with a diagnosis of M-D and did not obviously differ between mutation negative and mutation bearing families. Also, the rare symptom of laryngeal myoclonus in one familial (family 6) and a sporadic case (family 9) has recently been described in an SGCE mutation positive M-D family.

Haplotype analysis at the SGCE locus was performed in the mutation negative families 3–5 for which there was DNA on more than one member. Assuming reduced penetrance, inheritance was compatible with linkage to this locus in these families. Notably, in family 5, there is a 50% chance for the available affected members in three successive generations to share the same haplotype. In addition, the mode of transmission was incompatible with paternal expression/maternal imprinting of SGCE in all three families because the disease was maternally transmitted in several cases (fig 2), arguing against involvement of the SGCE gene in these families. This is particularly true for family 5, with two cases inheriting the disease from their mother. Although no definite conclusion on linkage to the SGCE region could be drawn in our three relatively small, mutation negative families, combined with the mutation and sequence analysis, it seems unlikely that their disease is due to mutations in this gene.

![Figure 2](Mutation negative families 3 to 7 with results of haplotype analysis below the respective individual for the SGCE region (left) and the locus on 18p11 (right) in families 3 to 5. Shared, possibly disease associated haplotypes are highlighted. Markers indicated by an asterisk flank the SGCE gene.)
<table>
<thead>
<tr>
<th>Family</th>
<th>FH</th>
<th>Pedigree number</th>
<th>Age of onset (years)</th>
<th>Sex</th>
<th>Myoclonic symptoms</th>
<th>Dystonic symptoms</th>
<th>Unusual features</th>
<th>Response to alcohol</th>
<th>Genetic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>III.1</td>
<td>4</td>
<td>F</td>
<td>Left arm, head, and to a minor degree of right arm</td>
<td>Laterocollis, foot dystonia</td>
<td>None</td>
<td>Not tested</td>
<td>966delT (SGCE)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III.2</td>
<td>9</td>
<td>F</td>
<td>Head and upper extremities (L&gt;R)</td>
<td>Laterocollis</td>
<td>None</td>
<td>Not tested</td>
<td>966delT (SGCE)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II.3</td>
<td>10</td>
<td>F</td>
<td>Mild to moderate; right arm</td>
<td>None</td>
<td>None</td>
<td>Not tested</td>
<td>None available</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II.4</td>
<td>9</td>
<td>F</td>
<td>Right leg; mild action myoclonus of both arms</td>
<td>Right foot dystonia</td>
<td>Prominent leg involvement</td>
<td>Not tested</td>
<td>None available</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II.5</td>
<td>13</td>
<td>F</td>
<td>Head during stress</td>
<td>None</td>
<td>None</td>
<td>Not tested</td>
<td>None available</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I.1</td>
<td>15</td>
<td>M</td>
<td>Head; action myoclonus of both arms</td>
<td>Laterocollis, foot dystonia</td>
<td>None</td>
<td>Not tested</td>
<td>966delT (SGCE)</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>III.2</td>
<td>14</td>
<td>F</td>
<td>Both hands; prominent myoclonus of both legs</td>
<td>Right foot dystonia</td>
<td>Prominent leg involvement</td>
<td>Positive</td>
<td>None available</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III.3</td>
<td>10</td>
<td>M</td>
<td>Both arms; action myoclonus of both legs</td>
<td>Right laterocollis; dystonia of the right hand (particularly the thumb)</td>
<td>None</td>
<td>Positive</td>
<td>179 A&gt;G (SGCE)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II.5</td>
<td>15</td>
<td>M</td>
<td>Mild; left hand (right hand amputated)</td>
<td>Right laterocollis with bilateral hand dystonia</td>
<td>None</td>
<td>Positive</td>
<td>None available</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>II.1</td>
<td>3</td>
<td>M</td>
<td>Head and hands</td>
<td>Laterocollis, dystonic movements of abdominal muscles</td>
<td>None</td>
<td>Negative</td>
<td>No mutation detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I.2</td>
<td>22</td>
<td>M</td>
<td>Head and arms</td>
<td>None</td>
<td>None</td>
<td>Positive</td>
<td>No mutation detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I.3</td>
<td>11</td>
<td>F</td>
<td>Rare; head; jerky postural hand tremor</td>
<td>None</td>
<td>None</td>
<td>Not tested</td>
<td>No DNA available</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I.5</td>
<td>14</td>
<td>F</td>
<td>None</td>
<td>Laterocollis, followed by writer’s cramp</td>
<td>None</td>
<td>Not tested</td>
<td>No DNA available</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>III.2</td>
<td>13</td>
<td>F</td>
<td>Frequent; irregular; proximal; upper limbs, with deterioration on action</td>
<td>Both arms and right foot</td>
<td>None</td>
<td>Positive</td>
<td>No mutation detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I.2</td>
<td>22</td>
<td>F</td>
<td>Generalised</td>
<td>Discrete dystonic posturing of upper extremities</td>
<td>None</td>
<td>Not tested</td>
<td>No mutation detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I.5</td>
<td>NA</td>
<td>F</td>
<td>Hands</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Not tested</td>
<td>No DNA available</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>III.2</td>
<td>7</td>
<td>F</td>
<td>None</td>
<td>Writer’s cramp and intermittent lateral deviation of head while walking</td>
<td>Left laterocollis, axial rotational dystonia</td>
<td>None</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II.1</td>
<td>7</td>
<td>F</td>
<td>Proximal; upper extremities with action-provoked deterioration</td>
<td>None</td>
<td>Positive</td>
<td>No mutation detected</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>II.4</td>
<td>11</td>
<td>M</td>
<td>Postural and action myoclonus of both arms</td>
<td>Axial and right arm dystonia</td>
<td>None</td>
<td>Not tested</td>
<td>No DNA available</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I.2</td>
<td>24</td>
<td>F</td>
<td>Head (when turning to the right)</td>
<td>Laterocollis</td>
<td>None</td>
<td>Not tested</td>
<td>No mutation detected</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>III.1</td>
<td>12</td>
<td>M</td>
<td>Head, trunk, and proximal muscles of both arms (R&gt;L)</td>
<td>Left laterocollis with torsion of the trunk to the right and mild dystonia of right leg</td>
<td>None</td>
<td>Positive</td>
<td>No mutation detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I.1</td>
<td>10</td>
<td>F</td>
<td>Head; laryngeal myoclonus</td>
<td>None</td>
<td>None</td>
<td>Positive</td>
<td>No DNA available</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>II.2</td>
<td>20</td>
<td>F</td>
<td>Head and both arms (L&gt;R)</td>
<td>Left torticollis and dystonia of left hand</td>
<td>None</td>
<td>Positive</td>
<td>No mutation detected</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II.3</td>
<td>7</td>
<td>F</td>
<td>Head and both arms</td>
<td>None</td>
<td>None</td>
<td>Positive</td>
<td>No DNA available</td>
</tr>
<tr>
<td>8</td>
<td>–</td>
<td>19</td>
<td>M</td>
<td>Both arms</td>
<td>Torticollis, dystonia of both arms</td>
<td>None</td>
<td>Negative</td>
<td>No mutation detected</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>–</td>
<td>5</td>
<td>F</td>
<td>Head, both arms (R&gt;L), laryngeal myoclonus, with infrequent action myoclonus of legs</td>
<td>Writer’s cramp of right hand</td>
<td>None</td>
<td>Positive</td>
<td>No mutation detected</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>–</td>
<td>9</td>
<td>M</td>
<td>Mild in head and prominent, predominantly proximal in both arms (R&gt;L)</td>
<td>Writer’s cramp (initial symptom), mild dystonia of left hand</td>
<td>None</td>
<td>Positive</td>
<td>No mutation detected</td>
<td></td>
</tr>
</tbody>
</table>

Index patients are in bold. FH, family history; NA, not available.
Haplotype analysis at the new M-D locus (18p11) was compatible with linkage in family 3 but does not narrow the previously linked region. The recombination event between the two markers GATA185C06 and D18S452 flanking the putative gene does not allow for a final statement on linkage status in family 4. In family 5, linkage was excluded to 18p11 and highly unlikely to the SGCE region. These findings and lack of mutations in the DYT1 and DRD2 genes raise the possibility that an as yet unidentified gene causes M-D in this family.

In conclusion, SGCE mutations appear to account for only a proportion of clinically ascertained M-D cases. Genes other than the three tested and the locus on chromosome 18 may contribute to the etiology of M-D in our set of mutation negative familial and sporadic M-D cases, supporting the notion that M-D is genetically heterogeneous.

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