SHORT REPORT

Genetic heterogeneity in ten families with myoclonus-dystonia

B Schüle, N Kock, M Svetel, N Dragasevic, K Hedrich, P de Carvalho Aguiar, L Liu, K Kabakci, J Garrels, E-M Meyer, I Berisavac, E Schwinger, P L Kramer, L J Ozelius, C Klein, V Kostic

J Neurol Neurosurg Psychiatry 2004;**75**:1181–1185. doi: 10.1136/jnnp.2003.027177

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.[2–9] 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.

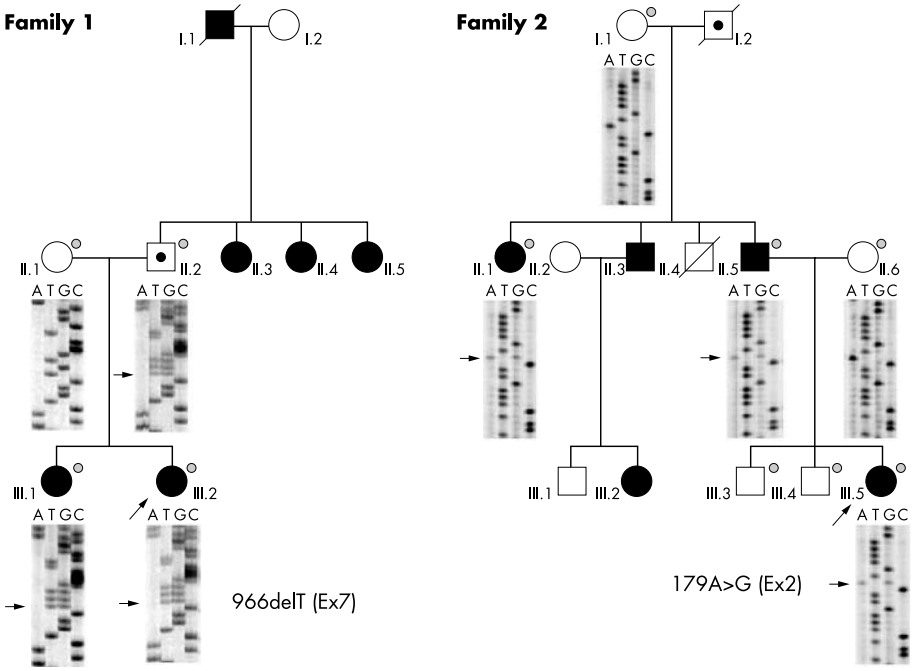

Figure 1 Pedigrees of M-D families. Affected individuals are shaded in black. Obligate carriers are indicated by a black dot. Index patients are marked by an arrow. Individuals from whom DNA samples were available are marked by a grey dot above the pedigree symbol. Families 1 and 2 with results of sequence analysis showing the *SGCE* mutations (966delT and 179 A>G; indicated by a black arrow). In family 1, affected sisters III.1 and III.2 and their unaffected father carried the heterozygous deletion. In family 2, the index patient (III.5), her affected father (II.5), and affected aunt (II.1) carried a heterozygous missense mutation.

automated sequencing machine (LI-COR, Lincoln, NE, USA) and haplotypes reconstructed manually.

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 the 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 case 8, and unknown in family 1. Signs of M-D did not respond to various antiepileptic drugs including clonazepam, valproate, and gabapentin.

Mutational analysis

In family 1, two affected females and their unaffected father carried a mutation in the *SGCE* gene (966delT), causing frame shift (321FS333X).[3] In family 2, all three affected individuals available for genetic testing carried a previously undescribed missense mutation in the *SGCE* gene (179A>G; H60R; fig 1). This alteration was absent in 100 chromosomes of CEPH controls. Gene dosage studies of exon 6 identified a ratio of the concentration of *SGCE* to a reference gene (β globin) of 0.8–1.2 (normal range) in all index patients. Therefore, no deletion or multiplication of exon 6 was detected, and large genomic alterations were excluded. 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.

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). For chromosome 18p11, both affected individuals studied in family 3 shared a common haplotype. In family 4, two affected and one unaffected member carried the same haplotype at the markers D18S54 to GATA185C06 with a recombination event between GATA185C06 and D18S452. Only two of the three affected shared a common haplotype at 18p11 in family 5 (fig 2).

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.[9] 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.[13] However, given the occurrence of pseudosporadic cases due to reduced penetrance[3] 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

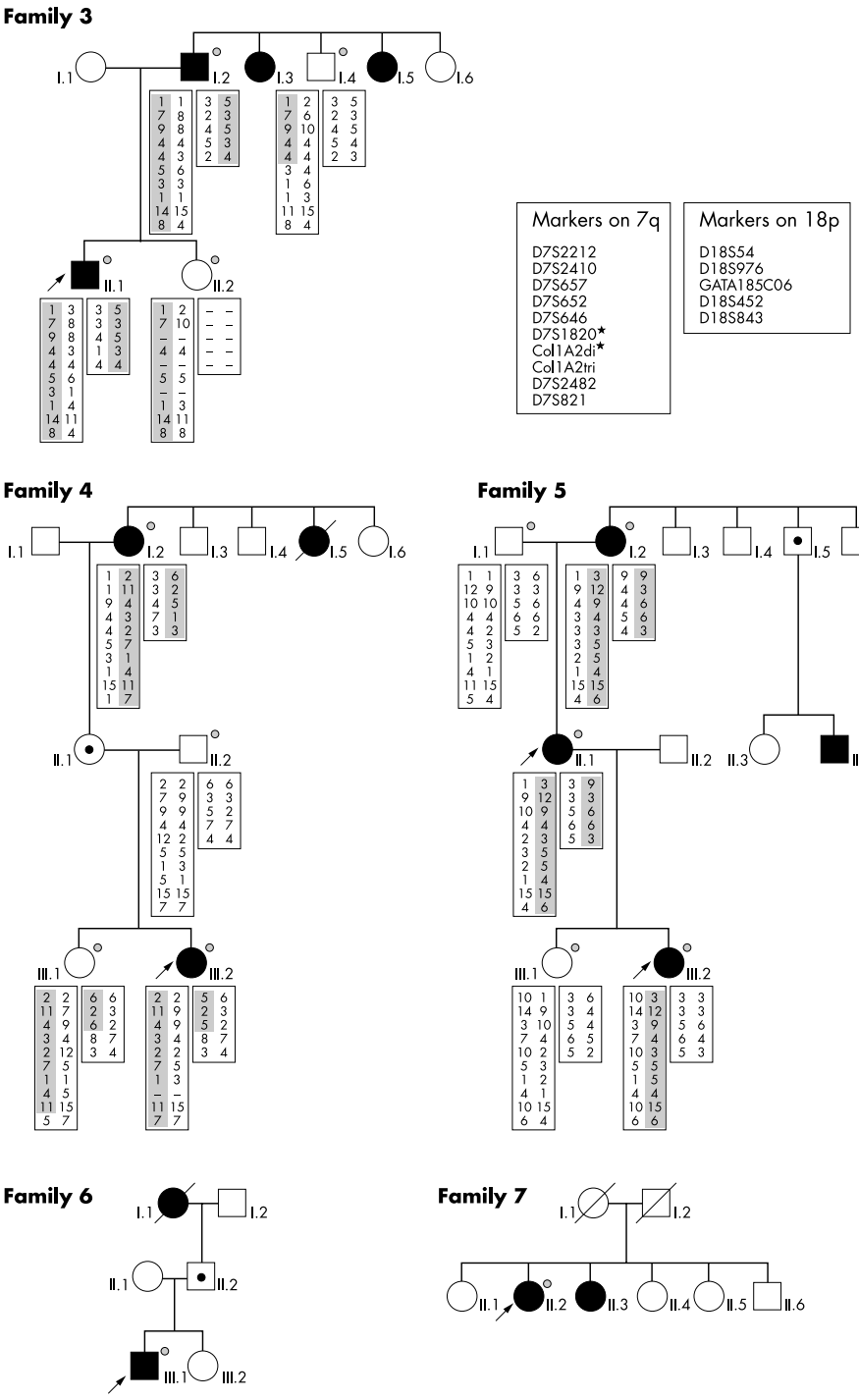

Figure 2 Mutation negative families 3 to 7 with results of haplotype analysis below the respective individual for the *SGCE* region (left) and the locus on 18p11 (right) in families 3 to 5. Shared, possibly disease associated haplotypes are highlighted. Markers indicated by an asterisk flank the *SGCE* gene.

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.[8]

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.

Table 1 Clinical findings in affected individuals from M-D families 1–10

Family	FH	Pedigree number	Age of onset (years)	Sex	Myoclonic symptoms	Dystonic symptoms	Unusual features	Response to alcohol	Genetic findings
1	+	III.1	4	F	Left arm, head, and to a minor degree of right arm	Laterocollis, foot dystonia	None	Not tested	966delT (*SGCE*)
		III.2	9	F	Head and upper extremities (L>R)	Laterocollis	None	Not tested	966delT (*SGCE*)
		II.3	10	F	Mild to moderate; right arm	None	None	Not tested	No DNA available
		II.4	9	F	Right leg; mild action myoclonus of both arms	Right foot dystonia	Prominent leg involvement	Not tested	No DNA available
		II.5	13	F	Head during stress	None	None	Not tested	No DNA available
		I.1	~15	M	Head; action myoclonus of both arms	Scoliosis (axial dystonia)	None	Not tested	No DNA available
2	+	III.2	14	F	Both hands; prominent myoclonus of both legs	Right foot dystonia	Prominent leg involvement	Positive	No DNA available
		III.5	7	F	Both hands	None	None	Positive	179 A>G (*SGCE*)
		II.1	12	F	Mild; both arms	Right laterocollis; dystonia of the right hand (particularly the thumb)	None	Positive	179 A>G (*SGCE*)
		II.3	~10	M	Both arms; action myoclonus of both legs	Right laterocollis with bilateral hand dystonia	None	Positive	No DNA available
		II.5	15	M	Mild; left hand (right hand amputated)	None	None	Positive	179 A>G (*SGCE*)
3	+	**II.1**	3	M	Head and hands	Laterocollis, dystonic movements of abdominal muscles	None	Negative	No mutation detected
		I.2	22	M	Head and arms	None	None	Positive	No mutation detected
		I.3	11	F	Rare; head; jerky postural hand tremor	None	None	Not tested	No DNA available
		I.5	14	F	None	Laterocollis, followed by writer's cramp	None	Not tested	No DNA available
4	+	**III.2**	13	F	Frequent; irregular; proximal; upper limbs, with deterioration on action	Both arms and right foot	None	Positive	No mutation detected
		I.2	22	F	Generalised	Discreet dystonic posturing of upper extremities	None	Not tested	No mutation detected
		I.5	NA	F	Hands	Unknown	Unknown	Not tested	No DNA available
5	+	III.2	7	F	None	Writer's cramp and intermittent lateral deviation of head while walking	None	Positive	No mutation detected
		II.1	7	F	Proximal; upper extremities with action-provoked deterioration	Laterocollis, axial rotational dystonia	None	Positive	No mutation detected
		II.4	11	M	Postural and action myoclonus of both arms	Axial and right arm dystonia	None	Not tested	No DNA available
		I.2	24	F	Head (when turning to the right)	Laterocollis	None	Not tested	No mutation detected
6	+	**III.1**	12	M	Head, trunk, and proximal muscles of both arms (R>L)	Left laterocollis with torsion of the trunk to the right and mild dystonia of right leg	None	Positive	No mutation detected
		I.1	~10	F	Head; laryngeal myoclonus	None	None	Positive	No DNA available
7	+	**II.2**	20	F	Head and both arms (L>R)	Left torticollis and dystonia of left hand	None	Positive	No mutation detected
		II.3	7	F	Head and both arms	None	None	Positive	No DNA available
8	–		19	M	Both arms	Torticollis, dystonia of both arms	None	Negative	No mutation detected
9	–		5	F	Head, both arms (R>L), laryngeal myoclonus, with infrequent action myoclonus of legs	Writer's cramp of right hand	None	Positive	No mutation detected
10	–		9	M	Mild in head and prominent, predominantly proximal in both arms (R>L)	Writer's cramp (initial symptom), mild dystonia of left hand	None	Positive	No mutation detected

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 aetiology of M-D in our set of mutation negative familial and sporadic M-D cases, supporting the notion that M-D is genetically heterogeneous.

ACKNOWLEDGEMENTS

We would like to thank the patients and family members who participated in this study. This work was supported by a research grant from the Fritz Thyssen Foundation (JG, CK, NK), a research grant from the University of Lübeck (BS), and the Myoclonus Research Foundation (LO, PdA, LL).

Authors' affiliations

B Schüle, N Kock, K Hedrich, K Kabakci, J Garrels, E-M Meyer, C Klein, Department of Neurology, University of Lübeck, Lübeck, Germany
B Schüle, N Kock, K Hedrich, K Kabakci, J Garrels, E-M Meyer, E Schwinger, C Klein, Department of Human Genetics, University of Lübeck, Lübeck, Germany
B Schüle, Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA
N Kock, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA
M Svetel, N Dragasevic, I Berisavac, V Kostic, Department of Neurology, University of Belgrade, Belgrade, Yugoslavia
P de Carvalho Aguiar, L Liu, L J Ozelius, Department of Human Genetics, Albert Einstein College of Medicine, Bronx, NY, USA
P L Kramer, Department of Neurology, Oregon Health and Science University, Portland, OR, USA

Correspondence to: Dr C Klein, Department of Neurology, University of Lübeck; Ratzeburger Allee 160, 23538 Lübeck, Germany; klein_ch@neuro.mu-luebeck.de

Received 1 September 2003
Revised 28 October 2003
Accepted 1 November 2003

REFERENCES

1 **Klein C**. Myoclonus and Myoclonus-Dystonias. In: Pulst SM, ed. *Genetics of Movement Disorders*. San Diego, USA: Academic Press/Elsevier Science, 2003:449–69.
2 **Zimprich A**, Asmus F, Leitner P, *et al*. Mutations in the gene encoding epsilon-sarcoglycan cause myoclonus-dystonia syndrome. *Nat Genet* 2001;**29**:66–9.
3 **Müller B**, Hedrich K, Kock N, *et al*. Evidence that paternal expression of the epsilon-sarcoglycan gene accounts for reduced penetrance in myoclonus-dystonia. *Am J Hum Genet* 2002;**71**:1303–11.
4 **Asmus F**, Zimprich A, Tezenas Du Montcel S, *et al*. Myoclonus-dystonia syndrome: epsilon-sarcoglycan mutations and phenotype. *Ann Neurol* 2002;**52**:489–92.
5 **Klein C**, Liu L, Doheny D, *et al*. Epsilon-sarcoglycan mutations found in combination with other dystonia gene mutations. *Ann Neurol* 2002;**52**:675–9.
6 **Hjermind LE**, Werdelin LM, Eiberg H, *et al*. A novel mutation in the epsilon-sarcoglycan gene causing myoclonus-dystonia syndrome. *Neurology* 2003;**60**:1536–9.
7 **Marechal L**, Raux G, Dumanchin C, *et al*. Severe myoclonus-dystonia syndrome associated with a novel epsilon-sarcoglycan gene truncating mutation. *Am J Med Genet* 2003;**119B**:114–17.
8 **DeBerardinis RJ**, Conforto D, Russell K, *et al*. Myoclonus in a patient with a deletion of the epsilon-sarcoglycan locus on chromosome 7q21. *Am J Med Genet* 2003;**121A**:31–6.
9 **Han F**, Lang AE, Racacho L, *et al*. Mutations in the epsilon-sarcoglycan gene found to be uncommon in seven myoclonus-dystonia families. *Neurology* 2003;**61**:244–6.
10 **Grimes DA**, Han F, Lang AE, *et al*. A novel locus for inherited myoclonus-dystonia on 18p11. *Neurology* 2002;**59**:1183–6.
11 **Hedrich K**, Kann M, Lanthaler AJ, *et al*. The importance of gene dosage studies: mutational analysis of the parkin gene in early-onset parkinsonism. *Hum Mol Genet* 2001;**10**:1649–56.
12 **Klein C**, Friedman J, Bressman S, *et al*. Genetic testing for early-onset torsion dystonia (DYT1): introduction of a simple screening method, experiences from testing of a large patient cohort, and ethical aspects. *Genet Test* 1999;**3**:323–8.
13 **Valente EM**, Misbahuddin A, Brancati F, *et al*. Analysis of the epsilon-sarcoglycan gene in familial and sporadic myoclonus-dystonia: evidence for genetic heterogeneity. *Mov Disord* 2003;**18**:1047–51.