ORIGINAL ARTICLE

Epsilon sarcoglycan mutations and phenotype in French patients with myoclonic syndromes

S Tezenas du Montcel*, F Clot*, M Vidailhet, E Roze, P Damier, C P Jedynak, A Camuzat, A Lagueny, L Vercueil, D Doummar, L Guyant-Maréchal, J-L Houeto, G Ponsot, S Thobois, M-A Cournelle, A Durr, F Durif, B Echenne, D Hannequin, C Tranchant, A Brice, the French Dystonia Network**

*Both authors have contributed equally to this work
See end of article for authors' affiliations

Correspondence to: Sophie Tezenas du Montcel, Service de Biostatistiques et Information Medicale, Groupe Hospitalier Pitie-Salpetriere, 47–83 Bd de l'Hopital, 75651 Paris Cedex 13, France; sophie.tezenas@psl.aphp.fr

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Background: Myoclonus dystonia syndrome (MDS) is an autosomal dominant movement disorder caused by mutations in the epsilon-sarcoglycan gene (*SGCE*) on chromosome 7q21.
Methods: We have screened for *SGCE* mutations in index cases from 76 French patients with myoclonic syndromes, including myoclonus dystonia (M-D), essential myoclonus (E-M), primary myoclonic dystonia, generalised dystonia, dystonia with tremor, and benign hereditary chorea. All coding exons of the *SGCE* gene were analysed. The *DYT1* mutation was also tested.
Results: Sixteen index cases had *SGCE* mutations while one case with primary myoclonic dystonia carried the *DYT1* mutation. Thirteen different mutations were found: three nonsense mutations, three missense mutations, three splice site mutations, three deletions, and one insertion. Eleven of the *SGCE* index cases had M-D and five E-M. No *SGCE* mutations were detected in patients with other phenotypes. The total number of mutation carriers in the families was 38, six of whom were asymptomatic. Penetrance was complete in paternal transmissions and null in maternal transmissions. MDS patients with *SGCE* mutation had a significantly earlier onset than the non-carriers. None of the patients had severe psychiatric disorders.
Conclusion: This large cohort of index patients shows that *SGCE* mutations are primarily found in patients with M-D and to a lesser extent E-M, but are present in only 30% of these patients combined (M-D and E-M).

Myoclonus dystonia syndrome (MDS), an autosomal dominant disorder, is characterised by myoclonic jerks mainly involving the arms and axial muscles in combination with dystonia. It often develops during childhood or early adolescence. Myoclonus is the predominant feature, with ''lightning jerks'' that are often responsive to alcohol. Dystonia classically appears later in the course of the disease and is often more focal and less spectacular than the myoclonus.[1]

In 1999, the MDS gene locus was linked to a 28 cM region of chromosome 7q21–q31,[2] and in 2001, the gene was identified as the ϵ-sarcoglycan gene (*SGCE*).[3] Epsilon-sarcoglycan (SGCE) is a transmembrane glycoprotein homologous to α-sarcoglycan. Sarcoglycan proteins (α, β, γ, and δ) are components of the dystrophin-glycoprotein complex, which links the muscle cytoskeleton to the extracellular matrix,[4] and are involved in autosomal recessive muscular dystrophies.[5 6] The SGCE protein is expressed in many human tissues: muscle, lung, liver, kidney, spleen, testis, and brain.[7] The *SGCE* gene is composed of 12 exons that span 71 kb. Exon 10 is alternatively spliced and is missing in the majority of transcripts. Recently, a new exon, 11b, was identified in a transcript from mouse brain.[8] Maternal imprinting of the *SGCE* gene has been demonstrated in mice[9] and later in humans,[10 11] and is responsible for the reduced penetrance observed in individuals who inherited the mutated allele from their mother. Since 2001, 24 mutations have been reported (table 1). In this study, we have evaluated the frequency of *SGCE* mutations and the corresponding phenotype in a large cohort of French patients with myoclonic syndromes, but also with clinically related disorders such as dystonia with tremor, generalised dystonia, or benign hereditary chorea (BHC).

METHODS

Families and patients

We included 76 index patients with myoclonus-dystonia (M-D; n = 49), essential myoclonus (E-M; n = 5), and primary myoclonic dystonia (n = 13), as well as dystonia associated with tremor (n = 5), generalised dystonia (n = 3), and BHC (n = 1) to broaden the phenotypic spectrum. All patients were French and of Caucasian origin.

M-D was defined by the presence of lightning jerks (myoclonus) and dystonia in patients.[1] E-M was defined by isolated myoclonus.[21 22] Primary myoclonic dystonia was defined by primary dystonia with superimposed jerky movements. These patients had predominant dystonia, and their jerks were not lightning jerks but the slower jerks commonly seen in primary dystonia. Dystonia and tremor was defined by the association of a postural, localised, and irregular tremor (dystonic tremor) superimposed on more sustained dystonic spasms.[23] Idiopathic generalised dystonia was characterised by involuntary sustained muscle contractions, causing twisting and repetitive movements or abnormal postures, involving at least one leg and the trunk and any other segment.[24] BHC was defined by the presence of early onset chorea with or without other movement disorders in patients with a positive family history but without the CAG

Abbreviations: BHC, benign hereditary chorea; E-M, essential myoclonus; M-D, myoclonus dystonia; MDS, myoclonus dystonia syndrome; PCR, polymerase chain reaction

repeat expansion responsible for Huntington's disease.[25] In all cases, history and investigations suggested a primary cause of the disease. All patients were interviewed and examined by a neurologist expert in the field of movement disorders. All index cases were tested for the GAG deletion in the *DYT1* gene (c.946delGAG mutation). When possible, relatives were also investigated blind to the genetic results. After written informed consent, a standardised questionnaire, based on an interview and a clinical examination, was filled out for each individual (index case or relative) and a blood sample was obtained for DNA extraction.

PCR amplification and sequence analysis of the *SGCE* gene

Genomic DNA was extracted from peripheral leucocytes by standard methods (phenol/chloroform). The 12 exons and the exon-intron junctions were amplified by the polymerase chain reaction (PCR). In addition, the 11b exon recently identified in a transcript from mouse brain was also amplified by PCR. PCR primers for amplification of the *SGCE* gene and the amplification conditions are shown in table 2. The amplified fragments were sequenced on a ABI 3730 automated sequencer using the BigDye Version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). Ninety unrelated control subjects were screened for the three new missense and three splice site mutations to exclude possible polymorphisms.

RT-PCR analysis of the c.232 +2T>C mutation

Total RNA was extracted from immortalised lymphocytes of a patient in family 1 with the c.232 +2T>C splice site mutation using the RNeasy Mini kit (Qiagen, Valencia, CA). RNA was reverse transcribed using oligo(dT) primer (ImProm-II Reverse Transcriptase; Invitrogen, Carlsbad, CA), and the full length *SGCE* cDNA was amplified by PCR using the forward primer 5'GAATCGAGGACGGACGGC3' in the promoter region and reverse 12R primer shown in the table 2. The PCR products corresponding to the expected 1437 bp cDNA were extracted from 1.5% agarose gel with a commercial kit (Qiaquick PCR purification kit; Qiagen) and sequenced with both forward and reverse primers.

Statistical analysis

Data are expressed as mean ± SD (min−max) or % (n). To increase the power of the comparison between *SGCE* mutation carriers and non-carriers, we added four previously published French families with *SGCE* mutations recruited with the same inclusion criteria as the patients reported in

Table 1 Characteristics of the published patients with *SGCE* mutations

Nucleotide or amino acid change	Localisation in gene	Affected (n)	Origin	Age at onset (year)*	Myoclonus (distribution)	Dystonia (distribution)	Alcohol sensitivity	References
Truncating mutations								
p. 97R>X	Exon 3	3	German	3.5	NK	H, N	NK	Zimprich *et al*[8]
p. 97R>X	Exon 3	1	NK	0.3	A, L, N	N	Yes	Valente *et al*[12]
p. 97R>X	Exon 3	1	NK	3	A, N	A, N	No	Valente *et al*[12]
p.102R>X	Exon 3	7	German	4	NK	H, N	NK	Zimprich *et al*[8]
p.102R>X	Exon 3	8	German	8	NK	N	NK	Zimprich *et al*[8]
p.102R>X	Exon 3	5	German	5–18	A, N, T	H, N	Yes	Asmus *et al*[13]
p.102R>X	Exon 3	3	German	2–6	A, L, N, T	H, L	Yes	Asmus *et al*[13]
p.102R>X	Exon 3	3	French	0.5–6	A, F, N, T	A, H, N	Yes	Asmus *et al*[13]
p.102R>X	Exon 3	13	Canadian	Childhood	A, F, N	N	Yes	Han *et al*[14]
p.102R>X	Exon 3	2	Canadian	1–14	A, N	None	NK	Han *et al*[14]
p.102R>X	Exon 3	1	German	7	A	H	Yes	Hedrich *et al*[15]
p.102R>X	Exon 3	1	German	13	A, N	Segmental	NK	Hedrich *et al*[15]
p.286Q>X	Exon 7	3	French	Childhood	A, N, T	A, L, T	NK	Asmus *et al*[13]
p.372R>X	Exon 9	1	NK	3	A, L, N, T	A, L, N	Yes	Valente *et al*[12]
p.372R>X	Exon 9	1	NK	11	A, L, N	A, L	Yes	Valente *et al*[12]
Missense mutations								
p.60H>P	Exon 2	1	German	7	A, F, T	N	Yes	Hedrich *et al*[15]
p.60H>P	Exon 2	5	Serbian	7–15	A, H, L	T, H, L; none for 2	Yes	Schule *et al*[16]
p.196L>R	Exon 5	2	German/ English/Welsh	2–10	A, F, L, V	A, H	Yes	Klein *et al*[17]
Splicing mutations								
c.233−1G>A	Intron 2	3	French	3–10	A, N	G	Yes	Asmus *et al*[13]
c.391−3T>C	Intron 3	1	NK	30	A, N	None	NK	Valente *et al*[12]
c.463+6T>C	Intron 4	1	United Kingdom	3	A, N, T	A, L	Yes	Asmus *et al*[13]
c.662+5G>A	Intron 5	3	German	1.5–12	N, A or G	H, N	Yes	Asmus *et al*[13]
c.825+1G>A	Intron 6	4	German	4	NK	N	Yes	Zimprich *et al*[8]
Deletions								
c.164delG	Exon 2	5	Welsh/Czech	<6	A, F, N	N	Yes	Hedrich *et al*[15]
c.276delG	Exon 3	1	German	4	A, L, N	None	Yes	Asmus *et al*[13]
c.391_405del	Exon 4	4	German	7	NK	N	NK	Zimprich *et al*[8]
c.402–405del	Exon 4	6	French	2–30	NK	A, H, L	Yes	Marechal *et al*[18]
c.483delA	Exon 5	11	German	4–12	NK	H, N	Yes	Zimprich *et al*[8]
c.564_576del	Exon 5	1	NK	2	A, N	A, N	Partial	Valente *et al*[12]
c.734_737delAATT	Exon 6	2	French	8–20	A, T	N	Yes	Asmus *et al*[13]
c.832_836delAAAAC	Exon 7	6	Canadian	Childhood	A, F, L, N	A	Yes	Han *et al*[14]
c.835_839delACAAA	Exon 7	8	German/ English/Welsh	2–16	A, F, H, L, T, V	A, L, N , T	NK	Klein *et al*[17]
c.966delT	Exon 7	2	German	4–9.5	NK	NK	NK	Muller *et al*[11]
c.966delT	Exon 7	6	Serbian	4–15	A, H, L	T, L; none for 2	NK	Schule *et al*[16]
c.974delC	Exon 7	9	Danish	1–4	L, N, T, V	A, H, L	Yes	Hjermind *et al*[19]
Insertions								
c.625insG	Exon 5	1	German	2	NK	NK	NK	Muller *et al*[11]
c.885insT	Exon 7	4	German	15–17	A, F, H, N	N, T	NK	Foncke *et al*[20]

A, arm; F, face; G, generalised; H, hand; L, leg; N, neck; NK, not known; S, shoulders; T, trunk; V, voice.
*Range of age at onset. When only one value is given, it is the mean age at onset in the family.

the present study.[13] Age of onset was compared using Student's *t* test. Alcohol responsiveness and distribution of the symptoms were compared using the χ^2 test or the Fisher exact test when appropriate. Sensitivity and specificity, that is the probability of a correct diagnosis based on phenotype given the diagnosis based on genotype, were also computed. Only index cases were used for these analyses. All statistical tests were two tailed. Statistical significance was defined as $p<0.05$. Statistical analyses were performed with the use of the SAS statistical package, version 8.2 (SAS Institute, Cary, NC).

RESULTS

Patients with mutations in the *SGCE* gene

In the 76 index patients tested, we found 13 different mutations in the *SGCE* gene in 16 patients. Eleven index cases had M-D and five E-M, two of whom had at least one relative with myoclonus dystonia (M-D). The total number of mutation carriers in the families was 38 (16 index cases and 22 relatives), six of whom were asymptomatic (table 3).

Seventeen of the 32 affected patients had M-D and 15 had E-M. Their mean age at onset was 7.2 (SD 6.7) years (range: 1–24), whereas the mean age at examination of the asymptomatic carriers was 40.2 (SD 8.8) (range: 30–53). In most patients (69%), myoclonus was the first symptom. Seven of the 10 patients tested for a response to alcohol experienced a mean decrease in their symptoms of 79% (SD 23%). None of the patients reported alcohol addiction. No severe psychiatric disorders were detected: a careful interview of the patients and their families uncovered no past medical histories, no complaints of marked depression, or no patent obsessive or compulsive symptoms.

In 12 families (75%), at least one other member of the family had a myoclonic syndrome corresponding to M-D (10 families) or E-M (two families). There was no known family history for the remaining four. Information on the transmitting parent was available for 24 mutation carriers. All carriers who received the mutated allele from their father were affected (complete penetrance), whereas all carriers who received the mutated allele from their mother were asymptomatic (null penetrance).

Comparison of patients with and without *SGCE* mutations

The features of the 60 index patients without mutations in the *SGCE* gene are given in table 4. A family history of M-D or dystonia was found in 38% (n = 23) of the patients (family history unknown for four patients). The mother transmitted the disease in 11 cases, the father in the other 12. Interestingly, one patient with primary myoclonic dystonia that responded extremely well to alcohol carried the *DYT1* mutation. This patient developed writer's cramp at age 18 and myoclonus 4 or 5 years later. Both upper and lower limbs were affected. His mother, of Ashkenazi Jewish origin, suffered from writer's cramp without myoclonus.

Index patients without *SGCE* mutations were compared to those with *SGCE* mutations, including four previously published families recruited using the same inclusion criteria.[13] The four previously published families comprised 16 carriers (nine with M-D, two with E-M, and five asymptomatic subjects) who were added to our 38 carriers. M-D patients with *SGCE* mutations were significantly younger at onset than non-carriers (8.2 (SD 7.6) (0.5–38) years *v* 15.6 (SD 15.0) (0.25–64) years, respectively; $p<0.003$) and their symptoms were more frequently sensitive to alcohol (82% *v* 31%, respectively; $p<0.0007$). However, information on alcohol responsiveness was available for only 17 carriers and 32 non-carriers, but 40% of the patients where this information was not available were under the age of 18. Since *SGCE* mutations were only found in patients with M-D or E-M, the analyses were also performed after excluding all other phenotypes and the results were similar. When patients with M-D or E-M phenotypes with or without *SGCE* mutations were compared, the location of the myoclonus was similar in both groups, but the location of dystonia differed ($p<0.001$): 73% of mutation carriers had dystonia in upper limbs only, 12% in lower limbs only, and 15% in both upper and lower limbs, whereas 47% of non-carriers had dystonia in upper limbs only, 0% in lower limbs only, and 48% in both upper and lower limbs (5% had no dystonia).

Table 2 Primer sequences and PCR conditions of *SGCE* exons

Exons		Primers	Annealing temperature (C°)	Amplimer size (bp)
1	1F	TGCTGAACTGGCCAAGCTGG	64	301
	1R	AGAGAGGCTGGTGCCCAAAG		
2	2F	GGCGTATCTCATTATTTGTC	55	434
	2R	AGGTAGATCACTTGTCAGAG		
3	3F	CATGTGTGAAAATAACTGTC	53	311
	3R	GGTAACTTTAGTTTCAACAC		
4	4F	ATGAAAATGGAAAGAATGAC	55	290
	4R	AGTTATATTAGGTATGTGGC		
5	5F	CCAGGATTATGACAGAACTC	55	393
	5R	GCAATAGGCCATCTTCCATC		
6	6F	AGGGATGAGTCTAGTTAATC	57	343
	6R	CAAACGTTAACTCCAGCCAC		
7	7F	GAATGCTTTAGTGTATCCAG	53	348
	7R	GTTGTTATCTTAGCAGGATC		
8	8F	GCATATAGTCTTAATGTTCC	53	171
	8R	CACATGTATGGAGCATGATG		
9	9F	AATTGATGACCCATCAGGCT	55	341
	9R	CACAACAACAGAAAGCTCTG		
10	10F	CAGTTGCATTTGGCAGACC	52	574
	10R	TTCTGCATAGCCATTCCATC		
11	11F	TCATTCTAGTATGTCTGCTC	53	220
	11R	TTTGGTGAAGATAAAGCTTC		
11b	11bF	GGCATTGTGGTAGGGAAAC	58	304
	11bR	GCTTACAAAGTAGCACCAAC		
12	12F	GTATCCATGCCCTGACTAAC	55	158
	12R	AGCTCATGCATTATTGGAAG		

F, forward primer; R, reverse primer.

Table 3 Clinical features of carriers of *SGCE* mutations

Family no./ sex	Nucleotide change	Amino acid change	Exon	Familial trans-mission*	Diagnosis	Age of onset	Age at examination	First symptom	Dystonia (location)†	Myoclonus (location)†	Sensitivity to alcohol¶
Splicing mutations											
1/M‡	c.232 +2T>C	Splicing	2	NK	E-M	23	64	Myoclonus	–	NK	NK
1/F	c.232 +2T>C	Splicing	2	F	E-M	4	30	Myoclonus	–	N, T	NK
1/F	c.232 +2T>C	Splicing	2	F	E-M	24	25	Myoclonus	–	N, A, T	NK
1/M	c.232 +2T>C	Splicing	2	F	E-M	NK	34	Myoclonus	–	F, N, A	NK
1/M	c.232 +2T>C	Splicing	2	F	E-M	4	9	Myoclonus	–	NK	NK
372/M	c.233 −1G>T	Splicing	3	NK	Unaffected	–	37	–	–	–	–
372/F‡	c.233 −1G>T	Splicing	3	F	M-D	4	13	Myoclonus	A, T	N, A	NK
372/F	c.233 −1G>T	Splicing	3	F	E-M	4	6	Myoclonus	–	L	NK
462/M‡	c.232 +1G>A	Splicing	2	F	M-D	6	12	M-D	A	N, A	NK
Nonsense mutations											
302/M	c.1114 C>T	R372X	9	NK	E-M	Adolescence	73	Myoclonus	–	F, A, T	No
302/M‡	c.1114 C>T	R372X	9	F	E-M	Childhood	43	Myoclonus	–	NK	NK
302/F	c.1114 C>T	R372X	9	F	M-D	Childhood	44	Myoclonus	A	A	NK
414/M	c.300 G>A	W100X	3	NK	Unaffected	–	47	–	–	–	–
414/M	c.300 G>A	W100X	3	F	E-M	2	14	Myoclonus	–	N, A	NK
414/F‡	c.300 G>A	W100X	3	F	M-D	2	4	Myoclonus	L	A, L	NK
565/M‡	c.300 G>A	W100X	3	NK	E-M	3	20	Myoclonus	–	N, A, L, T	Yes (0.5)
598/M	c.208 G>T	E70X	2	NK	M-D	5	49	NK	N, A, T	N, A	Yes (0.8)
598/F	c.208 G>T	E70X	2	F	M-D	12	28	Myoclonus	A	N	NK
598/F‡	c.208 G>T	E70X	2	F	E-M	10	20	Myoclonus	–	N, A	Yes (1)
664/F‡	c.300 G>A	W100X	3	NK	E-M	3	20	Myoclonus	–	N, A, L, T	Yes (1)
Missense mutations											
127/M‡	c.812 G>A	C271Y	6	NK	M-D	1.5	9	Dystonia	F, A, L, T	A, L	NK
429/F	c.344 A>G	Y115C	3	NK	E-M	NK	68	Myoclonus	–	F	NK
429/M	c.344 A>G	Y115C	3	F	M-D	Adolescence	43	Dystonia	A	A	No
429/F‡	c.344 A>G	Y115C	3	F	M-D	5	39	Dystonia	N, A, T	N, A	No
429/F	c.344 A>G	Y115C	3	M	Unaffected	–	39	–	–	–	–
429/F	c.344 A>G	Y115C	3	M	Unaffected	–	32	–	–	–	–
429/M	c.344 A>G	Y115C	3	M	Unaffected	–	30	–	–	–	–
429/F	c.344 A>G	Y115C	3	F	M-D	12	19	Dystonia	N, A	N, A	NK
429/F	c.344 A>G	Y115C	3	F	E-M	1	5	Myoclonus	–	N, F , A	NK
632/F‡	c.274 T>C	M92T	3	F	M-D	1.75	9	Subacute ataxia	L	A, L	NK
Insertion											
294/F‡	c.745_746insTGTA	S249fsX250	6	F	M-D	Before 6	46	Myoclonus	L	N, A	Yes
Deletions											
6/F‡	c.832_836delAAAAC	K278fsX295	7	F	M-D	2	10	Dystonia	N, L	N, A	NK
6/F	c.832_836delAAAAC	K278fsX295	7	F	M-D	7	7	M-D	A	A	NK
138/M	c.444_447delTAAT	N149fsX149	4	NK	Unaffected	–	53	–	–	–	–
138/F‡	c.444_447delTAAT	N149fsX149	4	NK	M-D	18	23	Myoclonus	N, T	N	Yes (1)
138/F	c.444_447delTAAT	N149fsX149	4	NK	E-M	6	27	Myoclonus	–	F, N, T	NK
486/F‡	c.444_447delTAAT	N149fsX149	4	NK	M-D	3	20	Dystonia	N, A	N, A, L	Yes (0.7)
562/F‡	c.221delA	Y74fsX85	2	NK	M-D	3	29	Myoclonus	A	N, A, L	NK

E-M, essential myoclonus; F, female; M, male; M-D, myoclonus-dystonia; NK, not known.
*F, father; M, mother; †A, arm; F, face; G, generalised; L, leg; N, neck; NK, not known; T, trunk; V, voice; ‡index cases; ¶percentage of decrease in symptoms after alcohol intake is shown in parentheses.

Which patients should be tested for *SGCE* mutations to optimise screening

All patients with *SGCE* mutations had an M-D or E-M phenotype, indicating a sensitivity of 100%. However, 63% of the patients without *SGCE* mutations also had an M-D or E-M phenotype, indicating a specificity of only 37%. Conversely, 30% of M-D or E-M patients had *SGCE* mutations, whereas no mutations were found in patients with other phenotypes. Taking into account the age at onset and genetic parameters improves specificity. If patients with mother to child transmission of the disease were excluded, the specificity of the mutation screen increased to 43%. In addition, if only patients with onset before the age of 25 were tested, the specificity increased to 55%. In both cases, sensitivity was still 100%.

Molecular genetics

Mutation analysis

Eleven of the 13 different *SGCE* mutations identified were novel (table 3). The two previously reported mutations were a splice site mutation in intron 2 (c.233 –1G>A, French family from a different region)[13] and a deletion in exon 7 (c.832_836delAAAAC, Canadian family).[14] The types of mutation found in the 16 index carriers were five nonsense mutations, three missense mutations, three splice site mutations, four deletions, and one insertion. These mutations are all likely to be causative. All the deletions and the insertion caused a frameshift in the coding region that introduced a premature stop codon resulting in a truncated protein, as for the five nonsense mutations. The three putative splice site mutations affected major bases of the consensus splice site sequence. One (c.233 –1G>A) was previously reported, the other (c.232 +2T>C) has been validated by RT-PCR (see below). The two new putative splice mutations were not present on 180 chromosomes from Caucasian controls. The *SGCE* missense mutations were all non-conservative amino acid changes affecting conserved amino acids and were also absent from the control chromosomes.

In addition, three variants were identified in the alternative exon 10 in our patients. The A to C substitution at nucleotide 40 of exon 10 had already been reported in a genomic database (www.ensembl.org), the other two were G to A substitutions at nucleotides 41 and 43 of exon 10. These variants were also found in 156 unrelated controls, indicating that they are most likely to be polymorphisms.

Identification of alternative exon 2 splicing in *SGCE* transcripts

Lymphocytes were available from only one patient with the c.232 +2T>C splice site mutation (fig 1). RT-PCR amplification of mRNA extracted from the cells produced a transcript that was smaller (1314 bp) than the expected size (1437 bp). Sequence analysis showed that this fragment lacked exon 2, resulting in the loss of 41 amino acids (p.37–77) without interrupting the reading frame. In the lymphocytes of a control subject, both the 1437 bp transcript and the 1314 bp transcript were detected, suggesting that exon 2 can be alternatively spliced, at least in lymphoblasts.

DISCUSSION

In this study, we screened the *SGCE* gene in 76 index patients with myoclonic syndromes or phenotypically related disorders, the largest series analysed so far. Mutations in the *SGCE* gene were found only in patients with typical M-D or E-M phenotypes. Although most were familial cases, four patients with apparently sporadic M-D also had *SGCE* mutations. This can be explained by maternal imprinting or because a family history was not available. This suggests that sporadic cases should be screened for *SGCE* mutations when the phenotype is consistent with M-D.

In both the familial and sporadic patients, the initial symptom of the disease was either dystonia or myoclonus that could affect any part of the body. Unlike the patients of Valente *et al*,[12] some of our patients had lower limb involvement. The syndrome developed during childhood or early adolescence and started before the age of 25 in all patients. This is in agreement with previous reports (table 1). There was no information about sensitivity to alcohol for 28 cases, but 40% of these were under the age of 18. When asked, however, several patients reported no response to alcohol. Unlike previous reports,[18 26] no major psychiatric disturbances were found in our patients by careful clinical examination. Although we did not used standardised algorithms for the diagnosis of mental disorders or obsessive compulsive rating scales, the patients did not complain of alcohol addiction, severe depression, or major obsessive or compulsive disorders.

Molecular analysis detected 13 different *SGCE* mutations distributed along the gene between exons 2 and 9, which corresponds to the sarcoglycan domain (tables 1 and 3). However, no mutations were found in exon 8. This might be explained by alternative splicing of exon 8, as in mouse brain,

Table 4 Clinical features of non-carriers of *SGCE* mutations

Diagnosis	No. of cases	Mean age at onset	Myoclonus (distribution)	Dystonia (distribution)	Sensitivity to alcohol	Family history
Myoclonus dystonia	35 (M:15/F:20)	13.7±15.5	UB: 20 UB+LB: 14 LB: 1	UB: 19 UB+LB: 15 UK: 1 (618)	Yes: 5 No: 10 NK: 20	AD(P): 3 AD(M): 4 AD(NK): 1 None: 24 NK: 2
Essential myoclonus	3 (M:2/F:1)	26.0±17.3	UB+LB: 2 UB: 1	None	Yes: 1 No: 2	AD(P): 1 None: 2
Primary myoclonic dystonia	13 (M:6/F:7)	18.7±15.8	UB+LB: 3 UB: 10	UB+LB: 8 UB: 5	Yes: 3 No: 7 NK: 3	AD(P):2 AD(M):4 ASP: 1 None: 6
Dystonia with tremor	5 (M:3/F:2)	16.1±13.6	None	UB+LB: 2 UB: 3	Yes: 1 No: 2 NK: 2	AD(P):1 AD(M):1 ASP: 1 None: 1
Generalised dystonia	3 (F:3)	4, 14	None	UB+LB	No: 1 NK: 2	AD(M): 1 ASP: 1
BHC	1 (M)	Childhood	UB	UB+LB	NK	AD(P)

AD(M), autosomal dominant with maternal inheritance; AD(P), autosomal dominant with paternal inheritance; AD(UK), autosomal dominant with transmitting parent unknown; ASP, two or more affected sibs; BHC, benign hereditary chorea; F, female; LB, lower body; M, male; NK, not known; UB, upper body.

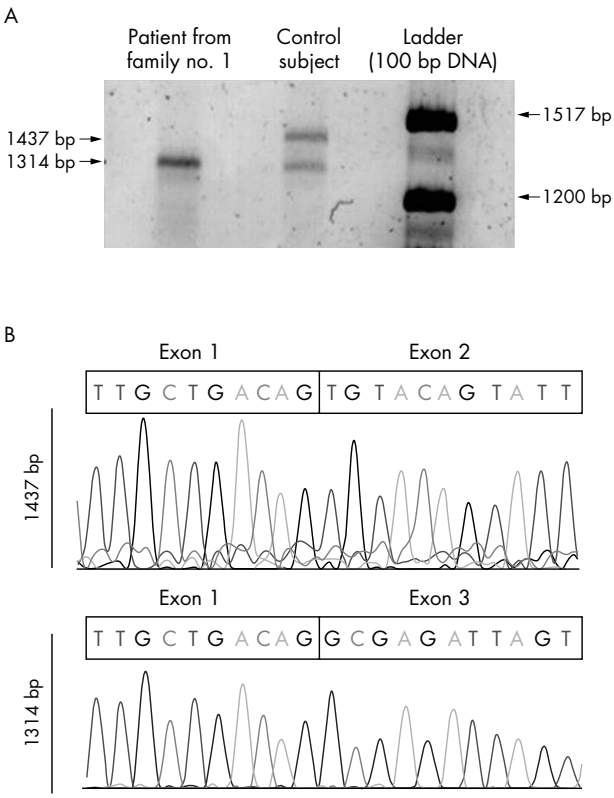

Figure 1 Alternative splicing of exon 2 in *SGCE* transcripts. (A) RT-PCR amplification of *SGCE* produced evidence of two *SGCE* transcripts in the lymphocytes of a control subject, a 1437 bp sequence and a 1314 bp sequence, visualised after agarose gel electrophoresis. Only the smaller fragment was amplified in a patient with the c.232 +2T>C splice site mutation. (B) Sequencing of the two transcripts showed that only the 1437 bp fragment contains exon 2.

where exon 11b is expressed.[8] However, our systematic screening of exon 11b, the first to be performed, did not detect mutations in this sequence either. All of the missense mutations identified affected amino acids that are conserved in other species, suggesting that they alter an important function. The W100X nonsense mutation in exon 3 was the most frequent mutation in our patients (3/16). Except for the R102X nonsense mutation found in nine families (six German, two Canadian, and one French), the majority of mutations are different in each family. All types of mutations have been found (tables 1 and 3): nonsense mutations (20%), missense mutations (14%), splice site mutations (20%), deletions (37%), and insertions (9%). The great majority (86%) give rise to aberrant or truncated proteins. There were no obvious differences, however, in the phenotypes (clinical features or age of onset) of patients with different mutations.

The phenotypes of 38 of the 60 patients without *SGCE* mutations were very similar to those of patients with mutations (that is, M-D and E-M). However, there were significant group differences. Mutation carriers had significantly earlier onset, more frequent responses to alcohol, and lower limb dystonia. The frequency of *SGCE* mutations increased when patients with onset after the age of 25 or who inherited the disease from their mother were excluded. The clinical pattern of myoclonus and dystonia does not predict the genetic status of the patients,[12 27] but more detailed analyses, including electrophysiological investigations, could increase specificity. This should be confirmed in future studies.

One of our patients of Ashkenazi origin with primary myoclonic dystonia, whose mother had writer's cramp, had a *DYT1* mutation. This patient had symptoms that responded to alcohol as do M-D patients. Since this patient was first examined at the age of 69, the age at onset given (18) might not be accurate. Although a myoclonic syndrome resembling M-D is relatively rare in patients suffering from early onset primary dystonia, it has been previously reported.[28–30] We tested one patient with BHC and found no mutation in this patient. A total of four patients with BHC have been tested negative for mutations in the *SGCE* gene.[12] The number of tested patients with primary myoclonic dystonia, dystonia associated with tremor, generalised dystonia, and BHC is relatively small, so that only a frequent involvement of the *SGCE* gene in these diseases can be excluded.

In conclusion, this large cohort of patients has enabled us to show that *SGCE* mutations are exclusively found in patients with typical myoclonus-dystonia or E-M, but only in a subset (30%), indicating further genetic heterogeneity in this disease. Excluding patients with onset after the age of 25 and those who inherited the disease from their mothers would increase the specificity of the analysis. Furthermore, our data suggest that testing for *SGCE* mutations in other related phenotypes is likely to be negative. In *SGCE* negative families, especially when several members are affected, other as yet unidentified genes may be involved.

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ELECTRONIC-DATABASE INFORMATION

The Ensembl web site can be found at www.ensembl.org. Mutations are registered in the Human Gene Mutation Database at http://www.hgmd.cf.ac.uk

Authors' affiliations
S Tezenas du Montcel, Service de Biostatistique et Information Medicale, Hôpital Pitié-Salpêtrière, AP-HP, Paris, France
F Clot, M Vidailhet, A Camuzat, A Durr, A Brice, INSERM U679, Groupe Hospitalier Pitié-Salpêtrière, Paris, France
E Roze, Service de Neurologie, Hôpital Saint-Antoine, AP-HP, Paris, France
P Damier, Service de Neurologie, Hôpital Laennec, Nantes, France
C P Jedynak, Fédération de Neurologie et Département de Génétique Cytogénétique et Embryologie, Groupe Hospitalier Pitié-Salpêtrière, AP-HP, Paris, France
A Lagueny, Service de Neurologie, Hôpital du Haut-L'Evêque, Pessac, France
L Vercueil, Service de Clinique Neurologique, CHU de Grenoble, Grenoble, France
D Doummar, Service de Neuropédiatrie, Hôpital Armand Trousseau, AP-HP, Paris, France
L Guyant-Maréchal, D Hannequin, Unité de Génétique Clinique and Inserm U614, Hôpital Charles Nicolle, Rouen, France
J-L Houeto, Service de Neurologie, Hôpital La Milétrie, Poitiers, France
G Ponsot, Service de Neuropédiatrie, Hôpital Saint Vincent de Paul, Paris, France

S Thobois, Service de Neurologie, Hôpital Pierre Wertheimer, Lyon, France
M-A Cournelle, Service de Pédiatrie, Centre Hospitalier du Pays d'Aix, Aix en Provence, France
F Durif, Service de Neurologie, Hôpital Gabriel Montpied, Clermont-Ferrand, France
B Echenne, Service de Neuropédiatrie, Hôpital Gui de Chauliac, Montpellier, France
C Tranchant, Département de Neurologie, Hôpital Civil, Strasbourg, France

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