Myoclonus–Dystonia Syndrome: ε-Sarcoglycan Mutations and Phenotype

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Mutations in the gene for ε-sarcoglycan (*SGCE*) have been found to cause myoclonus-dystonia syndrome. We now report clinical and genetic findings in nine additional European families with myoclonus-dystonia syndrome. The clinical presentation in 24 affecteds was homogeneous with myoclonus predominantly of neck and upper limbs in 23 of them and dystonia, presenting as cervical dystonia and/or writer's cramp, in 13 cases. Six novel and one previously known heterozygous *SGCE* mutations were identified. *SGCE* deficiency seems to be the common pathogenetic mechanism in myoclonus-dystonia syndrome.

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Myoclonus-dystonia syndrome (MDS) is an autosomal-dominant disorder characterized by brief, frequently alcohol-responsive myoclonic jerks with onset in childhood or early adolescence.[1] Usually neck and upper limbs are more involved than legs and gait. Cervical or brachial dystonia accompanies myoclonus in many patients. Psychiatric symptoms (obsessive-compulsive disorder, panic attacks, or alcohol dependence) have been reported in several families.[2,3] Laboratory and magnetic resonance imaging findings are typically normal.

The locus for MDS has been mapped to the 7q21 region.[4–6] Using a positional cloning approach, we previously identified five different heterozygous mutations in the gene for ε-sarcoglycan.[7] Reduced penetrance on maternal transmission of the disease allele was observed, suggesting maternal genomic imprinting, which also has been shown for the mouse ε-sarcoglycan gene.[8]

In this study, we sequenced all 11 exons and the splice variant of exon 9 of the *SGCE* gene in nine families with MDS from different genetic backgrounds. Six novel and one previously known mutations were identified. Here, we present the clinical and genetic findings of patients with genetically proven MDS.

Patients and Methods

Patients

This study was approved by the local ethics committee. We selected nine families from Germany, France, and the United Kingdom with index patients showing an MDS phenotype according to published criteria.[1] In three pedigrees (1, 7, and 8) linkage to the 7q21 region had been shown previously.[5,9] After obtaining informed consent, index patients and family members were examined systematically. Venous whole blood samples were taken, and DNA was extracted using standard protocols.

DNA Sequence Analysis

DNA was prepared from peripheral blood lymphocytes. Polymerase chain reaction (PCR) products amplified from 50ng genomic DNA were sequenced using the dideoxy cycle technique with the AmpliSequence Kit (Perkin-Elmer, Norwalk, CT) after purification with Qiaquick PCR Purification Kit (Qiagen, Chastworth, CA). Primer sequences and PCR conditions are available at htttp://www.neurogenetik.de/mds/primer/index.html.

Genotype Analysis of the SGCE *Locus*

In six subjects from Pedigrees 1, 2, and 8, carrying a common mutation (R102X, exon 3), haplotype analysis of microsatellite markers around the *SGCE* locus was performed. PCR products of D7S1513, an anonymous CA repeat marker in intron 3 (69292), and D7S1489 were analyzed using automated sequencers (for marker details see Zimprich and colleagues[7]).

Results

Mutational Screening

We sequenced all 11 exons and the splice variant of exon 9 in patients of each pedigree. Seven different mutations could be identified and are summarized in Table 1.

Two different mutations were detected in exon 3.

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The R102X mutation had been previously described in two German families.[7] We now detected this mutation in one French and two additional German families. No evidence of haplotype sharing was found (data not shown), indicating that these mutations arose independently.

In Family 3, a guanosine-deletion at position 276 (exon 3) and in Family 9 a 4bp deletion at position 733 (exon 6) were detected. These mutations cause a frameshift and a premature termination of protein translation.

Three different splice site mutations occurred in Pedigrees 4, 5, and 6, respectively. These sequence changes are located at splice acceptor/donor sites and segregate with the MDS phenotype. Mutations at intronic position +5 or +6 could not be detected in 120 control chromosomes. As shown, for example, in β-thalassemia[10] these splicing mutations lead to aberrant RNA processing and presumably to a loss of function of ε-sarcoglycan by nonsense-mediated mRNA decay. However, this remains to be proven by functional studies.

Phenotype of Patients with SGCE Mutations

Affecteds of all nine families presented with a spectrum of abnormal movements characteristic for inherited myoclonus-dystonia syndrome, as suggested previously[1] (Table 2). Mean age at onset for myoclonus was 5.4 years (n = 23; range, 0.5–20 years). Myoclonus predominantly affected neck, trunk, and upper extremities. In Families 2, 3, and 4, the lower limbs also were affected. Dystonia presented mostly in a focal distribution, that is, cervical dystonia and/or writer's cramp. The mean age at onset of dystonia was 8.8 years (n = 13; range, 1–38 years), often occurring in parallel with worsening of myoclonus and therefore most likely reflecting progression of MDS until the patients' early 20s. No patient experienced further progression of symptoms. Only in Family 7 did all three members have myoclonus and postural tremor accompanying upper limb dystonia.

Myoclonus is improved by alcohol ingestion in 21 of 24 affecteds. Notably this effect is dose dependent with improvement in some cases only after heavy drinking. Mainly male affecteds in Family 1 and 4 have severe alcohol problems with periodical excessive drinking.

Five affecteds of three families had a history of panic attacks, depression, and agoraphobia.

Only one patient from Family 2, aged 33 years, showed additional generalized athetoid movements and slight cognitive impairment. Magnetic resonance imaging scans in this case showed global brain atrophy, probably as a consequence of complicated delivery. Therefore, his phenotype is likely to represent comorbidity with infantile cerebral palsy.

Other neurological abnormalities were not detected, including signs of muscular dystrophy, polyneuropathy, and dysautonomia. Electrophysiological studies in seven patients showed normal nerve conductance times and no signs of chronic denervation.

Gender-specific Reduction of Penetrance and Genomic Imprinting

Pedigree analyses identified eight maternal transmissions of SGCE mutations in five families. Seven of these mutation carriers were clinically asymptomatic. This observed pattern of inheritance is indicative of genomic imprinting and confirms our previous observations in four German MDS pedigrees.[7]

Table 1. Mutations of the SGCE *Gene in Myoclonus–Dystonia Syndrome*

Family No.	Origin	Exon	Mutation	Number of Affecteds	Myoclonus Mean Age at Onset in Years (range)	Myoclonus Distribution	Dystonia Mean Age at Onset (range)	Dystonia Distribution	Alcohol Sensitivity	Additional Symptoms
1	D	3	R102X	5	8 (5–18)	Neck, trunk, UL	12 (6–18)	TC, WC	+	Alcohol abuse, panic attacks
2	D	3	R102X	3	4.5 (2–6)	Neck, trunk, UL and LL	4 (2–6)	WC, LL	+	Panic attacks, comorbidity with infantile cerebral palsy in one case
3	D	3	276 del G	1	4	Neck, UL, LL	—	—	+	—
4	D	5	1037 + 5G → A	3	3 (1.5–12)	Neck, shoulders, generalized	4.5 (3–6)	TC, WC	+	Alcohol abuse, panic attacks, agoraphobia
5	UK	4	463 + 6T → C	1	3	Neck, trunk, UL	15	UL, LL	+	—
6	F	3	233 − 1G → A	3	3 (n = 1)	Neck, shoulders, UL	5.5 (3–10)	Multifocal, generalized	+	—
7	F	7	Q286X	3	Childhood	Neck, trunk, UL	14.5 (12–17)	UL, trunk, LL	n.t	Postural tremor UL (n = 3)
8	F	3	R102X	3	3.5 (0.5–6)	Face, neck, trunk, UL	15 (1–38)	TC, WC, UL	+	Postural tremor UL (n = 1)
9	F	6	733delAATT	2	14 (8–20)	Trunk, UL	8 (n = 1)	TC	+	—

D = Germany; UK = United Kingdom; F = France; TC = torticollis; WC = writer's cramp; UL = upper limb; LL = lower limb; n.t. = not tested.

Table 2. Clinical Characteristics of 24 Myoclonus–Dystonia Syndrome Patients from Nine European Pedigrees

	At Onset		At Examination	
Clinical Signs	n = 24	%	n = 24	%
Myoclonus	19	79	23	96
Face/voice	2/—	8/—	2/2	8/8
Neck/axial/shoulders	11	46	19	79
UL	16	67	21	88
LL	2	8	3	13
Dystonia	9	38	13	54
Neck	9	38	11	46
Axial/shoulder	—	—	5	21
UL/writer's cramp	8	33	13	54
LL	2	8	4	16
Postural tremor	2	8	4	16

UL = upper limb; LL = lower limb.

Discussion

Our data confirm the key role of heterozygous mutations of ε-sarcoglycan in the pathogenesis of MDS. Clinical analysis of 24 patients from nine families with proven SGCE mutations indicates a relatively homogeneous clinical picture:

Most patients showed the typical, very brisk, "lightning-like,"[11] myoclonic jerks mainly affecting neck, trunk, and upper limbs. In approximately half of the patients (54%), additional focal or segmental dystonia was found, presenting as cervical dystonia and/or writer's cramp. In contrast to primary generalized dystonia,[12] involvement of lower limbs was rare and usually did not occur at onset, but as part of the progression of the disease.

Only one mutation carrier, from Family 8, showed no myoclonus but cervical dystonia accompanied by postural tremor with a late age at onset of 38 years. This phenotype is unusual for MDS but similar to sporadic cervical dystonia. Further studies will be necessary to determine whether SGCE mutations are pathogenic in a significant proportion of patients with this phenotype.

Psychiatric manifestations have been reported in MDS families[2] and were examined in detail by Saunders-Pullman and colleagues.[3] In our study, panic attacks and alcohol abuse were self-reported only by four patients in three pedigrees. Because we did not conduct systematic psychiatric interviews, the true prevalence of these symptoms may be underestimated.

Markedly reduced penetrance of the MDS phenotype upon maternal inheritance of the mutated allele has been noticed in many families[5–7,9]and also has been observed in this study (for pedigree example see Fig). Inactivation of the maternal SGCE allele ("genomic imprinting"), probably by methylation, has been suggested to account for this phenomenon. However, the apparent suppression of the phenotype by maternal inheritance is incomplete. As in our previous study,[7] approximately 10% of affecteds had inherited the mutated allele from their mother. The mechanism for this "reversal" is unclear. It could be speculated that either a variation in the expression levels of the intact paternal allele or a deviation from the expected differential imprinting pattern could be responsible.

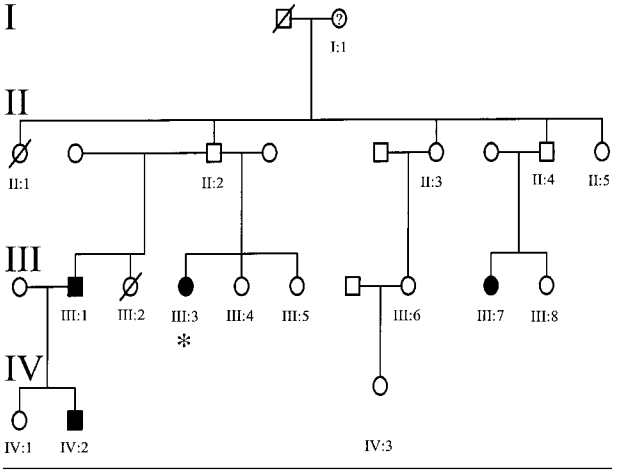
In Pedigrees 3 and 5, family history was negative. Assuming "genomic imprinting," this has to be expected in a proportion of cases, because maternal transmission may obscure a positive family history. Therefore, mutation screening in patients with a typical MDS phenotype but apparently negative family history may be useful.

ε-Sarcoglycan is a member of the sarcoglycan protein family, but in contrast with α-, β-, γ- and δ-sarcoglycan, mutations in this gene have not been reported to cause muscular dystrophy.[13]

Widespread expression of ε-sarcoglycan has been shown in embryonic and adult tissues,[14] including the outer cell membrane of Schwann cells.[15] In our study, patients did not show any clinical or electrophysiological signs of peripheral nerve disease or dysautonomia. Therefore, in Schwann cells loss of ε-sarcoglycan may be compensated, possibly by upregulation of another member of the sarcoglycan family.

SGCE mRNA has been detected in various parts of the brain including basal ganglia and cerebellum,[7] regions commonly implicated in the pathogenesis of movement disorders. However, the subcellular localiza-

Fig. Myoclonus–dystonia syndrome Pedigree 6 with a heterozygous splice site mutation (233-1G→A, exon 3) in the gene for ε-sarcoglycan. Note that Subjects I:1, II:2, and II:4 are obligate mutation carriers but show no MDS symptoms, putatively by inactivation of the mutated maternal SGCE allele ("genomic imprinting"). (filled symbols) *MDS phenotype;* (asterisk) *affected only by family history;* (question mark) *asymptomatic by family history.*



tion and function of ε-sarcoglycan in neurons have not been investigated, and the mechanism by which ε-sarcoglycan deficiency causes MDS is unknown.

Interestingly, in a patient with early-onset dystonia with myoclonic features, a 18bp deletion in the torsin A gene has been reported.[16] Further studies will be needed to clarify whether mutations of SGCE and torsin A act independently to cause MDS or if they are functionally connected.

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Preserved Visual Function in a Case of Occipitoparietal Microgyria

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A 20-year-old man with bilateral parasagittal occipitoparietal polymicrogyria and epilepsy, from whom normal functional magnetic resonance imaging and electroencephalogram responses to visual stimuli were obtained, was found to have no visual perceptual deficits. This suggests that microgyric cortex can perform normal visual functions, despite its gross structural abnormalities.

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Microgyria is a disorder in cortical layering and gyration probably caused by local ischemia before the end of neuronal migration, as suggested by neuropathological and imaging observations in humans[1,2] and by experimental work in animals.[3–6]

Electrophysiological and imaging observations in animals[7] and in man[8] indicate that the microgyric cortex can be activated by normal stimuli and preserves much of its normal functional characteristics. However, it may become hyperexcitable or epileptic.[9–11] Therefore, it is unclear what the consequences of microgyria

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