Mutations in the gene encoding ε-sarcoglycan cause myoclonus–dystonia syndrome

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The dystonias are a common clinically and genetically heterogeneous group of movement disorders. More than ten loci for inherited forms of dystonia have been mapped, but only three mutated genes have been identified so far. These are *DYT1*, encoding torsin A[1] and mutant in the early-onset generalized form, *GCH1* (formerly known as *DYT5*), encoding GTP–cyclohydrolase I and mutant in dominant dopa-responsive dystonia[2], and *TH*, encoding tyrosine hydroxylase and mutant in the recessive form of the disease[3]. Myoclonus–dystonia syndrome (MDS; *DYT11*) is an autosomal dominant disorder characterized by bilateral, alcohol-sensitive myoclonic jerks involving mainly the arms and axial muscles[4,5]. Dystonia, usually torticollis and/or writer's cramp, occurs in most but not all affected patients and may occasionally be the only symptom of the disease[6,7]. In addition, patients often show prominent psychiatric abnormalities, including panic attacks and obsessive–compulsive behavior[8–10]. In most MDS families, the disease is linked to a locus on chromosome 7q21 (refs. 11–13). Using a positional cloning approach, we have identified five different heterozygous loss-of-function mutations in the gene for ε-sarcoglycan (*SGCE*), which we mapped to a refined critical region of about 3.2 Mb. *SGCE* is expressed in all brain regions examined. Pedigree analysis shows a marked difference in penetrance depending on the parental origin of the disease allele. This is indicative of a maternal imprinting mechanism, which has been demonstrated in the mouse ε-sarcoglycan gene[14].

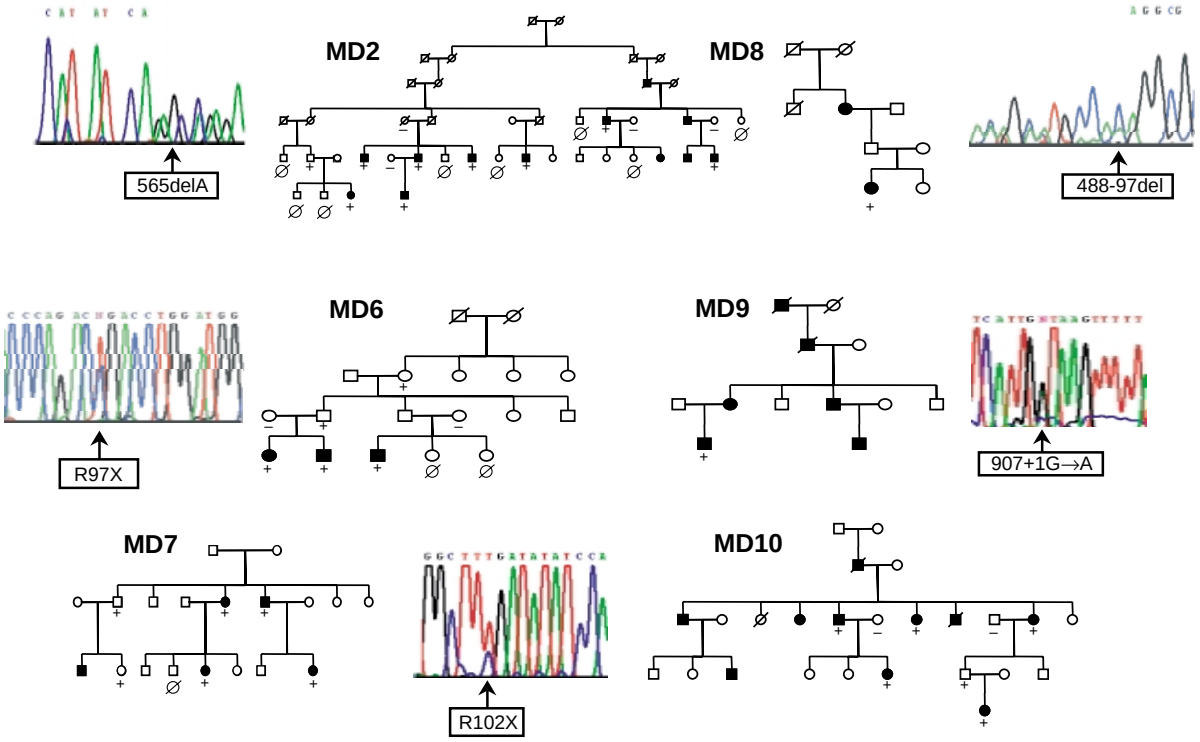

Fig. 1 *SGCE* mutations in MDS families. (+), *SGCE* mutation confirmed by sequencing; (–), mutation excluded by sequencing. Of 11 sequenced asymptomatic at-risk family members, 3 were positive and 8 were negative. Pedigrees have been reduced for greater clarity of presentation. (∅), the genetic status of these individuals is not shown, to protect their privacy.

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Table 1 • Clinical characteristics of families

Family	Origin	Number of living affected patients	M	F	Mean age at onset (yr)	Mc	Tc	Wc	Psychiatric disturbances (number of affected patients)
MD2	North Germany, coastal area	11	9	2	8.7	+	+	+	panic attacks (3)
MD6	East Bavaria	3	2	1	3.5	+	+	+	Obsessive–compulsive disorder (1)
MD7	North Bavaria	7	4	3	4	+	+	+	anxiety disorder (1)
MD8	Bavaria	4	2	2	7	+	+	–	n. a.
MD9	Bavaria	4	3	1	4	+	+	–	panic attacks (1) alcohol abuse (1)
MD10	Prussia	8	3	5	8	+	+	–	n. a.

M, male; F, female; Mc, myoclonus; Tc, torticollis; Wc, writer's cramp; n. a., data not available.

In this study, we included index patients from six German families with MDS (Fig. 1 and Table 1). We established linkage to chromosome 7q21 in families MD2, MD6, MD7 and MD10 by genotyping polymorphic CA repeat markers[13]. In two families (MD8 and MD9), only index patients were available. The critical region had been defined previously by a proximal recombination with *D7S652* (ref. 13) and a distal recombination with *D7S821* (ref. 12). We used an anonymous CA repeat on bacterial artificial chromosome clone AC003078 to reduce the critical region further to approximately 3.2 Mb (Fig. 2). Genomic sequences across this region were available through the Human Genome Project. Analysis of finished and unfinished sequences in the critical region showed 15 genes within this region (14 known and 1 novel) and 2 pseudogenes (transcript map available at http://ihg.gsf.de/chr7/index.html). For mutation screening, we used genomic DNA from index patients of the six families. By complete sequencing of ten of the identified genes, we found seven silent polymorphisms in three genes and one polymorphism leading to an amino acid change in the gene for PPP1R9A (Web Table A).

We detected loss-of-function mutations in *SGCE* in all index patients screened (Fig. 1): two nonsense changes in exon 3, R97X (289C→T) in family MD6 and R102X (304C→T) in families MD7 and MD10. Family MD2 showed a deletion (565delA) leading to a frameshift of the coding region, with a premature stop at codon 169. We identified a splice-site mutation at the 3′ exon–intron junction of exon 6 (907+1G→A) in family MD9 and a deletion of 97 bp, affecting intron 3 and 15 bp of exon 4 in family MD8 (488–97del). We found no sequence alterations in 72 control alleles in any of the 11 exons of the gene. Kindreds MD7 and MD10 have identical mutations. Although they are not known to be related, they share haplotypes for the two closest CA repeat markers typed, *D7S1513* and an anonymous CA repeat marker, located in intron 3 (69292). The mutations segregated with the disease in all four families with more than one affected family member available (Fig. 1). We found reduced penetrance, particularly in the offspring of affected females, indicating the possibility of maternal imprinting. Such a mechanism has been demonstrated for the orthologous mouse gene[14]. Further pedigree analysis in all families with known mutations in *SGCE* or linkage to chromosome 7q21 (MD2 and MD6–10 from our series, the family published by Nygaard *et al.*[11] and families C, E, F and G reported by Klein *et al.*[12]) confirmed that penetrance is highly dependent on the parental origin of the disease allele. Of 62 clinically affected individuals (40 males and 22 females) in the combined families, 49 inherited the disease from their father and only 4 from their mother. In nine cases, parental origin could not be determined. By contrast, we found a maternal origin of the affected allele in 14 of 18 clinically asymptomatic carriers (12 males and 6 females, carrier status being determined either by sequencing, in our families, or by pedigree position in the others), whereas paternal transmission occurred in only 3. In one case, parental origin could not be determined. This inheritance pattern deviates significantly from the expected equal distribution of parental alleles (2×2 contingency table, χ^2 test: $P<10^{-8}$), supporting the assumption that the differential expression of parental alleles contributes to disease penetrance. Four other families with MDS published in the literature[8,15–17] also show a pedigree structure compatible with this mechanism, indicating a lack of locus heterogeneity in this disorder.

SGCE is known to be widely expressed in embryonic and adult tissues[18] and has been localized to smooth muscle[19], to the outer Schwann cell membrane of peripheral nerve[20] and to the brain[19]. However, its distribution in different cell types of the central nervous

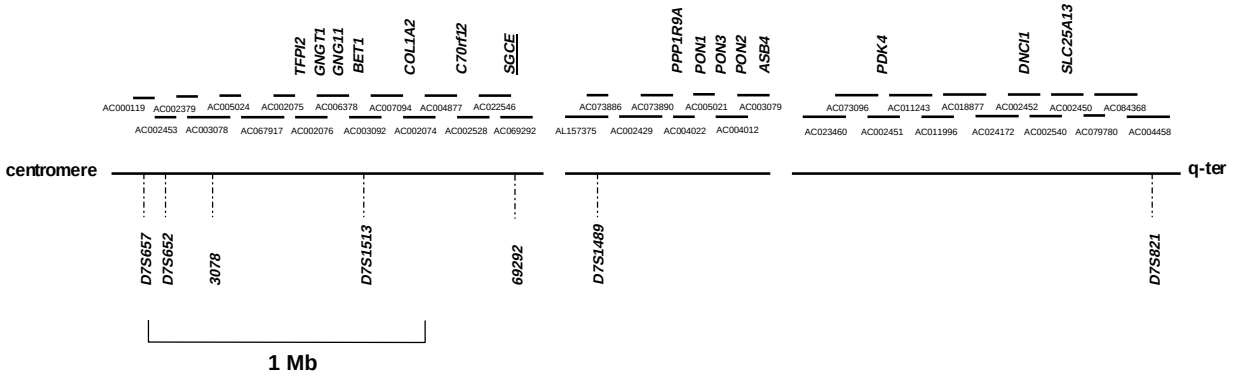

Fig. 2 Physical map of the *SGCE* critical region. The position of DNA markers and BACs are drawn 'to scale' as estimated by the unfinished sequence data. Gaps are shown between clones AC069292 and AL157375 and clones AC003079 and AC023460. The approximate positions of genes between markers 3078 and *D7S821* are indicated.

system had not been investigated. We found *SGCE* to be expressed in tumor cell lines of neuronal origin (Fig. 3*a*). In addition, northern blot analysis showed broad expression in different brain regions (Fig. 3*b*).

The molecular pathogenesis of abnormal movements is poorly understood. So far, investigations have focused on the role of neurotransmitters and their receptors, mainly because these abnormal movements can be induced pharmacologically through interference with dopaminergic and serotonergic neurotransmission. Furthermore, at least one variant, dopa-responsive dystonia, is caused by mutations in genes encoding enzymes of dopamine biosynthesis[2,3], and a disturbance of dopamine release has been suggested as a pathogenic mechanism in generalized dystonia[21]. Finally, a point mutation in the gene for the dopamine D2 receptor has been found in one family with MDS[9] but not in others[13,22,23].

The identification of *SGCE* mutations in MDS for the first time, to our knowledge, implicates a member of the sarcoglycan family, which until now was associated exclusively with muscular dystrophies, in the pathogenesis of a nondegenerative central nervous system disorder. *SGCE* is one of five known members of this family, encoding transmembrane components of the dystrophin–glycoprotein complex[20,24], which links the cytoskeleton to the extracellular matrix. Mutations in the genes encoding α-, β-, γ- and δ-sarcoglycan, which are expressed mainly in muscle, cause autosomal recessive limb-girdle muscular dystrophies[25]. It can be hypothesized that loss of *SGCE*, which is also expressed in brain, results in subtle changes in the neuronal architecture that give rise to the abnormal movements and psychiatric disturbances that are observed. Recent evidence indicates that changes in neuronal 'wiring', rather than in neurotransmitter metabolism, may constitute the underlying defect in mental disorders such as schizophrenia[26]. *SGCE* mutations may therefore have a role not only in more common forms of dystonia but also in a wider range of neuropsychiatric disorders.

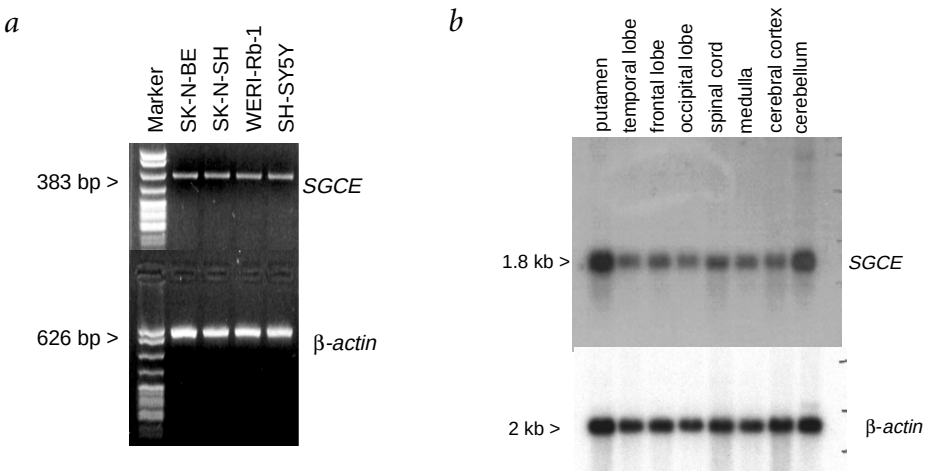

Fig. 3 Tissue expression pattern of *SGCE*. ***a***, RT-PCR analysis of total mRNA from three neuroblastoma cell lines and one retinoblastoma cell line with intron-spanning primers. Upper panel, *SGCE* expression; lower panel, β-actin expression (for comparison). ***b***, Northern blot analysis of different brain regions, hybridized with an *SGCE* cDNA fragment (bp 599–1270). Bottom, loading controls after hybridization with a β-actin probe.

Methods

Families. We identified a total of 37 living affected patients (14 women and 23 men) showing the typical signs of MDS. Patients developed proximal, bilateral, myoclonic jerks, usually involving the arms and axial muscles more than legs and gait, during the first or second decade of life (mean age of onset, 5 yr; range, 1.5–18 yr). Myoclonus was exquisitely responsive to alcohol in most cases. Pedigrees and a summary of clinical characteristics are in Table 1 and Fig. 1. Clinical features of four of the families are described in more detail elsewhere[7,13,27]. This study was approved by the local ethics committee. After giving informed consent, all patients and their relatives were systematically examined by neurologists trained in movement disorders (F.A., M.N., D.B., C. Kamm, K.S., M.B., J.W. and T.G.). The diagnosis of MDS was established according to published criteria[4]. We obtained 20 ml peripheral blood from each family member and extracted DNA following standard protocols.

Sequence analysis. Genomic sequences were obtained from GenBank and analyzed using an annotation tool. The results are available at http://ihg.gsf.de/chr7/index.html. The tool runs a series of sequence analysis programs on a DNA sequence, handling the various input and output formats required. We used three different gene-finding programs (GENESCAN, GRAIL and MZEF), electronic PCR of sequence-tagged sites and BLAST searches against databases of mRNAs, proteins and UniGene clusters. Genome-wide repeats were identified using REPEATMASKER (A.F.A. Smit and P. Green, unpublished data).

Mutation analysis. Dideoxy-cycle sequencing of PCR products amplified from genomic DNA was done with an Ampli Sequence sequencing kit (Perkin-Elmer) after purification with QIAquick PCR Purification Kit (Qiagen). Sequence products were analyzed on an ABI 3100 sequencer (PE-Applied Biosystems).

RT-PCR. Three neuroblastoma cell lines and one retinoblastoma cell line were obtained from American Type Culture Collection: SK-N-BE (CRL-2271), SK-N-SH (HTB-11), SH-SY5Y(CRL-2266) and WERI-Rb1(HTB-169). We extracted RNA with Trizol (Life Technologies) according to the manufacturer's instructions, and obtained first-strand cDNA from 5 μg total RNA using oligo-d$(T)_{12}$ random hexamers (Roche Molecular Biochemicals) and Moloney murine leukemia virus reverse transcriptase (Promega) at 42 °C, as specified by the supplier. To amplify *SGCE*, we designed cDNA primers in exons 2 and 5 (bp 153–536).

Northern blot analysis. Northern blots contained 2mg poly$(A)^+$ RNA from different brain regions (Human brain II; Clontech). For hybridization we used an *SGCE* cDNA fragment (bp 599–1,270) in Church buffer (BSA 1%, EDTA 1 mM, $NaPO_4$ 0.5M, pH 7.5, SDS 7%) at 65 °C and washed blots with 0.01×SSC at 60 °C.

Note: Supplementary information is available on the Nature Genetics web site (http://genetics.nature.com/supplementary_info/).

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