Short Report

Incomplete nonsense-mediated decay facilitates detection of a multi-exonic deletion mutation in SGCE


Mutations in SGCE represent the major cause of the myoclonus-dystonia syndrome (DYT11), an autosomal dominant disorder of reduced penetrance. Virtually all affected individuals have myoclonus, which is concentrated in the upper extremities, neck and trunk. Over half of patients have dystonia, usually affecting the neck or arms. SGCE is maternally imprinted. Of the more than 70 SGCE mutations reported in the literature, 18 are large deletions disrupting at least one exon. Therefore, testing for exonic deletions should be considered in individuals with a classic phenotype in whom Sanger sequencing is unrevealing. However, standard methodologies for detection of exonic deletion mutations are expensive, labor intensive and can produce false negatives. Herein, we report the use of cDNA derived from leukocyte RNA to identify a deletion mutation (exons 4 and 5) of SGCE in a family with DYT11. Residual RNA from incomplete nonsense-mediated decay permitted reverse transcription to cDNA. Breakpoints of the 8939 bp heterozygous deletion were then defined with long-range polymerase chain reaction and Sanger sequencing. Use of cDNA generated by reverse transcription of leukocyte RNA can reduce the costs associated with diagnostic genetic testing and can facilitate detection of deletion mutations.

Conflict of interest

The authors have no conflicts of interest to declare.

Myoclonus-dystonia (M-D) due to mutations in SGCE (M-D, DYT11, OMIM 159900) is a movement disorder characterized by variable combinations of myoclonus and dystonia. Myoclonus is manifest as brief, repetitive, involuntary contractions of a muscle or group of muscles. Many patients with M-D also develop dystonia affecting one or more anatomical segments, particularly the neck and arms (1). A definitive etiological diagnosis is valuable given that myoclonus, dystonia, and the combination of myoclonus and dystonia are clinically and genetically heterogeneous. For example, myoclonus and/or dystonia have been reported as motor signs in epilepsy, Parkinson disease, multiple sclerosis, Huntington disease, and DYT1 or TOR1A dystonia.

To date, over 70 distinct SGCE mutations have been reported in patients with MDS. However, Sanger sequencing fails to detect SGCE mutations in an important percentage of patients with M-D (1–4). Some of these cases are due to large deletion mutations in SGCE (4–8). The detection of large deletion mutations can be labor intensive, expensive and insensitive. A broad array of technologies including quantitative polymerase chain reaction (PCR) of individual exons, fluorescent in situ hybridization, quantitative multiplex PCR of short fluorescent fragments, high-density single nucleotide polymorphism arrays, array comparative genomic hybridization, and multiplex ligation-dependent probe amplification have been used...
Detection of a multi-exonic deletion mutation in SGCE

to interrogate genomic DNA (gDNA) for SGCE deletion mutations. Unfortunately, additional downstream analyses are typically required to confirm the deletions and define exact breakpoints in gDNA. Here, we report an M-D pedigree with a multi-exonic SGCE deletion identified by sequencing cDNA generated via reverse transcription of residual leukocyte RNA which escaped nonsense-mediated decay (NMD).

Clinical report

The proband, a 28-year-old female, reported the onset of upper facial myoclonus at age 7. The involuntary facial movements were present throughout the day and disappeared while sleeping. The myoclonus progressed in severity and anatomical distribution until approximately 24 years of age at which point it stabilized. In her early twenties, she was diagnosed and treated for depression. She was a below average to average student during elementary and high school, and was unable to consistently pass college course work. There was no history of seizures. General medical problems have included allergic sinusitis, asthma, gastroesophageal reflux disease and supraventricular tachycardia. She has never consumed alcoholic beverages. As a child, she was misdiagnosed with Tourette syndrome and had been unsuccessfully treated with haloperidol. Her myoclonus showed only a modest response to levetiracetam. On clinical examination, myoclonus was noted to affect the head, face, arms, and trunk with less involvement of the lower extremities and toes. Dystonic posturing was seen in both arms, particularly during motor activities such as postural maintenance and writing. There was no cervical dystonia. Her myoclonus, and, to a lesser degree, dystonia, improved with tetrabenazine. She has no dysmorphic features. She has consistently manifested excessive laughter during clinic visits, somewhat out of proportion to stimuli and mood.

The proband’s 25-year-old brother had a clinical course similar to his sister’s, but also showed evidence of significant cervical and truncal dystonia. Although not apparent on neurological examination, occasional episodes of hip girdle and leg dystonia were reported by the patient. He reported a dramatic reduction in myoclonus after the consumption of alcohol and was successfully treated with tetrabenazine. The proband’s mother and 26-year-old sister are healthy without evidence of neurological or psychiatric disease. Although the proband’s father has no reported history of myoclonus or dystonia, he does have long-standing psychiatric disease and had been diagnosed with bipolar disorder.

Methods and results

Genetic and phenotypic analyses and their publication were approved by the University of Tennessee Health Science Center Institutional Review Board (#01-07346-XP). After informed consent, whole blood was drawn for subsequent isolation of DNA and RNA. DNA was extracted using Roche’s (Indianapolis, IN) DNA Isolation Kit for Mammalian Blood. The LeukoLOCK™ Total RNA Isolation System (Life Technologies, Carlsbad, CA) and TRI Reagent® (Life Technologies) were used to isolate RNA from peripheral blood leukocytes. Leukocyte RNA was also extracted from six race-, age- and gender-matched neurologically-normal controls. DNA and RNA quantity and quality were analyzed with a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE), Quant-iT™ PicoGreen® dsDNA or Quant-iT™ RiboGreen® RNA Assay Kit (Life Technologies), and agarose gel electrophoresis or RNA Nano chips (Agilent Technologies, Santa Clara, CA). With Primer3 (frodo.wi.mit.edu), PCR primers were placed on flanking intronic or untranslated regions (UTRs) to encompass the coding regions of all 12 SGCE exons (Table S1). Sanger sequencing was used to exclude pathogenic sequence variants in coding, splice-site, 5’UTR, 3’UTR, and promoter regions. PCR amplicons were generated with the following conditions: 95°C for 15 min; 35 cycles at 94°C for 15 s, 60°C for 30 s, and 72°C for 45 s; and then 72°C for 10 min. After confirmation of amplicon size with agarose gel electrophoresis, PCR products were cleaned with ExoSAP-IT® (United States Biochemical, Cleveland, OH). Then, 1–2 μl of the purified PCR products were sequenced in the forward and reverse directions using the Applied Biosystems (Foster City, CA) 3130XL Genetic Analyzer.

For relative quantitative reverse transcription-PCR (QRT-PCR), RNA was first reverse transcribed (RT) to cDNA with Ambion’s RETROscript™ kit (Life Technologies) using 500 ng of total RNA as template. The reaction mixture was incubated at 44°C for 1 hr and then at 92°C for 10 min. QRT-PCR conditions were the same as those for PCR except that the total number of cycles was increased to 45. QRT-PCR was performed using the Roche LightCycler® 480 with SGCE specific primers and Universal ProbeLibrary (Roche) probes. Among a panel of six endogenous controls (β-actin, β-tubulin, TATA binding protein, hypoxanthine-guanine phosphoribosyltransferase, cyclophilin D, and S19), cyclophilin D showed the highest efficiency and the smallest sample-to-sample variance. All samples were run in triplicate.

Quantitative PCR (qPCR) of genomic DNA was performed under the following conditions: 95°C for 10 min; 45 cycles at 94°C for 10 s, 60°C for 10 s; and 72°C for 20 s. ALB and RPPH1 which have two copies in the diploid human genome were used as internal controls. Primers were designed to broadly flank deleted exons and long-range PCR was performed with the SeqQualPrep™ Long PCR Kit (Life Technologies) under the following conditions: 95°C for 2 min; 10 cycles at 94°C for 20 s, 60°C for 30 s, and 68°C for 10 min; 25 cycles at 94°C for 20 s, 60°C for 30 s, and 68°C for 10 min with addition of 20 s/cycle; and 72°C for 10 min.

Genetic analysis began with Sanger sequencing of the proband’s gDNA. No exonic, splice site or UTR mutations were identified. However, RT-PCR using leukocyte RNA and an extended 45 cycles of amplification exposed shortened and less abundant
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Fig. 1. SGCE deletion mutation. (a) Reverse-transcription-polymerase chain reaction of SGCE in the proband and a neurologically-normal unrelated control (NC, negative control; P, proband; and C, normal control). Primer pairs 1, 2 and 3 as indicated in Table S1 and illustrated in panel C. Primer pair 1 was used for amplification of exons 1–12 (1414 and 1142 bp with E1F/E12R in a normal control and the proband, respectively). Primer pairs 2 (325 bp with p62F1/p60R4) and 3 (886 bp with q71R5/E12R) were used for amplification of exons 1–4 and exons 5–12, respectively. An arrow points to the smaller and fainter mutant band that escaped nonsense-mediated decay. The fainter bands of appropriate size obtained with primer pairs 2 and 3 reveal normal transcripts consequent to leaky imprinting. (b) Long-range PCR of SGCE in the proband and controls using primer pairs LongF/LongR illustrated in panel C (10,266 and 1327 bp in a normal control and the proband, respectively. (c) The proband’s exon 4 and 5 deletion mutation in the context of other large deletion mutations reported in the literature. A TC dinucleotide is common to the 5′ and 3′ breakpoints (see tc and TC presented with shaded font). Locations are approximated and represented with bars, because a few previous publications identified breakpoints. The predicted effects of mutations on translation are shown above each bar. *Separate pedigrees with deletion of the same exons but with different breakpoints and deletion sizes. The p.M1_P462del mutation nomenclature is used to represent seven distinct pedigrees with deletions of 1.09 Mb–8.78 Mb.

transcripts in the proband (Fig. 1a). Sanger sequencing of the largest of these bands exposed an SGCE deletion mutation that results in elimination of exons 4 and 5 (Fig. 1a). Deletion of exons 4 and 5 was confirmed with qPCR. Then, long-range PCR (Fig. 1b) and Sanger sequencing were used to define the breakpoints of the 8939 bp heterozygous deletion in SGCE (Fig. 1c).

QRT-PCR showed minimal overall leukocyte SGCE expression in the proband (0.7% compared with normal controls). These low abundant transcripts were derived from both the imprinted and mutant alleles (Table S2). The proband’s brother was found to have the same SGCE deletion mutation. DNA was not available from the proband’s father or other family members.
Table 1. Clinical details for probands with large SGCE deletion mutations

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Deleted exons (size)</th>
<th>Age (year)/gender</th>
<th>Race</th>
<th>Age of onset (years)</th>
<th>Family history</th>
<th>Myoclonus distribution</th>
<th>Dystonia distribution</th>
<th>Alcohol sensitivity</th>
<th>Additional symptoms</th>
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<tr>
<td>DeBerardinis et al. (5)</td>
<td>Exons 1–12 (15.5 Mb)</td>
<td>2.7/M</td>
<td>Caucasian</td>
<td>1.3</td>
<td>No</td>
<td>N, UL</td>
<td>None</td>
<td>NA</td>
<td>GR, HF, MR, SK</td>
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<td>Asmus et al. (7)</td>
<td>Exons 1–12 (2.63 Mb)</td>
<td>47/F</td>
<td>Caucasian</td>
<td>1.5</td>
<td>No</td>
<td>N, UL</td>
<td>N, UL</td>
<td>Yes</td>
<td>GR, SK, HL</td>
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<tr>
<td></td>
<td>Exons 1–12 (4.99 Mb)</td>
<td>59/F</td>
<td>Caucasian</td>
<td>5</td>
<td>No</td>
<td>Generalized</td>
<td>N, UL</td>
<td>Yes</td>
<td>GR, SK</td>
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<td></td>
<td>Exons 1–12 (8.78 Mb)</td>
<td>9/M</td>
<td>Caucasian</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>GR, MR, SK, HL</td>
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<td>Grünewald et al. (4)</td>
<td>Exons 1–12 (1.09 Mb)</td>
<td>4/F</td>
<td>Caucasian</td>
<td>1.5</td>
<td>Yes</td>
<td>+</td>
<td>+</td>
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<td>Exons 1–12 (1.35 Mb)</td>
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<td>Caucasian</td>
<td>3</td>
<td>No</td>
<td>+</td>
<td>+</td>
<td>Yes</td>
<td>SK</td>
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<tr>
<td>Bonnet et al. (15)</td>
<td>Exons 1–12 (3.4 Mb)</td>
<td>7/F</td>
<td>Caucasian</td>
<td>1</td>
<td>No</td>
<td>N, UL</td>
<td>N, T, UL, LL</td>
<td>NA</td>
<td>HF</td>
</tr>
<tr>
<td>Saugier-Veber et al. (16)</td>
<td>Exons 2–12 (1.98 Mb)</td>
<td>17/M</td>
<td>Caucasian</td>
<td>12</td>
<td>Yes</td>
<td>T, UL, LL</td>
<td>Generalized</td>
<td>NA</td>
<td>GR, MR, HF, SK</td>
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<tr>
<td>Huang et al. (17)</td>
<td>Exons 2–12 (NA)</td>
<td>32/M</td>
<td>Asian</td>
<td>10</td>
<td>Yes</td>
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<td>N, T, UL</td>
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<td>Grünewald et al. (4)</td>
<td>Exon 2 (size NA)</td>
<td>5/F</td>
<td>Caucasian</td>
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<td>+</td>
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<td>Han et al. (8)</td>
<td>Exons 2–3 (18,339 bp)</td>
<td>NA/F</td>
<td>NA</td>
<td>7.5*</td>
<td>Yes</td>
<td>F, N, UL, T</td>
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<td>Exons 2–5 (13,556 bp)</td>
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<td>NA</td>
<td>2*</td>
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<td>NA</td>
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<td>Luciano et al. (18)</td>
<td>Exon 2–5 (NA)</td>
<td>17/M</td>
<td>Caucasian</td>
<td>12</td>
<td>No</td>
<td>N, T, UL, LL</td>
<td>T, UL, LL</td>
<td>NA</td>
<td>HF, GR, SE</td>
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<td>Asmus et al. (6)</td>
<td>Exon 5 (15,020 bp)</td>
<td>NA/F</td>
<td>Caucasian</td>
<td>14</td>
<td>Yes</td>
<td>H, N, UL, LL</td>
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<td>Yes</td>
<td>TR</td>
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<td>Luciano et al. (18)</td>
<td>Exon 5 (NA)</td>
<td>12/F</td>
<td>Caucasian</td>
<td>7</td>
<td>No</td>
<td>UL, LL</td>
<td>UL</td>
<td>NA</td>
<td>TR</td>
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<tr>
<td>Asmus et al. (6)</td>
<td>Exon 6 (6572 bp)</td>
<td>NA/F</td>
<td>Caucasian</td>
<td>1</td>
<td>Yes</td>
<td>N, T, UL, LL</td>
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<tr>
<td>Ritz et al. (12)</td>
<td>Exons 6–9 (NA)</td>
<td>NA/NA</td>
<td>NA</td>
<td>14</td>
<td>No</td>
<td>N, T, UL, LL</td>
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<td>NA</td>
<td>PB</td>
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<tr>
<td>Asmus et al. (19)</td>
<td>Exon 11 (NA)</td>
<td>9/F</td>
<td>Caucasian</td>
<td>1</td>
<td>No</td>
<td>N, T, UL, LL</td>
<td>UL</td>
<td>NA</td>
<td>None</td>
</tr>
<tr>
<td>This study</td>
<td>Exons 4–5 (8939 bp)</td>
<td>28/F</td>
<td>Caucasian</td>
<td>7</td>
<td>Yes</td>
<td>H, N, F, UL, LL</td>
<td>UL</td>
<td>NA</td>
<td>PB</td>
</tr>
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</table>

F, face; GR, growth retardation; H, head; HF, head and face abnormal features; HL, hearing loss; LL, lower limbs; MR, mental retardation; N, neck; NA, data not available or unclear; PB, psychiatric or behavioral symptoms; SE, seizures; SK, skeleton abnormalities; T, trunk; TR, tremors; UL, upper limbs; +, present without details.

*Mean ages of onset for several family members.
Discussion

Our M-D pedigree is similar to many described in the literature. Myoclonus is more common in proximal muscles of the arms and neck with less common involvement in the legs. Dystonia, usually affecting the neck or arms, is observed in the majority of patients, and can rarely be the only symptom of the disorder. Age of onset is usually prior to 20 years. Symptoms often respond to alcohol, sometimes leading to alcoholism (9). A significant percentage of patients manifest psychiatry co-morbiddities, particularly depression and anxiety. Inheritance patterns are consistent with maternal imprinting, and most patients inherit the mutant allele from their fathers. Only 5% of affected individuals inherit the mutant allele from their mothers and phenotypes are often milder in these cases (1, 10). The proband’s father had a psychiatric disorder without overt myoclonus or dystonia which may reflect maternal inheritance of his presumably mutant allele.

M-D genotype–phenotype correlations have not been apparent given that most reported pathological sequence variants have been nonsense mutations and small insertions or deletions leading to frameshift and premature termination codons and, consequently, NMD (1, 11). Similar to other familial forms of dystonia, broad intrafamilial and interfamilial phenotypic variability is seen in M-D. However, analysis of index cases with large SGCE deletion mutations shows that most patients exhibit dystonia (16/19) and myoclonus tends to be generalized (Table 1). Age of onset in subjects with large deletions (6.3 ± 4.9 years) is similar to individuals with missense and nonsense mutations (1, 12). However, patients with very large deletions that extend beyond the boundaries of SGCE often manifest mental retardation and extra-neural features such as growth retardation, facial dysmorphism and skeletal abnormalities.

Our study highlights the utility of leukocyte RNA and associated cDNA for the molecular diagnosis of M-D and other neurological disorders. The vast majority of genes expressed in brain and the peripheral neuromuscular system are also expressed in peripheral blood. Mutational analysis of cDNA rather than gDNA is particularly attractive for very large multi-exonic genes. In addition, RT-PCR facilitates detection of mutations that generate premature termination codons and trigger NMD. For example, DYSF is a 55 exon gene that encodes dysferlin, a protein associated with limb-girdle muscular dystrophy. Rather than performing a Western blot of skeletal muscle, RT-PCR of leukocyte RNA has been used for mutation detection in individuals with a clinical diagnosis of a dysferlinopathy (13). If reverse transcription fails because of complete NMD, monocytes can be treated with an NMD inhibitor such as cycloheximide prior to RNA extraction (14).

Supporting Information

The following Supporting information is available for this article:
Table S1. Polymerase chain reaction primers.

Table S2. SGCE mRNA expression in leukocytes.

Additional Supporting information may be found in the online version of this article.

Acknowledgements

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References