Autosomal recessive cortical myoclonic tremor and epilepsy: association with a mutation in the potassium channel associated gene CNTN2

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We characterize a consanguineous Egyptian family with an autosomal recessively inherited familial cortical myoclonic tremor and epilepsy. We used multipoint linkage analysis to map the causative mutation to a 12.7 megabase interval within 1q31.3–q32.2 with a log of odds score of 3.6. For further investigation of the linked region in an efficient and unbiased manner, we performed exome sequencing. Within the suspected region we identified a homozygous single base pair deletion (c.503_503delG) leading to a frameshift in the coding region of the sixth exon of CNTN2 alias TAG-1 (p.Trp168fs), which segregated in the respective family. Many studies point towards an important role of the CNTN2 product contactin 2 in neuronal excitability. Contactin 2, a glycosylphosphatidylinositol-anchored neuronal membrane protein, and another transmembrane protein called contactin associated protein-like 2 (CNTNAP2 alias CASPR2) are together necessary to maintain voltage-gated potassium channels at the juxtaparanodal region. CNTN2 knockout mice were previously reported to suffer from spontaneous seizures and mutations in the CNTNAP2 gene have been described to cause epilepsy in humans. To further delineate the role of CNTN2 in patients with epilepsy, we sequenced the coding exons in 189 Caucasian patients with epilepsy. No recessive mutation was detected and heterozygote carriers of rare CNTN2 variants do not seem to be predisposed to epilepsy. Given the severity of the mutation and the proposed function of the gene, we consider this mutation as the most likely cause for cortical myoclonic tremor and epilepsy in this family.

Keywords: cortical myoclonic tremor and epilepsy; whole-exome sequencing; CNTN2
Introduction

Familial cortical myoclonic tremor with epilepsy, familial adult myoclonic epilepsy, benign adult familial myoclonic epilepsy, and autosomal dominant cortical myoclonus and epilepsy are rare, but well described overlapping clinical syndromes (van Rootselaar et al., 2005; Striano et al., 2010; Coppola et al., 2011). Familial cortical myoclonic tremor with epilepsy is characterized by an autosomal dominant inheritance, cortical myoclonic tremor, and occurrence of epileptic seizures. Nevertheless, marked clinical heterogeneity is observed. Age at onset may vary considerably, with most cases starting within the second decade of life. Usually action- and posture-induced myoclonic tremor is the presenting symptom, characterized by tremulous finger movements and myoclonic jerks of the limbs. In a minority of patients, epileptic seizures are the presenting symptom; both complex partial as well as generalized tonic clonic seizures are described. Some patients exhibit mild cognitive impairment, and recently a relatively high psychiatric comorbidity was described (Coppola et al., 2011). Although a slight worsening of symptoms in advanced age is reported, the majority of patients seem to show a benign non-progressive course on anti-epileptic therapy. Both, clinical and electrophysiological features suggest cortical hyperexcitability, which might be consistent with a channelopathy; however, so far the underlying pathophysiology remains speculative (van Rootselaar et al., 2005; Coppola et al., 2011). To date, different families in Europe and Japan and three autosomal dominant inherited loci have been described, indicating genetic heterogeneity [8q24 (MIM 601068), 2p11.1–q12.2 (MIM 607876), 5p15.31–p15.1 (MIM 613608)] (Mikami et al., 1999; Guerrini et al., 2001; Depienne et al., 2010). A causative gene could not be detected (Mori et al., 2011; Crompton et al., 2012). We investigated a consanguineous Egyptian family exhibiting a clinical phenotype showing marked similarities with familial cortical myoclonic tremor with epilepsy, however segregating as an autosomal recessive trait.

Subjects and methods

Clinical data

We investigated a consanguineous Egyptian family with an autosomal recessive mode of inheritance (Fig. 1), in which five siblings of healthy parents had a similar clinical phenotype of cortical myoclonic tremor and epilepsy (Table 1). The parents were known to be second cousins, as the father’s grandmother and the mother’s grandfather were siblings. All affected siblings are under medical treatment at the University of Ain Shams, Cairo, Egypt. All patients and parents gave written informed consent; the study was approved by the local ethics committee.

For the screening of possible mutations of CNTN2 in a larger sample of patients with epilepsy we selected 170 Austrian patients with temporal lobe epilepsy and 19 Italian patients, exhibiting in part focal epilepsies with auditory features and in part myoclonic seizures. All patients are under treatment at the Medical University of Vienna, Austria, and at the Medical University of Florence, Italy, respectively. Written informed consent was obtained from all study participants; the study was approved by the local ethics committee.

Genetic data

We performed genome-wide linkage analysis in all five affected family members (Patients V-1, V-3, V-4, V-6 and V-7) using Affymetrix GeneChip Human Mapping 10K Xba142 2.0 arrays. Data for linkage analysis were prepared with a modified version of Alohohora (Ruschendorf and Nurnberg, 2005). Multipoint linkage analysis was achieved using Allegro (version1.1d) (Gudbjartsson et al., 2000). We assumed an autosomal recessive model. The frequency of the deleterious allele was set to 0.001, and the penetrance to 99% (q = 0.001; f1 = 0.0, f2 = 0.0; f3 = 0.99).

Whole exome sequencing was performed for two affected family members (Patients V-3 and V-6). Libraries for the two exomes were prepared following standard protocols using the SureSelect whole Exome Assay. Sequencing of post-enrichment libraries was carried out on an Illumina Genome Analyzer Ix as 54-bp paired-end runs. For each sample, two lanes of a flow cell were sequenced. Image analysis and base calling was carried out using the Genome Analyzer Pipeline version 1.5 with default parameters. Alignment of the reads to the hg19 reference sequence was performed with the

The control individuals for testing detected putative pathogenic variants consisted of 366 healthy Austrian blood donors (145 females, 221 males, mean age 40 years) and 148 healthy control individuals from different regions of North Africa (36 individuals from Egypt, 95 individuals from Algeria, 14 individuals from Morocco and three individuals from Tunisia; 80 females, 68 males, mean age 50 years). All study participants gave written informed consent; the study was approved by the local ethics committee.
BWA software (v.0.5.8) using mainly default parameters. Reads were trimmed of low quality bases at the end (-q) using a cut-off quality of 15. A small number (3–4%) of duplicated reads, which were indicated by identical outer co-ordinates of mapped mate pairs, were removed. The percentage of reads overlapping targeted regions and coverage statistics of targeted regions were calculated using Perl scripts. Mapped reads that directly overlapped the targeted regions were used for variant identification. About 6.5–7.0 Gb of map able sequence data were generated per individual with 34–39% of reads mapping to the target regions. On average, \(4^{98}\%\) of the targeted bases were covered at least once, and \(4^{75}\%\) of bases were covered 20-fold or more. Initially, \(29\,185\) and \(29\,755\) variants for the two samples including single nucleotide polymorphisms and indels using SAMtools (v.0.1.7) were called, respectively. For the variant filter part of SAMtools default parameters with the exception of setting a maximum read depth of 9999 (parameter-D) was used. Additional filters were applied to exclude low confidence variants: median base quality of the variant base of at least 15, a minimum of 15% of reads showing the variant base and that the variant base is indicated by at least 5% of reads coming from different strands. For indels it was required that at least 10% of reads covering this position to indicate the indel. Variant annotation was performed using custom Perl scripts. For prioritization, known single nucleotide polymorphisms from dbSNP build 130, from eight sequenced exomes of Hap Map samples (Ng et al., 2010) and from 72 control exomes of the in-house database were excluded (EXOME control database, Helmholtz Centre Munich). Finally, the analysis was confined to the linkage region chr1: 197\,386\,847–210\,109\,286 (UCSC genome build hg19). All variants that were shared between the two samples were selected.

TaqMan® allelic discrimination assays were developed for all three variants derived from the Exome sequencing and genotyped in (i) seven members of the Egyptian family (Patients IV-1, IV-2, V-1, V-3, V-4, V-6 and V-7); (ii) 170 Austrian and 19 Italian patients with epilepsy; and (iii) 366 Austrian and 148 North African healthy control subjects. Genotyping was achieved using Type-it™ Fast SNP Probe PCR Kit using standard protocols and reagents.

We performed capillary sequencing of all 22 coding exons of CNTN2 in 170 Caucasian patients with epilepsy. The sequencing reaction was carried out using BigDye® Terminator Cycle Sequencing kit v.3. Primer design was achieved using Primer3 software. Accurate analysis and alignment was achieved using Staden Package v.1.5. Each chromatogram was visually inspected for the presence of variants.

Seven novel exonic non-synonymous heterozygous variants resulting from capillary sequencing of CNTN2 in 189 Caucasian patients with epilepsy were genotyped with TaqMan® allelic discrimination assays in 402 control individuals (366 Austrian and 36 Egyptian healthy control subjects). DNA of the respective variant carrier served as positive control.

### Table 1 Clinical details of affected individuals

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Onset</th>
<th>Clinical features</th>
<th>Diagnostics</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>V-1</td>
<td>M</td>
<td>39</td>
<td>11</td>
<td>GTCS Cortical tremor</td>
<td>CCT normal</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>V-3</td>
<td>F</td>
<td>37</td>
<td>14</td>
<td>CPS GTCS Cortical tremor Myoclonic jerks</td>
<td>MRI normal EEG temporal IEDs EMG tremor 10–12 Hz Bursts &lt;50 ms SEP normal IQ = 85</td>
<td>Carbamazepine Propanolol negative</td>
</tr>
<tr>
<td>V-4</td>
<td>F</td>
<td>29</td>
<td>11</td>
<td>Aura CPS GTCS Cortical tremor Myoclonic jerks</td>
<td>MRI normal EEG normal</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>V-6</td>
<td>F</td>
<td>24</td>
<td>12</td>
<td>Aura CPS GTCS Cortical tremor Myoclonic jerks Neuropsychiatric symptoms</td>
<td>MRI bilateral mS EEG temporal IEDs</td>
<td>Carbamazepine Lamotrigine</td>
</tr>
<tr>
<td>V-7</td>
<td>F</td>
<td>21</td>
<td>11</td>
<td>Aura CPS GTCS Cortical tremor Neuropsychiatric symptoms</td>
<td>MRI normal EEG temporal IEDs</td>
<td>Carbamazepine</td>
</tr>
</tbody>
</table>

CPS = complex partial seizure; GTCS = generalized tonic clonic seizure; IED = interictal epileptiform discharges; mS = mesial sclerosis; CCT = cranial computed tomography; SEP = somatosensory evoked potentials.
Results

Clinical data

Pedigree and clinical data of the described family are shown in Fig. 1 and Table 1. All five siblings had experienced disease onset with seizures in adolescence. Four out of five patients (Patients V-3, V-4, V-6 and V-7) presented with focal seizures, suggestive of temporal lobe origin. Auditory features, complex hallucinations including vivid scenes as well as smelling a bad odour were reported as auras from three siblings (Patients V-4, V-6 and V-7). Most patients showed good seizure control on carbamazepine, except Patient V-6 who had resistant seizures on carbamazepine and lamotrigine add-on treatment. Brain MRI revealed bilateral mesial temporal sclerosis in Patient V-7, and no structural changes in the remaining patients. EEG showed temporal epileptiform discharges in three patients (Patients V-3, V-6 and V-7). During follow-up all five patients developed cortical myoclonic tremor, manifested as nodding head tremor, as well as a shivering-like twitching of the hands and fingers during posture and action. Propranolol treatment was ineffective on cortical myoclonic tremor in Patients V-3 and V-4, no treatment was necessary in the remaining patients (video of Patient V-3 and spiral drawing of Patients V-3 and V-6 available on request). Three siblings also had occasional myoclonic jerks of the limbs (Patients V-3, V-4 and V-6). EMG showed synchronous bursting of agonist and antagonist muscles with a frequency between 10–14 Hz and burst duration <50 ms. Somatosensory evoked potentials were normal in Patients V-3 and V-6. Two siblings (Patients V-6 and V-7) exhibited depressive symptoms as well as borderline intelligence, whereas Patients V-3 and V-4 showed average neuropsychological test results (Supplementary material).

Genetic data

Linkage analysis and whole exome sequencing

Assuming an autosomal recessive model, significant linkage with a lod of odds (LOD) score of 3.6 was obtained on chromosome 1q31.3–q32.2 between rs927510 and rs724054 (Supplementary Fig. 1). This 12.7 Mb region contains 153 annotated known and predicted genes (UCSC genomes build hg19, chr1: 197 386 847–210 109 286). To identify the disease causing variant, we selected two siblings (Patients V-3 and V-6) for whole exome sequencing. We filtered called variants as described above. We assumed that any homozygous variant within the linked region would be a disease candidate. This approach left three homozygous non-synonymous variants shared by both affected individuals within the candidate region: (i) c.844G>A (p.Glu282Gln) in the DDX59 gene. The encoded protein DDX59 is a probable ATP-dependent RNA helicase; publicly available data show only very low expression in the brain (UCSC genome browser); (ii) c.506C>T (p.Ser169Tyr) in the TNNI1 gene. The encoded protein, TNNI1, is expressed in cardiac and skeletal muscle during early development, but is restricted to slow-twitch skeletal muscle fibres in adults. TNNI1 prevents muscle contraction by inhibiting calcium-mediated conformational changes in actin–myosin complexes (Kee and Hardeman, 2008); and (iii) c.503_503delG (p.Trp168fs), a single nucleotide deletion, which results in a frameshift downstream of this position in the CNTN2 gene (also known as TAG-1) (Fig. 1). The gene product contactin 2 is a glycosylphosphatidylinositol-anchored neuronal membrane protein that contributes in conjunction with another transmembrane protein called contactin associated protein-like 2 to the organization of axonal domains at nodes of Ranvier, by maintaining voltage-gated potassium channels at the juxtaparanodal region (Poliak et al., 2003). All three variants of the three respective genes (DDX59, TNNI1 and CNTN2) were confirmed to be in a homozygous state in all affected subjects of the family and in a heterozygous state in the parents. None of these variants were present in the screened Austrian and Italian patients with epilepsy, nor in the Austrian and Egyptian control cohort. Considering expression as well as experimental data, which suggest an important role of CNTN2 in cortical neuronal hyperexcitability, CNTN2 seemed to be the most plausible candidate gene for a disorder like familial cortical myoclonic tremor with epilepsy.

Rare variants of CNTN2 among epilepsy cases and their frequency in control subjects

To assess the prevalence of other CNTN2 mutations among epilepsy cases, we screened all 22 coding exons of CNTN2 by capillary sequencing in 189 patients with various epilepsy syndromes. Seven novel variants, found in the patient cohort, and not present in dbSNP build 132, were genotyped in 402 control individuals. Furthermore we surveyed the exonic region of 1200 control individuals from an in-house database containing whole-exome sequencing data from 1200 individuals (EXOME control database, Helmholtz Centre Munich). Overall, in addition to c.503_503delG, 30 rare non-synonymous variants were identified. Rare was defined as present with a heterozygote allele frequency of <0.02 and/or no deposition in dbSNP build 132 (Supplementary Table 1). No homozygous variant was detected. We identified three different heterozygous non-synonymous variants only present in the patient group, five present in the patient and control groups and 22 only present in the EXOME control group. Eighty-five individuals (7.1%) in the EXOME control group and 10 individuals (5.3%) in the patient group carried a rare heterozygous non-synonymous variant. Taken together, neither the amount of rare non-synonymous variants nor the number of individuals carrying such a variant was higher in the patient group than in the EXOME control group.

Discussion

We investigated a consanguineous Egyptian family with an autosomal recessively inherited familial cortical myoclonic tremor with epilepsy. By performing linkage analysis and subsequent exome sequencing, we detected a single nucleotide deletion in exon 6 of CNTN2 (c.503_503delG) resulting in a frameshift mutation (p.Trp168fs), segregating in a recessive manner in the family.

The CNTN2 product contactin 2 is a well described neuronal cell adhesion molecule that belongs to the contactin subgroup of the immunoglobulin superfamily (Poliak and Peles, 2003; Traka et al.,...
The *CNTN2* gene is expressed by neurons and myelinating glial cells and is essential for the organization of juxtaparanodral regions in myelinated fibres (Poliak and Peles, 2003; Savvaki et al., 2008). Several studies show that contactin 2 and another transmembrane molecule called contactin associated protein-like 2 form a scaffold that enables the accumulation of voltage-gated potassium channels at the juxtaparanodes (Poliak et al., 2003; Horreosh et al., 2008). Voltage-gated potassium channels have been known for a long time to play a critical role in stabilizing membrane potential and modulating action potential frequency during repetitive firing (Smart et al., 1998). In *CNTN2*-deficient mice contactin associated protein-like 2 did not accumulate at juxtaparanodes, and the normal enrichment of voltage-gated potassium channels was severely disrupted (Poliak et al., 2003; Traka et al., 2003). Many studies point towards an important role of contactin 2 and associated proteins in cortical hyperexcitability and seizure susceptibility. *CNTN2*-deficient mice show spontaneous epileptic seizures, and increased seizure susceptibility to convulsant stimuli (Fukamauchi et al., 2001). Mice lacking voltage gated potassium channels (Kv1.1 and Kv1.2) exhibit increased seizure susceptibility and hyperexcitability in axons (Smart et al., 1998; Brew et al., 2007). There is also evidence for a critical role of this tripartite complex in cortical hyperexcitability in humans. Mutations in the gene encoding contactin associated protein-like 2 (*CNTNAP2*) were reported to cause recessive symptomatic focal epilepsy with mental retardation in Old Order Amish People (Strauss et al., 2006). Mutations in the *KCNA1* gene, encoding the voltage-gated potassium channel Kv1.1, are known to cause episodic ataxia type 1, and epileptic seizures show a significant over-representation in these families (Zuben et al., 1999).

Besides the described deletion in exon 6 of *CNTN2*, we detected two further homozygous missense variants within the putative linkage region 1q31.3–q32.2 (DDX59 p.Glu282Gln and TNNI1 p.Ser169Tyr). The respective encoded proteins are hardly expressed in the brain and their putative function does not suggest a relevant role in cortical neuronal hyperexcitability, which is the most likely pathophysiological background of cortical tremor and epilepsy. In contrast, given the expression and experimental data, as well as the impact of a loss of function mutation, we consider *CNTN2* as the most likely candidate gene. However, we cannot completely exclude an additional effect of these genes to the described phenotype, acting in combination with the mutated *CNTN2*.

The five affected patients showed a similar clinical picture with an onset of symptoms in adolescence, presenting with epileptic seizures, followed by appearance of a mild irregular tremor of the head and upper limbs. Clinical and EEG data suggested a focal, likely temporal origin of epileptic seizures. In one patient, brain MRI revealed bilateral mesial hippocampal sclerosis, previously not observed in this syndrome. Another patient in this family had severe seizures, although a progressive course of the syndrome was not apparent. Our patients exhibited cortical myoclonic tremor typical for familial cortical myoclonic tremor and epilepsy, described as irregular twitching of the fingers insensitive to beta-blocker therapy. Surface electromyographic investigations showed synchronous bursting of agonist and antagonist muscles with a frequency of 10–14 Hz and burst duration <50 ms, typical for a cortical origin of myoclonus. Somatosensory evoked potentials were normal, as also reported in other patients (Crompton et al., 2012). In line with previous observations in familial cortical myoclonic tremor with epilepsy, some family members exhibited borderline cognitive level and psychiatric symptoms (Coppola et al., 2011).

As *CNTN2* seemed to be a likely candidate gene for influencing neuronal excitability, to further delineate its role, we sequenced all coding exons in 189 patients with epilepsy. We did not detect homozygous mutations, and neither the amount of rare variants nor the number of individuals carrying rare heterozygote variants was higher in the patient group than in the control group. Thus, heterozygote carriers of rare *CNTN2* variants do not seem to be predisposed to the examined epilepsies.

The present study, therefore, presents an interesting, already known gene called *CNTN2* as the most likely underlying cause in a family with autosomal recessive cortical myoclonic tremor and epilepsy, showing marked similarities with the well-known phenotype of familial cortical myoclonic tremor with epilepsy. The crucial role of *CNTN2* in neuronal excitability makes this gene a plausible candidate for familial cortical myoclonic tremor with epilepsy. Its identification contributes to elucidating the pathogenesis of familial cortical myoclonic tremor with epilepsy and, considering that cortical myoclonic tremor is observed across a wide number of epileptic disorders (Grosse et al., 2003), may provide clues in understanding the molecular basis of more common types of epilepsy.

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**Supplementary material**

Supplementary material is available at *Brain* online.

**References**


