Congenital Myasthenic Syndrome With Episodic Apnea in Patients Homozygous for a CHAT Missense Mutation

Simone Kraner; Iris Laufenberg; Hans M. Straßburg, MD; Joern P. Sieb, MD; Ortrud K. Steinlein, MD

Background: The syndrome of congenital myasthenia with episodic apnea (CMS-EA) was previously found to be due to mutations in the choline acetyltransferase gene (CHAT).

Objective: To identify the mutations underlying CMS-EA in a Turkish multiplex family.

Design: Direct sequencing of the CHAT gene.

Patients: A consanguineous Turkish family with 2 siblings affected by muscular weakness and episodic respiratory distress.

Results: The sequencing of CHAT coding exons identified a previously unknown missense mutation that affected a highly conserved amino acid residue (I336T). The mutation was absent in 164 control chromosomes.

Conclusions: The high degree of conservation in different species strongly suggests that I336T is a functionally important amino acid residue. The absence of I336T from a large control sample further supports the pathogenic role of I336T in CMS-EA. This is the second report of CHAT mutations causing presynaptic CMS.

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IMPACT OF neuromuscular transmission can either be acquired or inherited. Congenital myasthenic syndromes (CMS) are due to gene mutations in proteins located in the presynaptic, synaptic, or postsynaptic part of the neuromuscular junction. Presynaptic defects are caused by mutations in the gene coding for the enzyme choline acetyltransferase (ChAT), while synaptic CMS was found to be associated with end plate acetylcholinesterase deficiency due to COLQ (collagen-like tail subunit of asymmetric acetylcholinesterase) mutations. In patients with postsynaptic CMS, mutations have been found in all 4 genes coding for subunits of the adult-type muscular nicotinic acetylcholine (ACh) receptor as well as in the gene coding for the ACh receptor–associated protein rapsyn.

Mutations in the CHAT gene have recently been identified as a cause of the frequently fatal congenital myasthenia with episodic apnea syndrome (CMS-EA), previously named familial infantile myasthenia. Congenital myasthenia with episodic apnea usually manifests at birth or in the neonatal period with hypotonia, ptosis, dysphagia, and respiratory insufficiency with apnea. If the patient survives this initial phase, the condition improves, but recurrent crises, such as infections, fever, vomiting, or overexertion, may result in sudden death or anoxic brain damage. Acetylcholinesterase inhibitors are effective in preventing or moderating these life-threatening crises. In CMS-EA, end plates show no morphologic abnormality. In vitro studies on intercostal muscle specimens have elucidated the electrophysiologic basis of this disorder of neuromuscular transmission. Prolonged stimulation of muscle bundles at 10 Hz results in an abnormal decrease of the amplitude of the miniature end plate potentials (MEPPs). This decrease of MEPP amplitude on prolonged stimulation suggests a progressive decrease in the acetylcholine content of the presynaptic vesicles. The recently described CHAT mutations reduce or abolish the synthesis of acetylcholine from acetyl coenzyme A and choline at the cholinergic synapses. We have screened the CHAT gene in a multiplex CMS-EA family of Turkish origin and have identified a previously unknown mis-
Healthy individuals of European descent. The control sample consisted of DNA from 82 unrelated

were corrected by edrophonium chloride, probably because
tal responses after rest. The EMG decrements, when present,
when the tested muscles were weak due to exercise. Low-
hepatitis B, probably due to co-natal infection, was present in

In both siblings, motor milestones were delayed. They had

abnormality during the pregnancy and birth of both children.

more severely affected sibling, a boy, had 3 episodes of
acute-onset respiratory distress with cyanosis during infancy.

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already shown increased fatigability as toddlers. They were

never able to keep up physically with their peers. Their exer-
tion tolerance decreased continuously during childhood. At
the age of 7 years, the boy had to stop walking approximately
every 10 m for a brief rest. He experienced repeated infections
that resulted in rapid decline of the remaining muscle force,
requiring ventilatory support. Although he improved signifi-
cantly while taking acetylcholinesterase inhibitors during

smoses from healthy controls.

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Polymerase chain reaction (PCR) was performed using primer
sets that amplified CHAT coding exons 2 to 14 and their adjac-
ent exon-intron boundaries. Polymerase chain reaction was

Figure 1. Pedigree of the Turkish family with congenital myasthenia with episodic apnea. A star marks the index patient. A double line indicates the consanguineous marriage. The genotypes of individuals available for analysis are given below the symbols, and examples of the sequencing results (reverse strand) are given for both I336T heterozygotes (upper part) and homozygotes. An arrow marks the mutation found in the present study.

Figure 1

The I336T mutation creates a site for the restriction endonu-
ase, Tsp4CI, allowing a rapid screening of controls. Exon 7 was
amplified using primers n1743 (5'-AGGGGCCACCAAGTA-
GACA-3') and n1744 (5'-GAAGGCCAATGTTTACAGAGCAT-3').
The amplified fragment contained 3 additional Tsp4CI restriction sites serving as internal controls. Five milliliters of the resulting
110–base pair (bp) PCR product were digested with Tsp4CI and
separated on a 3% agarose gel. The following bands were observed:
wild-type allele, 44 bp+68 bp+170 bp; mutant allele, 44 bp+68
bp+75 bp+95 bp.

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RESULTS

Polymerase chain reaction amplification and subsequent direct sequencing of CHAT exons from the DNA of the index patient revealed a homozygous thymine to cytosine nucleotide exchange within exon 7, leading to the substitution of isoleucine by threonine in amino acid position 336 (I336T; nucleotide numbering referring to the complementary DNA sequence of CHAT isoform M, accession number NM020549). Sequencing of additional family members showed that the affected sister was homozygous for the amino acid exchange 1336T, while both healthy parents were heterozygous for the mutation. The I336T mutation was not found in 164 chromosomes from healthy controls.

COMMENT

Choline acetyltransferase catalyses the synthesis of the neurotransmitter acetylcholine from acetyl coenzyme A and choline in central and peripheral neurons. A single gene with different promoter regions, which can produce several transcripts, encodes the enzyme. In humans, the M-type RNA has the capability to generate both large and small forms of ChAT proteins, while R- and N-type RNA generate only the small form. The deficiency of ChAT protein expression has been reported in different neurodegenerative conditions, such as Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis. Abnormalities of ChAT activity have also been described in schizophrenia and sudden infant death syndrome. In most of these diseases, the reason for the observed loss of ChAT activity is either unknown or sus-
activity causes a lethal phenotype.\textsuperscript{13} It is therefore most likely that the I336T mutation reported here reduces but does not abolish ChAT function. The residual ChAT activity is probably the reason why, despite the important function of ChAT in the brain, CMS-EA patients have no signs of central cholinergic dysfunction.

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**Author contributions:** Study concept and design (Drs Sieb and Steinlein); acquisition of data (Ms Laufenberg and Drs Straßburg and Steinlein); analysis and interpretation of data (Mss Karson and Laufenberg and Drs Sieb and Steinlein); drafting of the manuscript (Mss Karson and Laufenberg and Drs Sieb and Steinlein); critical revision of the manuscript for important intellectual content (Drs Straßburg and Steinlein); statistical expertise (Dr Steinlein); obtained funding (Drs Sieb and Steinlein); administrative, technical, and material support (Mss Karson and Laufenberg and Dr Straßburg); study supervision (Drs Sieb and Steinlein).

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### Table 1. Evolutionary Conservation of Amino Acid Residue I336 Within the Choline Acetyltransferase Family

<table>
<thead>
<tr>
<th>Species</th>
<th>Amino Acid Sequence</th>
</tr>
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<tbody>
<tr>
<td>Human</td>
<td>SEGDL—FTQLRK</td>
</tr>
<tr>
<td>Mouse</td>
<td>SEGDL—FTQLRK</td>
</tr>
<tr>
<td>Rat</td>
<td>SEGDL—FTQLRK</td>
</tr>
<tr>
<td>Sheep</td>
<td>SEGDL—FTQLRK</td>
</tr>
<tr>
<td>Caenorhabditis elegans</td>
<td>SYADYETGLAIAE</td>
</tr>
<tr>
<td>Neurospora crassa</td>
<td>TEKI—AINIQL</td>
</tr>
<tr>
<td>Mycoplasma pulmonis</td>
<td>DLSSL—NKTNFL</td>
</tr>
</tbody>
</table>

**Figure 2.** Evolutionary conservation of amino acid residue I336 within the family of eukaryotic acetyltransferases. Only parts of the protein sequences are shown. A box marks the position of I336.

**REFERENCES**