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

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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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Impairment 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.\(^1\) 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.\(^1\) Prolonged stimulation of muscle bundles at 10 Hz results in an abnormal decrease of the amplitude of the miniature end plate potentials (MEPPs).\(^4\) 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\(^3\) 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-

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Polymerase chain reaction (PCR) was performed using primer sets that amplified CHAT coding exons 2 to 14 and their adjacent exon-intron boundaries. Polymerase chain reaction was carried out in a total volume of 25 mL in a PTC (Peltier thermal cycler) 200 (MJ Research, Waltham, Mass), containing 50 ng of genomic DNA, 5 pmol of each forward and reverse primer, 200 mM of each deoxynucleotide triphosphated NTP, 1.5 mM of magnesium chloride, 50 mM of potassium chloride, 20 mM of Tris-hydrochloride (pH 8.3), and 0.1 U of Taq-DNA polymerase. The PCR parameters were as follows: denaturation at 95°C for 5 minutes followed by 33 cycles at 95°C for 30 seconds, annealing at 66° to 72°C for 30 seconds, extension times at 72°C, varying between 30 seconds and 80 seconds, followed by a final extension step of 5 minutes at 72°C. The PCR products were directly sequenced on an ABI 377 sequencer (Applied Biosystems, Foster City, Calif).

RESULTS

The 1336T mutation creates a site for the restriction endonuclease, Tsp4C1, allowing a rapid screening of controls. Exon 7 was amplified using primers n1743 (‘5′-ACGGGACCCCAACAAGT-GACA-3′), and n1744 (‘5′-AAAAGCCATGGGACAGGACT-3′). The amplified fragment contained 3 additional Tsp4C1 restriction sites serving as internal controls. Five milliliters of the resulting 110–base pair (bp) PCR product were digested with Tsp4C1 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.

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. 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 suspicious.

MUTATION SCREENING

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 strands) are given for both 1336T heterozygotes (upper part) and homozygotes. An arrow marks the mutation found in the present study.

SENSITIVE MUTATION

The siblings showed ptosis pronounced by sustained upward gaze but no ophthalmoparesis. Limb and girdle muscles fatigued easily. Additionally, the sister suffered from bilateral developmental hip dysplasia. Chronic aggressive hepatitis B, probably due to co-natal infection, was present in the brother. Pronounced decrements in 3-Hz stimulation were detected only when the tested muscles were weak due to exercise. Low-frequency stimulation failed to result in abnormal decremental responses after rest. The EMG decrements, when present, were corrected by edrophonium chloride, probably because this reversible cholinesterase inhibitor increases the amount of ACh available at the neuromuscular junction. Repeated tests for the presence of anti-ACh receptor antibodies were negative. The parents did not give consent for muscle biopsies. The control sample consisted of DNA from 82 unrelated healthy individuals of European descent.

METHODS

CMS-EA FAMILY AND CONTROLS

The pedigree of the CMS-EA family is shown in Figure 1. The ethical committee of the University Hospital Bonn (Bonn, Germany) approved the study, and informed consent was obtained from the participating individuals or their respective parents. The patients are 2 siblings from a consanguineous Turkish family (Figure 1). The parents did not report any abnormality during the pregnancy and birth of both children. The more severely affected sibling, a boy, had 3 episodes of acute-onset respiratory distress with cyanosis during infancy. In both siblings, motor milestones were delayed. They had already shown increased fatigability as toddlers. They were never able to keep up physically with their peers. Their exertion 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 significantly while taking acetylcholinesterase inhibitors during these exacerbations, recovery was delayed, and he was hospitalized for weeks at a time on many occasions. On examination, the siblings showed ptosis pronounced by sustained upward gaze but no ophthalmoparesis. Limb and girdle muscles fatigued easily. Additionally, the sister suffered from bilateral developmental hip dysplasia. Chronic aggressive hepatitis B, probably due to co-natal infection, was present in the brother. Pronounced decrements in 3-Hz stimulation were detected only when the tested muscles were weak due to exercise. Low-frequency stimulation failed to result in abnormal decremental responses after rest. The EMG decrements, when present, were corrected by edrophonium chloride, probably because this reversible cholinesterase inhibitor increases the amount of ACh available at the neuromuscular junction. Repeated tests for the presence of anti-ACh receptor antibodies were negative. The parents did not give consent for muscle biopsies. The control sample consisted of DNA from 82 unrelated healthy individuals of European descent.
Knockout mice, a complete failure of ChAT enzymatic 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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REFERENCES