

Mutation typing in patients with medium chain AcylCoA dehydrogenase deficiency (MCADD) and PCR based mutation screening in SIDS victims[☆]

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Abstract. We investigated 80 patients affected by medium chain AcylCoA dehydrogenase deficiency (MCADD) in a German population, and found the following frequencies of mutation: 985A>G (81.9%); 157C>T (3.1%), 799G>A (3.1%), 244–245 insT (3.1%), 362C>T (1.3%) as well as five rare mutations at frequencies below 0.6%. About 4.4% of the mutations in our patients remained unidentified. After having carried out a mutation typing procedure, we created rapid tests based on a PCR/electrophoresis technology and investigated the four most frequent mutations. Using these screening tests we identified one MCADD case among 409 SIDS victims. These investigations indicate that, in very few cases, MCADD may contribute to SIDS. © 2005 Published by Elsevier B.V.

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1. Introduction

MCADD was described in 1985 as an activity loss of the medium chain AcylCoA dehydrogenase (MCAD), causing serious hypoglycemiae [1]. After the tandem mass spectrometry method had been introduced into neonatal screening during the late 1990s, this disease was recognised as one of the most frequent inborn errors of metabolism. Emery et al. [2] believed that MCADD could probably play a role in the ensemble of multiple SIDS causalities. Kemp et al. [3] and Boles et al. [4] found several cases of MCADD in SIDS victims. However, the majority of relevant SIDS studies did not reveal MCADD cases. The approach of our investigation is to carry out a molecular MCADD screening in specimens of 409 SIDS cases. Only a rough estimate of about 50% of this population was submitted to a MCADD screening before during the neonatal period. Our PCR-mediated screening program presented here is based on the mutation typing results established by the investigation of 80 MCADD patients of our region.

2. Materials and methods

DNA was prepared from 80 neonatal screening blotters from confirmed MCADD patients and from liver specimens of 409 SIDS victims. The DNA was extracted by using the NucleoSpin Blood Kit and NucleoSpin Tissue Kit (Macherey and Nagel GmbH, Düren/Germany).

In a first step we screened the most common MCADD mutation 985A>G using the known PCR/*Nco*I test [5]. About 82% of all MCAD defects were found to belong to this mutation type. Specimens lacking this mutation and compound-heterozygous samples were submitted to a full dye terminator sequencing cycle sequencing procedure including all 12 exons. Initially, the sequencing templates for this procedure were generated using the PCR amplification technique. In addition to the known PCR/*Nco*I test, we created another PCR mediated screening test to detect the 157C>T mutation.

To produce amplicons containing an artificial *Nco*I site, we used the MC985F miss match primer and the perfect matching MC985R primer:

MC985F: ATACATTTATGCTGGCTGAAATGGCC[≡]ATG

MC985R: ACCAGAATCAACCTCCAAG

The PCR creates a *Nco*I restriction site (CCATGG) when the 985A>G mutation is present.

To generate amplicons containing an artificial *Bsr*G1 site, we used the perfect matching MC157F primer and the MC157R miss match primer:

MC157F: ACATACTGACTTCATAGGAC

MC157R: ATTCCTCTCTGGCAAATG[≡]TAC

The PCR creates a *Bsr*G1 restriction site (TGTACA) when the 157G>A mutation is present.

The described test for detecting the 985A>G mutation was used for mutation typing in 80 MCAD patients as well for screening 409 SIDS victims. DNAs from the latter group

Table 1
Mutation types and their frequency in 160 MCAD gene lesions

Mutation	<i>n</i>	Percent (%)	Mutation	<i>n</i>	Percent (%)
985A>G	131	81.9	340–432delAAG	1	0.6
157C>T	5	3.1	685T>G	1	0.6
799G>A	5	3.1	616C>T	1	0.6
244–245 insT	5	3.1	1148C>G	1	0.6
362C>T	2	1.3	1178A>T	1	0.6
Unidentified				7	4.4
Total				160	~100

were submitted to a full sequencing of the 12 MCAD exons when the 985A>G mutation was found in a heterozygous state. Furthermore, the whole SIDS sample was investigated using 157C>T mutation screening tool.

3. Results and discussion

Table 1 presents the type and number of mutations in a total of 160 MCAD gene defects. As described earlier [5] and due to a founder effect, the 985A>G transition is the prevalent mutation causing MCADD. In our population only three other gene lesions reach frequencies > 0.03: 244–245 insT, 157C>T and 799G>A. The latter two mutations have been reported before [6]. Owing to this situation, we think that it is advisable to screen MCADD risk groups for these mutations. Despite contrary publications we consider the SIDS population to be a risk group, and have screened them for two MCADD mutations to date: 985A>G and 157C>T. (Screening for 799G>A and 244–245 insT mutations will follow soon.) In our sample of 409 specimens of SIDS corpses, the 985A>G transition was found homozygous in one case and in combination with the wild type allele in 4 cases. These results confirm earlier observations that MCADD plays a role within the SIDS scene. Although undiscovered MCADD cases are unlikely to occur in the newborn population in Germany and many other highly developed countries as this disease is also detected with the tandem mass spectrometry screening method, we believe that MCADD screening tests are still needed for investigating cases of sudden unexpected death of elder children, adolescents and babies of migrant families.

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