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Short Communication

Molecular basis of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency: Identification of two new mutations

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Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) is catalysed by the mitochondrial trifunctional protein (MTP), which also contains enoyl-CoA hydratase and 3-ketothiolase activities (Carpenter et al 1992; Uchida et al 1992). The cDNAs encoding the α and β subunits were cloned by Kamijo et al (1994a). Many patients have been described with a defect in this enzyme complex and it appears that in most patients there is an isolated deficiency of the dehydrogenase activity of the MTP. We and others have reported a G1528C mutation in the gene coding for the α subunit of MTP, changing the codon for glutamate (510) into glutamine (IJlst et al 1994; Sims et al 1995). In a series of 34 LCHAD-deficient patients the G1528C mutation was found to be very frequent (87%), which corresponds to the situation observed in MCAD deficiency with the frequent G985A mutation. The G1528C mutation is directly responsible for the loss of dehydrogenase activity without changing the structure of the enzyme complex (IJlst et al 1996).

In a group of 46 LCHAD-deficient patients as studied enzymatically in our laboratory, we found 12 to be compound heterozygous for the common mutation. Here we describe two new mutations found in this compound heterozygous group.

MATERIALS AND METHODS

Screening for the common G1528C mutation on genomic DNA was done with the PCR-RFLP method using *Pst*I as previously described (IJlst et al 1996). Total RNA was prepared from frozen fibroblasts and used for cDNA synthesis exactly as previously described (IJlst et al 1994). PCR products (700–933bp) were sequenced with fluorescent labelled primers using the thermosequenase cycle sequencing kit (Amersham Life Science, Cleveland, OH, USA) and analysed on an ABI 377 sequencer (Applied Biosystems). The cDNAs encoding the α and β subunits were amplified using primers with an M13 extension as recommended by the manufacturer.

Table 1 Mutations in the gene coding for the α subunit of MTP found in two LCHAD-deficient patients

Patient	*Mutation*	*Codon change*	*Amino acid homology*[a]
LCHAD 1	C2129 ins	Phe-710 → stop733	
	G1528C	Glu-510 → Gln	Glu
LCHAD 2	T1025C	Leu-342 → Pro	Leu, Phe, Ile
	G1528C	Glu-510 → Gln	Glu

[a]Comparison of the mutated residue with the corresponding amino acid residue in: α subunit mitochondrial trifunctional protein (rat); FADB gene product (*E. coli*); FAOB gene product (*P. fragi*); peroxisomal trifunctional protein (human and rat)

RESULTS AND DISCUSSION

Using the PCR-RFLP method we found in all 46 LCHAD-deficient patients studied at least one copy of the mutated C1528 allele. The majority of patients investigated (34) are homozygous for this common mutation; only 12 patients were found to be heterozygous. Since the LCHAD activity is fully deficient in these patients, we expect that the second allele carries another mutation which also causes loss of activity. We earlier described one compound heterozygous mutation in this journal (IJlst et al 1995). To identify these unknown mutations we sequenced the complete cDNAs encoding the α and β subunits. The results obtained from two patients are summarized in Table 1.

In both patients we found a mutation in the cDNA coding for the α subunit of MTP and no differences in the cDNA coding for the β subunit. The MTP protein is a hetero-octamer which is composed of 4α and 4β subunits (Carpenter et al 1992; Uchida et al 1992). The C-insert at position 2129 as found in patient 1 changes the reading frame for the α subunit, thereby creating a premature stop codon (residue 733). This gives rise to a truncated protein which is probably unstable. From pulse chase experiments (Kamijo et al 1994b) and expression studies (IJlst et al 1996) it is known that the enzyme is only fully active and stable in an $\alpha_4\beta_4$ conformation. The β subunit of the enzyme complex harbours the thiolase activity. Normal subunit composition of the enzyme complex can be monitored by measuring the thiolase activity. LCHAD-deficient patients homozygous for the α-MTP:G1528C mutation have near-normal thiolase activity (60% of control) when measured with 3-ketohexadecanoyl-CoA as substrate. In contrast, the thiolase activity in fibroblast homogenates from patient 1 was found to be much lower (20%) than control values, giving further support to the hypothesis of an enzyme complex with decreased stability.

The missense mutation (T1025C) found in patient 2 changes the codon of leucine-342 to proline. So far little is known about the structure of the MTP protein. Leucine-342 is located in a predicted α-helix domain. Proline prevents the formation of an α-helix and dramatically changes the secondary structure of the enzyme complex, and it is likely that this also gives rise to an inactive enzyme.

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