J. Perinat. Med. 35 (2007) 76–78 • Copyright © by Walter de Gruyter • Berlin • New York. DOI 10.1515/JPM.2007.012

Short communications

Neither maternal nor fetal mutation (E474Q) in the α-subunit of the trifunctional protein is frequent in pregnancies complicated by HELLP syndrome

Sabine Mütze[1,2,*], Ines Ahillen[1], Sabine Rudnik-Schoeneborn[1], Thomas Eggermann[1], Brigitte Leeners[2,3], Peruka M. Neumaier-Wagner[4], Sabine Kuse[5], Werner Rath[2] and Klaus Zerres[1]

[1] Institute of Human Genetics, University Hospital of Aachen, Germany
[2] Department of Obstetrics and Gynecology, University Hospital of Aachen, Germany
[3] Department of Obstetrics and Gynecology, University Hospital of Zurich, Switzerland
[4] Department of Obstetrics and Gynecology, Institute of Human Genetics, Universíty Hospital of Aachen, Germany
[5] German Preeclampsia Society, Issum, Germany

Abstract

Objective: An association between maternal HELLP syndrome and fetal long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency has been proposed. LCHAD catalyzes the third step in the β-oxidation of fatty acids in mitochondria. Whereas about 75% of LCHAD-deficient patients carry a G-to-C mutation at nucleotide position 1528 (Glu474Gln, E474Q) on both chromosomes, compound heterozygosity for E474Q on one chromosome and a second different LCHAD mutation on the other can be observed in up to 25% of LCHAD-deficiency cases; only very few patients carry two mutations different from E474Q. Genetic analysis of the mother alone is insufficient in case of compound heterozygosity. Since information on the fetal carrier status of the E474Q mutation in maternal HELLP syndrome is rare, we investigated the frequency of the E474Q mutation in families where the mother had HELLP syndrome.

Methods: The occurrence of the E474Q mutation was analyzed by PCR and RFLP in 103 mothers with HELLP syndrome, in 82 children of affected pregnancies and in 21 fathers in families where fetal DNA was not available. In addition, 103 control women with only uncomplicated pregnancies were investigated.

Results: The mutation E474Q was not detected in the study population.

Conclusion: Neither maternal nor fetal heterozygosity for the E474Q mutation is a relevant factor of HELLP syndrome.

Keywords: Fatty acid oxidation; HELLP syndrome; inherited metabolic disease; 3-hydroxyacyl-CoA dehydrogenase (LCHAD).

*Corresponding author:
Dr. Sabine Mütze, MD
Universitätsklinikum der RWTH
Institut für Humangenetik, Universitätsklinikum der RWTH
Pauwelsstr. 30, D-52057 Aachen, Germany
Tel.: +49-241-80-88107
Fax: +49-241-80-82580
E-mail: smuetze@ukaachen.de

HELLP syndrome complicates 0.17–0.85% of all live births and is a multisystem disorder specific to pregnancy with a high maternal and perinatal morbidity and mortality [7]. The pathogenesis of HELLP syndrome remains incompletely understood, but in mothers of children suffering from a fatty acid oxidation disorder with a long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency, an increased incidence of HELLP syndrome or acute fatty liver of pregnancy (AFLP) has been observed [3, 9]. Ibdah et al. [3] published data on 19 pregnancies with LCHAD-deficient fetuses, 16% of which were complicated by HELLP syndrome and 63% by AFLP. These findings suggest that the fetal condition may contribute to the etiology of HELLP syndrome.

LCHAD catalyzes the third step in the β-oxidation of fatty acids in mitochondria. The active site of this enzyme is located in the C-terminal domain of each of the four α-subunits of the trifunctional protein that is associated with the inner mitochondrial membrane [10]. The gene encoding for the α-subunit is localized in the chromosomal region 2p23. Eighty-seven percent of LCHAD-deficient patients carry a G-to-C mutation at nucleotide position 1528, resulting in an E474Q (Glu474Gln) amino acid substitution [5]. The E474Q mutation inactivates LCHAD directly within the catalytic domain and heterozygosity reduces LCHAD activity in all organs by 50% [4, 8]. It was hypothesized that in the presence of the E474Q mutation, long-chain 3-hydroxyacyl metabolites produced by the fetus or placenta can accumulate in the mother and exert highly toxic effects on the liver, resulting in the typical histological pattern found in HELLP syndrome or AFLP [3, 4].

Most studies on the correlation between maternal liver disease in pregnancy and LCHAD deficiency focussed only on the maternal genotype with the exception of Yang et al. [11] who screened 27 women with AFLP and 81 with HELLP syndrome recruited in North-America and their offspring or partners for mutations in the TFP α-subunit. They identified E474Q as an important risk factor for AFLP (19% carriers) but not for HELLP syndrome (1.23%). The ethnic background of the study population was mixed and entry criteria were not strictly defined.

The aim of our study was to assess the frequency of the E474Q mutation in Caucasian families where the mother had been affected by HELLP syndrome but not AFLP in at least one pregnancy. We performed molecular genetic analysis in 103 Caucasian women with clearly defined HELLP syndrome, their offspring or partner. The diagnosis of HELLP syndrome was based on the presence of hemolytic anemia (serum haptoglobin levels <0.3 g/L or LDH >300 U/L), elevated liver enzymes (elevation of AST or ALT over norm), and evidence of thrombocytopenia, defined as a platelet count of $<100.000/\mu$L. In addition to the mothers, we tested either their children born from pregnancies complicated by HELLP syndrome ($n=82$) or, if the child's blood was not available, the fathers ($n=21$) for the E474Q mutation. In addition, 103 unrelated female probands of German origin with uncomplicated pregnancies served as controls. The study was approved by the Institutional Medical Ethics Committee (EK 934) and informed consent was obtained from adult subjects and parents of the children. Genomic DNA was isolated from venous blood or umbilical cord blood samples, respectively, as described by Miller et al. [6].

To allow rapid genotyping, a PCR-based restriction fragment length polymorphism (RFLP) assay for the variant E474Q, based on an *Msp*I digest, was established. Since the wildtype DNA sequence does not contain the respective restriction site, we constructed a mismatch primer. Primer sequences were as follows: sense primer is 5′-T-CCC-GTG-GAC-AAG-ATG-CAG-CTG-CC̲G-3′ (artificially created restriction site for *Msp*I is underlined), antisense primer sequence is 5′-CTG-CAG-CTC-TGT-TAT-ACA-GCC-3′. In case of the wildtype sequence, *Msp*I digests the 145 bp-product into two fragments (122 and 23 bp), in case of E474Q mutation this restriction site is destroyed and the amplification product remains undigested (145 bp). PCR and restriction analysis were carried out according to standard protocols. Digestion products were separated on 10% polyacrylamide gels, further details can be provided on request. Bands were visualized by silver staining.

None of the 103 HELLP patients, 82 fetuses, 21 fathers, and none of the 103 controls showed heterozygosity for the E474Q mutation. Our results thus confirm those of den Boer et al. [2] who found heterozygosity for the E474Q mutation in one mother among 113 Dutch women with a history of HELLP syndrome. Furthermore our data are in accordance with those reported by Yang et al. [11]. Among 81 North-American women with HELLP syndrome they detected one mother who was heterozygous for E474Q whereas no mutation was found in the offspring. The study population was mostly of Caucasian descent, but African American and Hispanic women were also included.

According to Ijlst et al. [5], about 75% of LCHAD deficient children are homozygous for the E474Q mutation while up to 25% carry a second mutation different from E474Q, and very few LCHAD patients carry two mutations other than E474Q. Thus, 50% of these compound heterozygotes cannot be identified by analysis of the mothers alone. We, therefore, examined either the offspring or the father, in addition to the mother, in order to study the prevalence of fetal LCHAD deficiency among women with HELLP syndrome. None of the children and none of the fathers showed the E474Q mutation. Following Hardy-Weinberg's law, a negative result for the E474Q mutation in the α-subunit of the TFP in both parents or the fetus excludes this mutation as a major genetic risk factor in an unselected obstetric population with HELLP syndrome.

The role of other LCHAD mutations in the pathogenesis of HELLP syndrome should be further elucidated. Mutation analysis among LCHAD-deficient patients revealed at least 10 other mutations, apart from the common E474Q mutation [3]. These non-E474Q mutations generate nonsense (premature termination) codons, either directly or by causing frame shifts in the α-subunit mRNA [8]. Yang et al. [11] screened the MTP α-subunit for E474Q and other mutations by SSCP and direct sequencing, but no further mutation, neither maternal nor fetal, were found in women affected by HELLP syndrome. Recently, a novel α-subunit mutation in exon 11 of the LCHAD domain (Q322K) was described. The mother who developed HELLP syndrome after delivery was found to be heterozygous for this mutation while the fetal genotype was completely normal [1]. Whether these non-E474Q mutations, which usually occur in combination with the common E474Q mutation as the second allele in LCHAD deficiency with the exception of the case mentioned above, play a crucial role in the development of HELLP syndrome in the general obstetric population has yet to be investigated.

The comparison of our study with the Dutch study performed by Den Boer et al. [2], both with a similar ethnic background, reveals a low prevalence of the E474Q mutation among women with HELLP syndrome (1:216). Thus, the carrier frequencies of this common LCHAD mutation do not differ significantly from the general population (1:783) [2]. To conclude, our data do not provide evidence for a relevant role of maternal or fetal heterozygous E474Q LCHAD mutations in the pathogenesis of

HELLP syndrome. Abnormal LCHAD function seems to represent only one of a variety of risk factors in the etiology of this specific disorder of pregnancy. Therefore, future studies will have to concentrate on other candidate genes that might be causative factors for HELLP syndrome. However, considering the clinical consequences it is important to remember the risk of LCHAD deficiency in a newborn of a mother with HELLP syndrome.

Acknowledgments

We thank the Medical Faculty of the Aachen University for their financial support (START program project grant 32/01) of this work. We also gratefully acknowledge the families who participated in the study.

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Received August 11, 2006. Revised October 13, 2006. Accepted October 13, 2006.