mutations, and several investigators have characterized eight private or rare mutations (24–26). Interestingly, a recent report describes a full HFE screening procedure using DHPLC methodology (27). Building on the same technology, these two approaches could be complementary in a full evaluation of the HFE involvement in HC. This would allow identification of iron-overloaded patients in whom HFE is not involved in the disease and who are therefore candidates for the exploration of other genes involved in iron-metabolism disorders.

References

New Nuclear Encoded Mitochondrial Mutation Illustrates Pitfalls in Prenatal Diagnosis by Biochemical Methods, Markus Schuelke,1,2 Anne Detjen,3 Lambert van den Heuvel,4 Christoph Korenke,5 Antoon Jansen,6 Arie Smits,7 Frans Trijbel8, and Jan Smeltink2 (1 Department of Neuropediatrics, Charité Virchow University Hospital, Augustenburger Platz 1, D-13353 Berlin, Germany; 2 Department of Pediatrics, Nijmegen Center for Mitochondrial Disorders, University Medical Center Nijmegen, PO Box 9101, NL-6500 HB Nijmegen, The Netherlands; 3 Department of Neuropediatrics, Children’s Hospital, Georg-August-Universität, Robert-Koch Strasse 40, D-37075 Göttingen, Germany; 4 Department of Human Genetics, University Medical Center, PO Box 9101, NL-6500 HB Nijmegen, The Netherlands; * author for correspondence: fax 49-30-4505-66920, e-mail markus.schuelke@charite.de)

A frequent etiology of congenital lactic acidosis is disturbed mitochondrial energy metabolism. Affected children generally present with neurologic symptoms, such as myopathy and epilepsy. Parents who have lost a child to mitochondrial disease often ask for prenatal diagnosis in subsequent pregnancies. The large number of possible mitochondrial or nuclear DNA mutations often makes the molecular defect unknown. In these cases, prenatal diagnosis rests solely on biochemical analysis. Here we report a possible pitfall in prenatal diagnosis of mitochondrialopathies by biochemical methods that might occur despite all precautions. It is illustrated by a patient with isolated mitochondrial complex I deficiency and her family in the light of a new mutation (632C→T) in 1 of the 36 nuclear encoded genes of complex I (NDUFEV1).

The girl (II.1 in Fig. 1A) was the first child of healthy Caucasian first-degree cousins. Postnatally she showed acrocyanosis, muscular hypotonia, and a pendular nystagmus. Fundoscopy revealed bitemporal retinal depigmentation. The latencies of the visual evoked potentials were pathologically increased. Lactic acidosis (pH 7.19) was noted, with a plasma lactate concentration of 24.1 mmol/L (reference interval, 0.5–2.2 mmol/L), a lactate-
to-pyruvate ratio of 57 (reference values <20), plasma alanine of 893 μmol/L (reference interval, 40–500 μmol/L), urine α-ketoglutaric acid of 1852 mmol/mol creatinine (reference interval, 159 ± 137 mmol/mol creatinine), urine lactate of 1713 mmol/mol creatinine (reference interval, 234 ± 165 mmol/mol creatinine), and cerebrospinal fluid lactate of 9.6 mmol/L (reference values <2 mmol/L). Cranial ultrasound and magnetic resonance imaging results were normal. Muscle histology revealed intracytoplasmic accumulation of glycogen. Mitochondria were ultrastructurally normal on electron microscopy.

We measured the respiratory chain complex I, II+III, and IV activities in a fresh muscle biopsy specimen and in cultured fibroblasts according to standard procedures (1) (see the data supplement available with the online version of this Technical Brief, at http://www.clinchem.org/content/vol48/issue5/). In both samples we found an isolated complex I deficiency (Table 1). The child died at the age of 4 weeks from respiratory failure in the course of an uncontrollable metabolic crisis.

At the time of the second pregnancy, the parents asked for prenatal diagnosis. Because of the high variability of the biochemical defect between the tissues, we try to increase the diagnostic safety by offering prenatal diagnosis only if the biochemical defect is present in the index patient’s muscle and cultured fibroblasts (2). To further increase the safety margin, we measure respiratory chain complex activities in native and cultured chorionic villi. This policy was adopted after we had another patient with complex I deficiency in whom the native chorionic villi had normal activities, whereas the cultured ones were clearly complex I-deficient. He died at 18 months from severe lactic acidosis and hypertrophic cardiomyopathy (unpublished observation). In the second pregnancy (II.2 in Fig. 1A), a chorionic villus biopsy was performed at 11 weeks of gestation. We measured respiratory chain enzyme activities in native as well as in cultured chorionic villus biopsy specimens. In the native specimen, complex I activity was normal, whereas it was pathologically decreased in the cultured specimen (Table 1). Because the parents opted for a high degree of security, the decision was made to terminate the pregnancy. Analysis of a muscle specimen from the aborted female fetus, however, revealed normal complex I activity.

At the time of the third pregnancy (II.3 in Fig. 1A), most of the human respiratory complex I genes had been cloned, and we were able to offer mutation screening. The consanguinity of the parents led us to suspect a mutation in one of the nuclear genes of complex I. We sequenced the open reading frames of the NDUFV1, NDUF54, NDUF57, and NDUF58 genes, in which we had detected mutations previously (3, 4). In the present case, we detected a homozygous C→T transition at nucleotide 632 in the patient’s NDUFV1 cDNA. The presence of the mutation was documented in genomic DNA by the gain of a restriction site within the 230-bp PCR fragment generated by the primer pair 5′-TgCAggtgCCATCCgAgAggC-3′ (forward) and 5′-CACAgTCgACCagggT-TAcg-3′ (reverse), which was cleaved into 131- and 99-bp fragments by Bsp1268I (Fig. 1B). The mutation was confirmed to be homozygous in the genomic DNA of the index patient, whereas her parents and siblings were heterozygous for this mutation. It was absent in 280 alleles from healthy controls and in nearly 100 expressed sequence tags from GenBank. This excludes a common polymorphism. The mutation changes an alanine to a valine at amino acid 211 within the FMN-binding domain.
(5), which is strictly conserved down to *Escherichia coli* (Fig. 1D). With 10 different mutations identified to date, *NDUFV1* seems to be a mutational hotspot in isolated complex I deficiency (3, 6).

The finding of heterozygosity and normal complex I activity in the muscular tissue of the aborted fetus (II.2) was unexpected, based on the biochemical results of the cultured chorionic villus specimen. Reanalysis of a second cultured chorionic villus specimen stored in liquid nitrogen confirmed the original result. Unfortunately, no homogenate of the original native chorionic villi was available for reanalysis.

To resolve the discrepancy between the biochemical results from native and cultured chorionic villi, we first investigated the genetic identity of the cultured chorionic villus specimen by analyzing nine different polymorphic short tetranucleotide repeat loci and the X-Y homologous gene amelogenin with the AmpFSTR™ Profiler Plus Kit (Applied Biosystems). The combination of these loci offers an average probability of identity of \( P \leq 1.04 \times 10^{-11} \) in Caucasians. We found that the cultured chorionic villus specimen was entirely of maternal, probably decidual, origin (Fig. 1C). This had happened despite all precautions, including the commonly performed microscopic check for maternal contamination. Milunsky and Cheney (7) detected the presence of maternal cells in 3 of 24 chorionic villus samples and concluded that microscopic examination of direct villi for apparent maternal cells is not rigorous enough, especially in the face of very sensitive PCR techniques.

The finding of maternal cell overgrowth does not explain the complex I deficiency of the cultured chorionic villus specimen, which should be normal because the mother is heterozygous for the mutation. Indeed, the cultured fibroblasts of both heterozygous parents had normal complex I activities (Table 1). We excluded a chromosomal aberration in the cultured maternal cells (8) as a possible cause for the biochemical abnormalities. To exclude a de novo mitochondrial DNA mutation as a possible cause for the complex I deficiency, we additionally sequenced all mitochondrially encoded tRNAs and ND genes of complex I in tissue DNA extracts from the mother (I.1), the index patient (II.1), and cultured chorionic villi (II.2). In all samples we found the following homoplasmic deviations from the standard GenBank sequence, NC_001807: MTND1, 3423T→C (Val→Val), 3480A→G; MTND2, 4769A→G; MTND3, 10398A→G; MTND4L, 10550A→G, 10632T→C (Leu→Leu); MTND4, 11299T→C, 11467A→G, 11719G→A; MTND5, 12372G→A; MTND6, 14167C→T; and MTTL2, 12308A→G. The underlined polymorphisms were not found in MITOMAP (http://www.gen.emory.edu/mitomap.html) but did not cause an amino acid exchange. All other polymorphisms were already known and recorded in the MITOMAP database.

We wished to determine whether the reference values for decidual cells differed from those of chorionic villi. We therefore prepared native decidual cells from healthy placentas and cultured decidual cells from chorionic villus biopsy specimens that had been obtained for purposes other than identifying mitochondrial diseases (see data supplement). We measured the respiratory chain enzyme activities in cultured as well as homogenized native decidual cells. The complex I activity of the native chorionic villus sample was within the reference interval for native decidual cells. The cultured decidual cells of the second pregnancy, however, still showed a clear complex I deficiency compared with control cultured decidual cells (Table 1). These results did not place the complex I activity of cultured cells from the second pregnancy within the reference interval. Despite all efforts, we could not find a satisfactory explanation for the results obtained in the second pregnancy of this family.

Because the mutation was now known, at the time of the third pregnancy we performed genetic and biochemical analyses with all available methods. This time the

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**Table 1. Respiratory chain enzyme activities** in fibroblasts, muscle tissue, chorionic villi, and decidual cells.

<table>
<thead>
<tr>
<th>Source</th>
<th>Material</th>
<th>Complex I</th>
<th>Complex II+III</th>
<th>Complex IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index patient (II.1)</td>
<td>Cultured fibroblasts&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51 (100–260)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>234 (210–440)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>941 (680–1190)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Muscle&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24 (84–273)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1748 (520–2080)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Second pregnancy (II.2)</td>
<td>Native chorionic villi&lt;sup&gt;a&lt;/sup&gt;</td>
<td>131 (84–263)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;f&lt;/sup&gt;</td>
<td>512 (271–922)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cultured decidual cells&lt;sup&gt;b&lt;/sup&gt;</td>
<td>120 (220–300)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>270 (190–530)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>830 (500–860)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Muscle (aborted fetus)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>108 (84–273)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1119 (520–2080)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Third pregnancy (II.3)</td>
<td>Native chorionic villi&lt;sup&gt;a&lt;/sup&gt;</td>
<td>160 (84–263)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;f&lt;/sup&gt;</td>
<td>550 (271–992)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cultured chorionic villi&lt;sup&gt;b&lt;/sup&gt;</td>
<td>290 (220–330)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>468 (190–530)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>505 (500–860)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Healthy controls (n = 5)</td>
<td>Native decidual cells&lt;sup&gt;a&lt;/sup&gt;</td>
<td>122–414&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;f&lt;/sup&gt;</td>
<td>122–208&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Healthy controls (n = 3)</td>
<td>Cultured decidual cells&lt;sup&gt;b&lt;/sup&gt;</td>
<td>220–300&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;f&lt;/sup&gt;</td>
<td>540–640&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mother (I.1)</td>
<td>Cultured fibroblasts&lt;sup&gt;b&lt;/sup&gt;</td>
<td>275 (100–260)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;f&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Father (I.2)</td>
<td>Cultured fibroblasts&lt;sup&gt;b&lt;/sup&gt;</td>
<td>218 (100–260)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;f&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;f&lt;/sup&gt;</td>
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<sup>a</sup> The reference values are not normally distributed; therefore, the 5th to 95th centile range is indicated in parentheses after the measured values.

<sup>b</sup> Activity measured in the mitochondrial enriched fraction.

<sup>c</sup> Activity measured in the tissue homogenate.

<sup>d</sup> Results expressed as mU/U cytochrome c oxidase activity.

<sup>e</sup> Results expressed as mU/U citrate synthase activity.

<sup>f</sup> ND, not determined.
results were consistent and showed conclusively that (a) both cultured and uncultured chorionic villi were of fetal origin, (b) the 632C→T transition was heterozygous (Fig. 1B), and (c) the complex I enzyme activities were normal (Table 1). A healthy boy was born after an uncomplicated pregnancy. He is now >3 years of age and does not show any symptoms of mitochondrial disease.

This case demonstrates the difficulties that can arise from prenatal diagnosis based on biochemical results alone. When results differ between native and cultured cells, no safe prediction can be made. Unidentified culturing artifacts might influence the biochemical activity of the cultured cells. Especially if the fetal cells are complex I deficient, a few “healthy” maternal cells may overgrow the fetal cells because of their advantage in oxidative energy metabolism. When interpreting the results of biochemical tests on cultured chorionic villi, one should be aware of the possibility of maternal cell contamination and exclude it by microsatellite marker analysis. When the fetus is male, a chromosome analysis might suffice.

We are grateful to the members of the family for participation in this study, to Dr. R. Knapp for providing the patient material, to Dr. H. A. Zondervan and Vera Mengers-Idenburg for providing the chorionic villi, to Christina Rovirosa and Loes van Moerkerken for technical assistance, and to Dr. C. Gerstenfeld for critical discussions about the manuscript. This study was supported by the Deutsche Forschungsgemeinschaft (SFB 577 TP B4) and the parents’ support group “Helft dem muskelkranken Kind” (Hamburg).

References

Complete Sequencing of a Genetic Polymorphism in NAT2 in the Korean Population, Soo-Youn Lee,1 Kyung-A Lee1, Chang-Seok Ki,2 O. Jong Kwon,2 Ho Joong Kim,2 Man Pyo Chung,2 Gee Young Suh,2 and Jong-Won Kim’ (1 Department of Clinical Pathology and Division of Pulmonary and Critical Medicine, 2Department of Medicine, Sungkyunkwan University School of Medicine and Samsung Medical Center, Seoul 135-710, Korea; * address correspondence to this author at: No. 50, Ilwon-dong, Kangnam-ku, Department of Clinical Pathology, Samsung Medical Center, Seoul 135-710, Korea; fax 82-2-3410-2719, e-mail jwonk@smc.samsung.co.kr)

N-Acetyltransferase 2 (NAT2) metabolizes arylamines and hydrazines. The substrates of NAT2 include many therapeutic drugs, such as isoniazid (INH), as well as chemicals and carcinogens (1–3). For that reason, N-acetylation activity is associated with drug effects or toxicities and susceptibility to various cancers. The ability of NAT2 to N-acetylate arylamines is subject to a genetic polymorphism in the NAT2 gene. The acetylation rate and NAT2 genotype distribution are quite different among various populations. The genetic polymorphism in NAT2 has not been studied extensively in the Korean population. Previous reports were based only on phenotyping or restriction fragment length polymorphism analysis, leading to possible misclassification of genotypes. We therefore decided to investigate NAT2 allelic variability and genotype distributions in the Korean population by complete sequencing. We also evaluated the relationship between genotype and phenotype to understand N-acetylation pharmacogenetics.

One thousand Korean individuals who visited the health promotion center at Samsung Medical Center were anonymously studied. An additional 23 healthy volunteers and 18 patients with pulmonary tuberculosis participated in this study. DNA was extracted from peripheral blood leukocytes. We amplified a 1211-bp fragment that included an 870-bp protein-coding region of the NAT2 gene and performed full sequencing analysis (4) on an ABI Prism 377 DNA Sequencer (Perkin-Elmer). We then checked nucleotide substitutions by combined use of allele-specific-PCR (AS-PCR) and restriction enzyme digestion. Specific primers (5) for the wild-type and mutant alleles were used in separate PCRs to detect C282T, T341C, and G590A substitutions. The nucleotides at positions 190, 481, 590, 803, and 857 were explored by digesting the 1211-bp PCR fragment or AS-PCR product carrying the wild-type or mutant allele with NciI, KpnI, TaqI, DdeI, and BamHI, respectively. For phenotyping, the healthy volunteers and patients gave informed consent and were given 300 mg of INH orally after an overnight fast. Participants were 18–65 years of age; had no underlying liver, kidney, or gastrointestinal diseases; did not have any drug history within the previous 2 weeks; and were not chronic alcoholics. Blood samples were obtained at 6 h, and urine samples were collected up to 6 h after INH administration. We measured urine and plasma concentrations of INH and acetylisoniazid (AcINH) by