Newborn Screening for 3-Methylcrotonyl-CoA Carboxylase Deficiency: Population Heterogeneity of MCCA and MCCB Mutations and Impact on Risk Assessment


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New technology enables expansion of newborn screening (NBS) of inborn errors aimed to prevent adverse outcome. In conditions with a large share of asymptomatic phenotypes, the potential harm created by NBS must carefully be weighed against benefit. Policies vary throughout the United States, Australia, and Europe due to limited data on outcome and treatability of candidate screening conditions. We elaborated the rationale for decision making in 3-methylcrotonyl-coenzyme A (CoA) carboxylase deficiency (MCCD), which afflicts leucine catabolism, with reported outcomes ranging from asymptomatic to death. In Bavaria, we screened 677,852 neonates for 25 conditions, including MCCD, based on elevated concentrations of 3-hydroxyisovalerylcarbamine (3-HIVA-C). Genotypes of MCCA (MCCC1) and MCCB (MCCC2) were assessed in identified newborns, their relatives, and in individuals (n = 17) from other regions, and correlated to biochemical and clinical phenotypes. NBS revealed eight newborns and six relatives with MCCD, suggesting a higher frequency than previously assumed (1:84,700). We found a strikingly heterogeneous spectrum of 22 novel and eight reported mutations. Allelic variants were neither related to biochemical nor anamnestic data of our probands showing all asymptomatic or benign phenotypes. Comparative analysis of case reports with NBS data implied that only few individuals (<10%) develop symptoms. In addition, none of the symptoms reported so far can clearly be attributed to MCCD. MCCD is a genetic condition with low clinical expressivity and penetrance. It largely represents as nondisease. So far, there are no genetic or biochemical markers that would identify the few individuals potentially at risk for harmful clinical expression. The low ratio of benefit to harm was pivotal to the decision to exclude MCCD from NBS in Germany. MCCD may be regarded as exemplary of the ongoing controversy arising from the inclusion of potentially asymptomatic conditions, which generates a psychological burden for afflicted families and a financial burden for health care systems. Hum Mutat 27(8), 748–759, 2006.
INTRODUCTION

An increasing number of screening centers in the United States, Australia, and Europe have implemented extended population newborn screening (NBS) programs using tandem mass spectrometry (MS/MS), which permits the simultaneous biochemical detection of more than 30 inborn errors of metabolism at a presymptomatic clinical stage. There is no consensus of national recommendations and policies as to which conditions should be mandated for NBS due to differences 1) of resources available, 2) in interpretations of conditions in relation to impact and treatability, and 3) in public advocacy by families and healthcare professionals. Recommendations range from expanded screening programs (29 conditions) including 3-methylcrotonyl-coenzyme A (CoA) carboxylase deficiency (MCCD; MIM 210200), as proposed by the American College of Medical Genetics (http://mchb.hrsa.gov/screening), to a restricted screening panel set up in the United Kingdom (four conditions) that excludes MCCD (www.ich.ucl.ac.uk/newborn). In Bavaria, South Germany, MCCD has been embedded into a NBS program since 1999. Here we present the data that led German authorities to exclude this disorder from mandated screening.

With the expansion of NBS, children with potentially mild or even asymptomatic disease are being increasingly identified. This is raising still unresolved questions with regard to justification of disorders selected for screening and proper prognostic counseling of families. MCCD is one of at least nine organic acidemias detectable by expanded NBS. The carnitine conjugate 3-hydroxyisovalerylcarnitine (3-HIVA-C) represents the sensitive, diagnostic hallmark in both NBS and high-risk screening programs [Röschinger et al., 1995; van Hove et al., 1995]. In addition, there is increased formation of 3-hydroxyisovaleric acid (3-HIVA) and 3-methylcrotonylglycine (3-MCG). 3-HIVA-C may also be a marker for deficiencies of holocarboxylase synthetase (HC-S), 3-methylglutaconyl-CoA hydratase (MG-H), and 3-hydroxy-3-methylglutaryl-CoA lyase (HMG-L). To our knowledge, no individual with HC-S has been identified so far after pilot screening of more than 5,000,000 newborns worldwide, implying an insufficient, albeit unknown, diagnostic sensitivity of 3-HIVA-C for detection by NBS. Only one individual with MG-H- and two with HMG-L-deficiency were reported. Individuals with 2-methylacetoacetyl-CoA cetoacetyl-CoA thiolase deficiency present with elevated 2-methyl-3-hydroxybutyrylcarnitine, which shares the same mass as 3-HIVA-C. They show tiglyl-carnitine as an additional marker.

3-Methylcrotonyl-CoA carboxylase is a biotin-dependent enzyme (MCC; EC 6.4.1.4) in the L-leucine degradation pathway (Fig. 1). The clinical course has been shown to vary considerably, ranging from entirely asymptomatic to death in infancy [Bannwart et al., 1992; Mourmans et al., 1993]. Severe and mild phenotypes are not clearly defined. Phenotypes are generally considered severe when they either show a fatal outcome or present with neurological symptoms such as considerable psychomotor retardation, seizures, or coma or with symptoms of cardiorespiratory failure. The vast majority of individuals with mild phenotypes is asymptomatic, while a subgroup displays mild unspecific symptoms like fatigue and weakness during catabolic episodes or mild developmental delay.

The enzyme consists of two subunits (MCCα, β) encoded by MCCA (MCCC1; MIM 609010) and MCCB (MCCC2; MIM #...
The MCC gene is located on chromosome 3q26–28 and consists of 19 exons, while the MCCB gene is located on chromosome 5q13 and consists of 17 exons (Fig. 2). MCCα has a biotin carboxylase domain containing the ATP and the bicarbonate binding sites, and a carboxyl terminal biotin carboxyl carrier domain. MCCβ contains a binding site for the acyl-CoA substrate within a carboxyltransferase domain that nearly spans the entire subunit. Both subunits possess amino terminal cleavable targeting signals, which direct them to the mitochondrial matrix in a membrane potential-dependent manner. The MCCα signals, which direct them to the mitochondrial matrix, are also shown in a schematic representation of the protein structure. The relative locations of the biotin carboxylase domain, the carboxyltransferase domain, and the mitochondrial signal peptides are also shown in a schematic representation of the protein structure.

Prior to NBS, MCCD had been considered an extremely rare inborn error of metabolism. However, expanded NBS revealed that MCCD is much more frequent than assumed from clinical observations. A birth incidence between 1:64,000 and 1:250,000 was estimated. However, expanded NBS revealed that MCCD is much more frequent than estimated from clinical observations. A birth incidence between 1:64,000 and 1:250,000 was expected.

This study had three objectives. The first was to prospectively ascertain a cohort of individuals with MCCD detected by NBS in Bavaria, South Germany, and to determine disease frequency and approximate allele frequency. The second was to describe genetic heterogeneity and to correlate genotypes to biochemical and clinical phenotypes by including an additional MCCD cohort. The third was to compare the group of individuals detected by NBS for potential differences in disease frequency and genotype-phenotype relationships.

The schematic representation of the genomic structure shows the number and approximate size of all coding regions of the MCCα and MCCβ genes. Downward arrows represent missense mutations. Upward arrows represent nonsense mutations, insertions, deletions, and splicing mutations. Mutations in bold letters are novel mutations (n = 22) identified in this study. The relative locations of the biotin carboxylase domain, the biotin carboxyl carrier domain, the carboxyltransferase domain, and the mitochondrial signal peptides are also shown in a schematic representation of the protein structure. The information used for this figure is derived from the Human Gene Mutation Database (HGMD) in Cardiff (www.hgmd.org), Baumgartner et al., 2001, Baykal et al., 2005, Gallardo et al., 2001, Holzinger et al., 2001, and the ExPASy Proteomics Server (MCCA/MCC1 Q96RQ3; MCCB/MCC2 Q9HCC0) (www.expasy.org).
with previously reported individuals showing clinical symptoms in order to 1) identify potential markers that may assist counseling of afflicted families and 2) find a rationale for deciding whether nationwide MCCD screening is justified in Germany.

**HUMAN SUBJECTS AND METHODS**

**Individuals With Allelic Variants in MCCA or MCCB**

A total of 28 probands from 23 families were analyzed. The individuals codes utilized were defined as follows: family (Families 01 to 23), sequence variation in MCCA or MCCB with changes in both alleles (A or B), family member identified by NBS (neonate, n), by family screening (sibling (s), mother (m)), and by high-risk screening (high risk, hr).

The study is based on a total population of 677,852 newborns tested within the Bavarian MS/MS-based NBS program, corresponding to 98.4% of all neonates born in Bavaria, South Germany, between January 1999 and December 2004, as assessed by demographic tracking [Liebl et al., 2002]. Eight neonates with biochemically confirmed MCCD were detected directly through NBS (Individuals 01-B-n, 02-A-n, 03-A-n1, 03-A-n2, 04-A-n, and 05-A-n; two follow-up samples were not available); additionally, two elder siblings were found by family screening after the identification of the index individual (Individuals 01-B-s and 05-A-s), and four afflicted mothers by follow-up investigation due to initially elevated 3-HIVA-C of their healthy newborns (Individuals 06-B-m, 07-B-m, and 08-B-m; one follow-up sample was not available).

In addition, specimens of 17 individuals from other parts of Germany (n = 13), Austria (n = 3), and South Australia (n = 1) were analyzed with 16 individuals identified directly or indirectly by NBS: eight neonates (Individuals 09-A-n, 10-B-n, 12-A-n, 13-B-n, 17-A-n, 18-B-n, 21-A-n, and 22-B-n), two elder siblings (Individuals 10-B-s1 and 10-B-s2), and six mothers (Individuals 11-A-m, 14-B-m, 15-A-m, 16-A-m, 19-A-m, and 20-A-m). One child was detected during diagnostic work-up by high-risk screening (Individual 23-A-hr).

**MS/MS-Based Newborn Screening for MCCD in Bavaria**

Acylcarnitines in dried blood spots obtained for NBS were analyzed as butyl-esters on a triple quadrupole tandem mass spectrometer with a TurboIon Spray source (API 365; MDS Sciex, Toronto, Ontario, Canada, www.mdssciex.com) as previously described [Fingerhut et al., 2001]. The analyte 3-HIVA-C was used as primary biochemical marker in the screening for MCCD. The secondary marker was the analyte ratio 3-HIVA-C/propionylcarnitine. The analyte and the analyte ratio were flagged automatically when their value exceeded a statistically determined technical decision limit (cutoff) based on data collected from reference populations of >10,000 newborns (mean plus four-fold standard deviation [SD] or the 99.5th percentile). Only elevations of both the primary marker 3-HIVA-C and the analyte ratio 3-HIVA-C/propionylcarnitine were utilized to label initial screening results as suspicious. This standardized biochemical NBS procedure resulted in an overall recall rate of 0.03% with a positive predictive value of 3.7% for the eight neonates identified.

All results with an abnormal acylcarnitine profile consistent with MCCD were verified in at least two separate analyses of the initial screening card. Further confirmatory investigations included acylcarnitine analyses of repeat dried blood spot samples, plasma acylcarnitines, and urinary organic acids. The measurement of the specific MCC enzyme activity was performed if fibroblasts were available.

**MCCA and MCCB Mutation Analysis**

After obtaining informed consent, mutation analysis was performed in individuals with a biochemically confirmed diagnosis of MCCD and from their parents if available.

We extracted RNA from skin fibroblasts using the QIAmp DNA Mini Kit (Qiagen). PCR amplification of the complete coding regions was performed with intron-located primers employing the HotStarTaq Master Mix Kit (Qiagen). The primers were designed to amplify exons, exon-intron boundaries, and short intron flanking stretches. Cycling conditions were as follows: initial denaturation/polymerase activation at 95 °C for 15 min followed by 34 cycles with denaturation of 94 °C for 45 s, annealing at 62 °C for 45 s, extension at 72 °C for 45 s, and final extension at 72 °C for 10 min. After purification, all PCR products were subjected to bidirectional direct cycle sequencing using the dRhodamine DNA Sequencing Kit and an ABI 310 capillary DNA sequencer (Applied Biosystems, Foster City, CA; www.appliedbiosystems.com). Sequencing primers were the same that had been used for PCR amplification. Recommendations of the Nomenclature Working Group were followed [Antonarakis, 1998]. Primer sequences, annealing temperatures, and fragment lengths of the PCR products are available upon request. cDNA mutation numbering is based on GenBank AF310972 (MCCA/MCCCI) and AF310971 (MCCB/MCCCI), with +1 as A of the ATG start codon; the genomic reference sequence for intronic mutations is GenBank NM_020166 (MCCA/MCCCI) and NM_022132 (MCCB/MCCCI), respectively.

**Enzyme Assay of MCC**

The assay involves the incorporation of [14C] bicarbonate (NaH [14C]O3) into 3-methylcrotonyl-CoA to give labeled nonvolatile products. Excess of labeled bicarbonate is removed by acidification by formic acid. These methods were described previously [Weyler et al., 1977; Tuchman et al., 1993]. All reactions were performed in duplicate both with and without supplementation of bovine. Specific activities of MCCD individuals and controls were expressed in picomoles of [14C] bicarbonate fixed per milligram of protein × min and the activities of the individuals were expressed in percentages of controls.

**RESULTS**

**MCCD Identified by MS/MS-Based Newborn Screening in Bavaria**

Eight newborns (five females, three males) with biochemically confirmed MCCD were detected, corresponding to a frequency of 1:84,700 (1:12,500 to 1:196,000; 95% confidence interval) in the population investigated (677,852 newborns). In addition, two
elder siblings (one female, one male) and four mothers were identified by family screening. For the primary biochemical marker 3-HIVA-C, the normal mean was 0.16 μmol/l (+4 SD: 0.72 μmol/l), for the analyte ratio 3-HIVA-C/propionylcarnitine 0.059 (+4 SD: 0.47). Compared with the 99.5th percentile of the entire population screened, all MCCD cases exceeded the values for both the primary marker 3-HIVA-C (0.51 μmol/l) and the ratio 3-HIVA-C/propionylcarnitine (0.33). Initial screening values of the MCCD individuals identified in Bavaria (Individual 01-B-n and Individuals 02-A-n through 05-A-n) ranged from 1.8 to 35.0 μmol/l for 3-HIVA-C, and from 2.2 to 17.6 for the ratio of 3-HIVA-C/propionylcarnitine.

**Spectrum of MCCA and MCCB Mutations**

Blood samples for mutation analysis (derived from NBS in Bavaria and sent from various children’s hospitals) were available from 28 out of 31 individuals with biochemically confirmed MCCD. All individuals revealed two mutations within either the MCCA or MCCB gene.

A spectrum of 30 different mutations was identified (15 in MCCA; 15 in MCCB), comprising seven splice-site mutations (three in MCCA; four in MCCB), five nonsense mutations (three in MCCA; two in MCCB), two small deletions in MCCA, two small insertions in MCCB, and 14 missense mutations (seven in MCCA; seven in MCCB). All novel (n = 22) and previously reported (n = 47) mutations in MCCA and MCCB are summarized in Figure 2. The mutations identified in all 28 individuals analyzed are strikingly heterogeneous and almost evenly distributed over the two genes, MCCA and MCCB, with no mutational hotspot.

Only 8 of the 30 mutations (five in MCCA; three in MCCB) have previously been described: six (c.559C>T, c.1604C>T, and c.2079delA in MCCA; c.512–1G>A, c.518dupT, and c.803G>C in MCCB) were reported in MCCD individuals with mild clinical phenotypes [Baumgartner et al., 2001; Holzinger et al., 2001]. The mutation c.1527C>A in MCCA was found in both mildly and severely affected individuals and the variant c.1155A>C in MCCA was found in two individuals (one severe, one case not reported in detail) [Baumgartner et al., 2001; Gallardo et al., 2001; Holzinger et al., 2001; Dantas et al., 2005]. The remaining 22 mutations (10 in MCCA; 12 in MCCB) are novel mutations (Table 1).

**Novel Mutations in the MCCA Gene**

The novel mutations in the MCCA gene include three splice-site mutations, two nonsense mutations, one small deletion, and four missense mutations (Table 1). The splice-site variants c.137–2A>G, c.1268–2A>G, and c.1681+5G>A alter the highly conserved splice consensus sequence affecting mRNA splicing. While c.1268–2A>G results in the skipping of exons 12 and 13, c.1681+5G>A leads to the loss of exon 14. For c.137–2A>G, exon skipping could not be verified, since RNA for transcript analysis was not available. c.945T>A and c.1225C>T are novel nonsense mutations. Both of them provoke an in-frame premature termination codon (PTC) (p.R315X, p.R409X). The small deletion c.250_251delAG causes a frameshift within the amino acid coding sequence and introduces a PTC in the reading frame of the altered protein nine amino acids after the deletion (p.R84KfsX9). As mRNA containing PTGs are identified by an mRNA quality control system leading to degradation by a nonsense mediated decay (NMD) pathway [Culbertson, 1999; Frischmeyer and Dietz, 1999], these sequence variations are generally considered to have a deleterious effect on protein biosynthesis. If small amounts of PTC-containing mRNA should escape the NMD pathway, it is most probable that the resulting truncated proteins will be degraded by intracellular proteases. Thus, these sequence variations are considered to result in functional null-mutations, characterized by negligible amounts of variant protein formed. The following four mutations are nonsense mutations and lead to exchanges of single amino acid residues. The alterations c.369G>C, c.375C>G, c.479T>G, and c.1135G>A concern amino acids within the biotin carboxylase domain (for MCCA/MCCC1 Q96RQ3; ExPASy Proteomics Server; www.expasy.org). Protein alignments across species revealed that all of these novel missense mutations affect amino acids that are highly conserved for example in Mus musculus, Glycine max (except for c.1135G>A), and Arabidopsis thaliana (data not shown).

**Novel Mutations in the MCCB Gene**

The novel mutations in the MCCB gene include three splice-site mutations, two nonsense mutations, one small insertion, and six missense mutations (Table 1). The splice-site variants c.281+5G>A, c.383+1G>T, and c.384–2A>G alter the highly conserved splice consensus sequences processing mRNA. RNA for transcript analysis was not available. Both novel nonsense mutations, c.538C>T and c.1465C>T, generate in-frame PTGs (p.R180X and p.Q489X), while c.243dupT is an insertion in exon 3, creating a PTC seven altered amino acids after the insertion (p.L81LfsX7). The carboxytransferase domain (ExPASy Proteomics Server; www.expasy.org for MCCC2/MCCB Q9HC0) nearly spans the complete MCCB subunit (amino acid residues 69–561) and PTGs in this domain result in loss of function of the protein subunit. Six missense mutations in the MCCB gene are novel (c.302C>T, c.578G>A, c.979A>T, c.1019A>T, c.1208A>C, and c.1663A>G) and generate substitutions of single amino acid residues. The first five alterations affect amino acid residues that are evolutionary highly conserved in Agrobacterium tumefaciens and Arabidopsis thaliana (data not shown).

While nonsense mutations, small deletions or insertions, and splice-site mutations are considered to be functional null-mutations resulting in a defective phenotype, the effect of missense mutations (on protein function) is at issue when expression analyses are not available. Applying the following indirect criteria, however, the reported missense mutations in MCCA and MCCB are likely to affect protein function, modifying the observed biochemical phenotype: 1) No additional mutations have been found by sequencing of the complete coding regions of both the MCCA and MCCB genes in all individuals. Mutations that might have escaped detection by our method such as promoter variations and large deletions are very rare. 2) The identified mutations segregated with the biochemical phenotype. Only individuals homozygous or compound heterozygous for allelic variants displayed an abnormal biochemical phenotype, whereas heterozygous individuals were unaffected. 3) All except one (c.1663A>G) novel missense mutation affect amino acid residues that are highly conserved in MCCA, β-proteins of other eukaryotic organisms (data not shown). The mutation c.1663A>G (p.K555E) in MCCB represents a significant amino acid substitution (exchange of a basic residue by an acidic one).

**Sequence Variations Related to Biochemical Phenotypes of MCCD Individuals**

The 28 probands reported in this study were divided into three groups and listed with decreasing concentrations of 3-HIVA-C in blood as measured at diagnosis (Table 2): group 1 consisted of 14
newborns (nine female, five male) identified by NBS (age range: 1.75–6.5 years). In group 2, four elder siblings (one female, three male) diagnosed through family screening were summarized (age range: 5.5–12.25 years) after identification of the index individual in NBS. In group 3, nine mothers (age range: 22–38 years) were identified by follow-up of an initially pathologic NBS sample of their healthy newborns. In addition, one 7-year-old boy was found through high-risk screening. More than two-thirds of their healthy newborns. In addition, one 7-year-old boy was diagnosed through family screening were summarized (age range: 8–22 years).

All individuals in our cohort showed a biochemical phenotype characteristic for MCCD with regard to 1) the accumulation of metabolites in blood (3-HIVA-C and the ratio 3-HIVA-C/propionylcarnitine) and 2) in urine (3-HIVA and/or 3-MCG), of metabolites in blood (3-HIVA-C and the ratio 3-HIVA-C/propionylcarnitine) and 2) in urine (3-HIVA and/or 3-MCG), of metabolites in blood (3-HIVA-C and the ratio 3-HIVA-C/propionylcarnitine) and 2) in urine (3-HIVA and/or 3-MCG).

The specific enzyme activity of each MCCD individual was directly compared with a normal control, both with and without supplementation of biotin. Activities in MCCD individuals ranged from 0 to 13% of control fibroblasts, with no response to biotin supplementation of biotin. Activities in MCCD individuals ranged from 0 to 13% of control fibroblasts, with no response to biotin supplementation of biotin. Activities in MCCD individuals ranged from 0 to 13% of control fibroblasts, with no response to biotin supplementation of biotin. Activities in MCCD individuals ranged from 0 to 13% of control fibroblasts, with no response to biotin supplementation of biotin.
### TABLE 2. Mutations in the MCCA and MCCB Gene, Biochemical Phenotypes, and Ethnic Origin of 28 Probands With MCCD*

<table>
<thead>
<tr>
<th>Proband</th>
<th>3-HIVA-C</th>
<th>3-HIVA-C/C3</th>
<th>MCC %</th>
<th>Nucleotide change; allele 1*</th>
<th>Location</th>
<th>Predicted consequence</th>
<th>Nucleotide change; allele 2*</th>
<th>Location</th>
<th>Predicted consequence</th>
<th>Current age</th>
<th>Ethnic Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>22-B-n</td>
<td>3.71</td>
<td>3.0 n.m.</td>
<td>c.384-2A&gt;G</td>
<td>IVS 04</td>
<td>(B)</td>
<td>Def. splicing</td>
<td>c.578G&gt;A</td>
<td>Ex 06</td>
<td>(B)</td>
<td>p.R139H</td>
<td>1 year, 9 months</td>
</tr>
<tr>
<td>02-A-n</td>
<td>35.0</td>
<td>16.0 2.5</td>
<td>c.1155A&gt;C</td>
<td>Ex 11</td>
<td>(A)</td>
<td>p.R385S</td>
<td>c.1681G&gt;A</td>
<td>IVS 14</td>
<td>(A)</td>
<td>p.Q123H</td>
<td>3 years, 1 month</td>
</tr>
<tr>
<td>21-A-n</td>
<td>21.9</td>
<td>29.2 n.m.</td>
<td>c.369G&gt;C</td>
<td>Ex 04</td>
<td>(A)</td>
<td>p.Q123H</td>
<td>c.369G&gt;C</td>
<td>Ex 04</td>
<td>(A)</td>
<td>p.C509X</td>
<td>5 years</td>
</tr>
<tr>
<td>12-A-n</td>
<td>24.0</td>
<td>5.7 n.m.</td>
<td>c.1527C&gt;A</td>
<td>Ex 13</td>
<td>(A)</td>
<td>p.C509X</td>
<td>c.1527C&gt;A</td>
<td>Ex 13</td>
<td>(A)</td>
<td>p.C509X</td>
<td>5 years</td>
</tr>
<tr>
<td>17-A</td>
<td>11.6</td>
<td>24.2 n.m.</td>
<td>c.1355G&gt;A</td>
<td>Ex 11</td>
<td>(A)</td>
<td>p.C509X</td>
<td>c.375G&gt;C</td>
<td>Ex 05</td>
<td>(A)</td>
<td>p.I125M</td>
<td>3 years, 1 month</td>
</tr>
<tr>
<td>18-B</td>
<td>11.6</td>
<td>19.7 n.m.</td>
<td>c.302C&gt;T</td>
<td>Ex 14</td>
<td>(B)</td>
<td>p.S101F</td>
<td>c.243dupT</td>
<td>Ex 03</td>
<td>(B)</td>
<td>p.L811fsX7</td>
<td>3 years, 11 months</td>
</tr>
<tr>
<td>10-B</td>
<td>11.0</td>
<td>11.2 5.8</td>
<td>c.803G&gt;C</td>
<td>Ex 08</td>
<td>(B)</td>
<td>p.R268T</td>
<td>c.803G&gt;C</td>
<td>Ex 08</td>
<td>(B)</td>
<td>p.R268T</td>
<td>3 years, 1 month</td>
</tr>
<tr>
<td>05-A-n</td>
<td>10.1</td>
<td>15.7 0.0</td>
<td>c.1604C&gt;T</td>
<td>Ex 14</td>
<td>(A)</td>
<td>p.S335F</td>
<td>c.2079delA</td>
<td>Ex 19</td>
<td>(A)</td>
<td>p.V694X</td>
<td>5 years, 8 months</td>
</tr>
<tr>
<td>03-A-n</td>
<td>7.4</td>
<td>2.3 n.m.</td>
<td>c.479T&gt;G</td>
<td>Ex 05</td>
<td>(A)</td>
<td>p.I125M</td>
<td>c.479T&gt;G</td>
<td>Ex 05</td>
<td>(A)</td>
<td>p.I125M</td>
<td>1 year, 9 months</td>
</tr>
<tr>
<td>01-B</td>
<td>5.8</td>
<td>2.2 3.0</td>
<td>c.1019A&gt;T</td>
<td>Ex 11</td>
<td>(B)</td>
<td>c.1645C&gt;T</td>
<td>Ex 15</td>
<td>(B)</td>
<td>c.489X</td>
<td>3 years, 9 months</td>
<td>German/Italian</td>
</tr>
<tr>
<td>13-B</td>
<td>3.2</td>
<td>0.5 n.m.</td>
<td>c.338C&gt;T</td>
<td>Ex 06</td>
<td>(B)</td>
<td>p.R180X</td>
<td>c.979A&gt;T</td>
<td>Ex 08</td>
<td>(B)</td>
<td>p.H266L</td>
<td>1 year, 9 months</td>
</tr>
<tr>
<td>04-A-n</td>
<td>3.2</td>
<td>3.0 2.3</td>
<td>c.1681G&gt;A</td>
<td>IVS 14</td>
<td>(A)</td>
<td>Loss of exon 14</td>
<td>c.1681G&gt;A</td>
<td>IVS 14</td>
<td>(A)</td>
<td>Loss of exon 14</td>
<td>3 years</td>
</tr>
<tr>
<td>09-A-n</td>
<td>2.6</td>
<td>5.1 12.0</td>
<td>c.599T&gt;C</td>
<td>Ex 06</td>
<td>(A)</td>
<td>p.S878P</td>
<td>c.1155A&gt;C</td>
<td>Ex 11</td>
<td>(A)</td>
<td>p.R385S</td>
<td>5 years, 7 months</td>
</tr>
<tr>
<td>03-A-n</td>
<td>1.8</td>
<td>17.6 0.0</td>
<td>c.479T&gt;G</td>
<td>Ex 05</td>
<td>(A)</td>
<td>p.I125M</td>
<td>c.479T&gt;G</td>
<td>Ex 05</td>
<td>(A)</td>
<td>p.I125M</td>
<td>6 years, 6 months</td>
</tr>
<tr>
<td>01-B-s</td>
<td>18.4</td>
<td>16.0 2.4</td>
<td>c.1019A&gt;T</td>
<td>Ex 11</td>
<td>(B)</td>
<td>c.1645C&gt;T</td>
<td>Ex 15</td>
<td>(B)</td>
<td>c.489X</td>
<td>7 years, 8 months</td>
<td>German/Italian</td>
</tr>
<tr>
<td>10-B-s</td>
<td>17.0</td>
<td>34.0 n.m.</td>
<td>c.803G&gt;C</td>
<td>Ex 08</td>
<td>(B)</td>
<td>p.R268T</td>
<td>c.803G&gt;C</td>
<td>Ex 08</td>
<td>(B)</td>
<td>p.R268T</td>
<td>5 years, 7 months</td>
</tr>
<tr>
<td>10-B-s1</td>
<td>14.0</td>
<td>23.3 n.m.</td>
<td>c.803G&gt;C</td>
<td>Ex 08</td>
<td>(B)</td>
<td>p.R268T</td>
<td>c.803G&gt;C</td>
<td>Ex 08</td>
<td>(B)</td>
<td>p.R268T</td>
<td>6 years, 11 months</td>
</tr>
<tr>
<td>05-A</td>
<td>9.4</td>
<td>28.0 n.m.</td>
<td>c.1604C&gt;T</td>
<td>Ex 14</td>
<td>(A)</td>
<td>p.S335F</td>
<td>c.2079delA</td>
<td>Ex 19</td>
<td>(A)</td>
<td>p.V694X</td>
<td>12 years, 3 months</td>
</tr>
<tr>
<td>11-A-m</td>
<td>40.5</td>
<td>44.1 13.0</td>
<td>c.12682A&gt;G</td>
<td>IVS 11</td>
<td>(A)</td>
<td>Loss of exons 12/13</td>
<td>c.12682A&gt;G</td>
<td>IVS 11</td>
<td>(A)</td>
<td>Loss of exons 12/13</td>
<td>38 years</td>
</tr>
<tr>
<td>15-A-m</td>
<td>37.5</td>
<td>93.8 9.2</td>
<td>c.1527C&gt;A</td>
<td>Ex 13</td>
<td>(A)</td>
<td>p.C509X</td>
<td>c.1527C&gt;A</td>
<td>Ex 13</td>
<td>(A)</td>
<td>p.C509X</td>
<td>26 years</td>
</tr>
<tr>
<td>07-B-m</td>
<td>34.2</td>
<td>67.6 0.0</td>
<td>c.3831G&gt;T</td>
<td>IVS 04</td>
<td>(B)</td>
<td>Def. splicing</td>
<td>c.3831G&gt;T</td>
<td>IVS 04</td>
<td>(B)</td>
<td>Def. splicing</td>
<td>29 years</td>
</tr>
<tr>
<td>19-A</td>
<td>25.4</td>
<td>65.1 n.m.</td>
<td>c.2502GdelAG</td>
<td>Ex 03</td>
<td>(A)</td>
<td>p.R84KfsX9</td>
<td>c.2502GdelAG</td>
<td>Ex 03</td>
<td>(A)</td>
<td>p.R84KfsX9</td>
<td>24 years</td>
</tr>
<tr>
<td>16-A</td>
<td>21.5</td>
<td>0.0 n.m.</td>
<td>c.947T&gt;A</td>
<td>Ex 09</td>
<td>(A)</td>
<td>p.Y315X</td>
<td>c.1225C&gt;T</td>
<td>Ex 11</td>
<td>(A)</td>
<td>p.R409X</td>
<td>30 years</td>
</tr>
<tr>
<td>08-B</td>
<td>14.5</td>
<td>17.2 n.m.</td>
<td>c.243dupT</td>
<td>Ex 03</td>
<td>(B)</td>
<td>p.L811fsX7</td>
<td>c.5122G&gt;A</td>
<td>IVS 05</td>
<td>(B)</td>
<td>Def. splicing</td>
<td>38 years</td>
</tr>
<tr>
<td>20-A</td>
<td>14.4</td>
<td>50.1 n.m.</td>
<td>c.1577-2A&gt;G</td>
<td>IVS 02</td>
<td>(A)</td>
<td>Def. splicing</td>
<td>c.1577-2A&gt;G</td>
<td>IVS 02</td>
<td>(A)</td>
<td>Def. splicing</td>
<td>22 years</td>
</tr>
<tr>
<td>06-B-m</td>
<td>10.5</td>
<td>7.5 n.m.</td>
<td>c.2811G&gt;A</td>
<td>IVS 03</td>
<td>(A)</td>
<td>Def. splicing</td>
<td>c.1220A&gt;C</td>
<td>Ex 13</td>
<td>(A)</td>
<td>p.N403T</td>
<td>38 years</td>
</tr>
<tr>
<td>14-B</td>
<td>1.4</td>
<td>1.5 n.m.</td>
<td>c.518dupT</td>
<td>Ex 06</td>
<td>(B)</td>
<td>p.S173HfsX4</td>
<td>c.1663A&gt;G</td>
<td>Ex 17</td>
<td>(B)</td>
<td>p.K555E</td>
<td>26 years</td>
</tr>
</tbody>
</table>

*All probands are asymptomatic except for Individuals 11-A-m and 06-B-m with mild exercise intolerance, and Individual 23-A-hr with mild speech delay. All showed marked elevations of the organic acids 3-HIVA and 3-MCG except for Individuals 09-A-n and 14-B-m, who showed moderate elevations, and Individual 08-B-m with mild exercise of only 3-MCG. Individual 20-B-m was not analyzed.

**A**-HIVA-C concentration in blood, expressed in μmol/l; 99.5th percentile = 0.51 μmol/l; 3-HIVA-C is significantly higher in mothers compared to neonates (P < 0.1).

†Ratio of 3-HIVA-C to propionylcarnitine concentrations in blood, each expressed in μmol/l; 99.5th percentile = 0.33.

‡Specific activity of MCC in skin fibroblast cultures (picomoles of [3H] bicarbonate fixed/mg protein × min), expressed as percentage of control.

§DNA mutation numbering is based on GenBank AF310972 (MCCA/MCCB) and AF310971 (MCCB/MCCC), with +1 as A of the ATG start codon; genomic reference sequence for intronic mutations is GenBank NM_020166 (MCCA/MCCB) and NM_022132 (MCCB/MCCC), respectively.

(A) represents MCCA; (B) represents MCCB.

Children from consanguineous parents.

3-HIVA, 3-hydroxyisovaleric acid; 3-MCG, 3-methylcrotonylglycine; 3-HIVA-C, 3-hydroxyisovalerylcarnitine; Ex, exon; Def, defective; n.m., not measured.
at the age of 33 days (cerebral edema, cardiopulmonary failure) [Baykal et al., 2005], 11 months (cardiopulmonary failure after a generalized seizure) [Bannwart et al., 1992], 26 months (cerebral edema) [Kobori et al., 1989; Jurecki and Packman, 1992], and 40 months (cause of death not reported) [Dodelson de Kremer et al., 2002], respectively, resulting in a lethality of about 10.0%. The death of one individual at the age of 6.5 months appears not directly related to MCCD (cardiac arrest after accidental obstruction of the endotracheal tube) [Wiesmann et al., 1998]. Nine children [Beemer et al., 1982; Kobori et al., 1989; Tsai et al., 1989; Jurecki and Packman, 1992; Mourmans et al., 1995; Pearson et al., 1995; Ihara et al., 1997] and one adult [Visser et al., 2000] did not show any clinical signs (27%), while a large subgroup developed mild, mostly neurological, symptoms.

The time points when first clinical symptoms arise are spread between the first day of life [Bannwart et al., 1992; Murayama et al., 1997; Wiesmann et al., 1998] and 4.7 years of age [Beemer et al., 1982]. Laboratory investigations during a catabolic decomposition due to febrile illness, gastroenteritis, or prolonged fasting may show hypoglycemia, a mild imbalance of blood gas analysis (pH 7.30–7.38; base excess –7.5 to –15.3 mmol/l), and sporadically hyperammonemia [Layward et al., 1989; Tsai et al., 1989]. A frequent finding is impaired consciousness, with the development of coma in single individuals [Beemer et al., 1982; Bartlett et al., 1984; Gitzelmann et al., 1987; Rolland et al., 1991; Pearson et al., 1995; Lehnert et al., 1996; Visser et al., 2000; Baykal et al., 2005].

Generally, neurological symptoms like muscular hypotonia, psychomotor retardation, or seizures are prominent. Two individuals necessitated intubation due to an acute apnea [Bartlett et al., 1984; Layward et al., 1989]. One girl presented with cardiomyopathy [Visser et al., 2000], four individuals showed brain atrophy [Murayama et al., 1997; Steen et al., 1999; Baumgartner et al., 2004; Baykal et al., 2005], and two children developed multiple foci of leukodystrophy [Dodelson de Kremer et al., 2002; Baykal et al., 2005]. Finally, single individuals were reported to have fatty infiltration of liver [Kobori et al., 1989; Layward et al., 1989] or muscle [Elpeleg et al., 1992].

**DISCUSSION**

Mass spectrometric technology enables expansion of NBS to a potentially large range of inherited conditions detectable simultaneously in single analytical runs. Thus cost-benefit considerations for inclusion of individual disorders are less important than outlined in traditional screening criteria. Nevertheless, in the context of population mass screening, there remains the potential to cause harm by NBS. Individual preventive benefits must be carefully weighed against the adverse effects of false-positive rates in a predominantly healthy population or unwanted detection of benign conditions that are prone to unnecessary intervention and/or stigmatization of individuals.

The criteria to select and prioritize disorders for inclusion in NBS vary considerably between countries. This is due to differing weighting of performance of testing procedures and the natural course and treatment options of specific novel conditions [Natowicz, 2005]. Some disorders have previously been underdiagnosed and NBS pilot programs revealed that the spectrum of many disorders, like MCCD, is much broader than anticipated. The disorder may include a large share of milder phenotypes of as yet unknown clinical significance. In Germany, expanded NBS

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**TABLE 3. Analysis of Published Data: Clinical Variability of 37 Individuals With Clinically Diagnosed MCCD, Not by NBS, Between the Years 1982 and 2005**

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>Number of patients</th>
<th>% of Patients (n = 37)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>No symptoms</td>
<td>10</td>
<td>27.0</td>
<td>Beemer et al. [1982]; Kobori et al. [1989]; Tsai et al. [1989]; Jurecki and Packman [1992]; Mourmans et al. [1995]; Pearson et al. [1995]; Ihara et al. [1997]; Visser et al. [2000]</td>
</tr>
<tr>
<td>Deceased</td>
<td>4</td>
<td>10.8</td>
<td>Kobori et al. [1989]; Bannwart et al. [1992]; Jurecki and Packman [1992]; Dodelson de Kremer et al. [2002]; Baykal et al. [2005]</td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>7</td>
<td>18.9</td>
<td>Bartlett et al. [1984]; Gitzelmann et al. [1987]; Kobori et al. [1989]; Layward et al. [1989]; Tsai et al. [1989]; Steen et al. [1999]; Baykal et al. [2005]</td>
</tr>
<tr>
<td>Mild imbalance of blood gas analysis</td>
<td>5</td>
<td>13.5</td>
<td>Beeper et al. [1982]; Bartlett et al. [1984]; Gitzelmann et al. [1987]; Kobori et al. [1989]; Layward et al. [1989]; Baykal et al. [2005]</td>
</tr>
<tr>
<td>Hyperammonemia</td>
<td>2</td>
<td>5.4</td>
<td>Layward et al. [1989]; Tsai et al. [1989]</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>5</td>
<td>13.5</td>
<td>Elpeleg et al. [1992]; Murayama et al. [1997]; Wiesmann et al. [1998]; Visser et al. [2000]; Dodelson de Kremer et al. [2002]</td>
</tr>
<tr>
<td>Muscular hypotonia</td>
<td>11</td>
<td>29.7</td>
<td>Bartlett et al. [1984]; Gitzelmann et al. [1987]; Bannwart et al. [1992]; Elpeleg et al. [1992]; Wiesmann et al. [1998]; Dodelson de Kremer et al. [2002]; Baykal et al. [2005]; Murayama et al. [1997]; Yap et al. [1998]; Steen et al. [1999]; Visser et al. [2000]; Dodelson de Kremer et al. [2002]; Baumgartner et al. [2004]; Baykal et al. [2005]</td>
</tr>
<tr>
<td>Psychomotor retardation</td>
<td>12</td>
<td>32.4</td>
<td>Bartlett et al. [1982]; Gitzelmann et al. [1987]; Kobori et al. [1989]; Rolland et al. [1991]; Bannwart et al. [1992]; Tuchman et al. [1993]; Pearson et al. [1995]; Lehnert et al. [1996]; Murayama et al. [1997]; Wiesmann et al. [1998]; Steen et al. [1999]; Visser et al. [2000]; Dodelson de Kremer et al. [2002]; Baykal et al. [2005]</td>
</tr>
<tr>
<td>Seizures</td>
<td>6</td>
<td>16.2</td>
<td>Tsai et al. [1989]; Bannwart et al. [1992]; Lehnert et al. [1996]; Murayama et al. [1997]; Steen et al. [1999]; Baumgartner et al. [2004]</td>
</tr>
<tr>
<td>Other neurological symptoms</td>
<td>18</td>
<td>48.6</td>
<td>Beemer et al. [1982]; Bartlett et al. [1984]; Gitzelmann et al. [1987]; Kobori et al. [1989]; Rolland et al. [1991]; Bannwart et al. [1992]; Tuchman et al. [1993]; Pearson et al. [1995]; Lehnert et al. [1996]; Murayama et al. [1997]; Wiesmann et al. [1998]; Steen et al. [1999]; Visser et al. [2000]; Dodelson de Kremer et al. [2002]; Baykal et al. [2005]</td>
</tr>
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</table>
pilot programs have been established since 1999 having voluntary participation and parental consent as prerequisite for participation.

In a prospective observational study in Bavaria, South Germany, 25 disorders were screened for 6 years with a participation rate of over 98% covering a total of 677,852 newborns. The data of this experience were instrumental for revising the initial large panel of disorders selected for NBS in pilot programs. German federal authorities recently recommended 12 conditions, including three organic acidemias, for nationwide, standardized NBS [Hess, 2005]. Here we present data and reasoning as to why MCCD was excluded.

In the prospective study, MCCD was identified at a higher frequency than previously suggested from rare clinical observations. We found eight individuals with a characteristic biochemical phenotype for MCCD indicating a frequency of 1:84,700 (1:12,500–1:196,000; 95% confidence interval).

Similar birth prevalences were reported in other NBS programs [Zytkovicz et al., 2001; Chace et al., 2002; Koebel et al., 2003; Schulze et al., 2003; Wilcken et al., 2003]. The most likely reason for divergent frequency estimates is that MCCD represents a disease with a functionally expression and low clinical penetrance. Follow-up observation of the MCCD neonates revealed that all individuals were healthy and were growing and developing normally up to an age of 6.5 years.

However, validation of such outcome measures in NBS remains limited, since all affected newborns were treated early on with mild protein restriction, carnitine supplementation, and by counseling of parents to prevent catabolic episodes. The natural course of the disease subsequent to NBS thus remains difficult to ascertain. Randomized studies were impossible to justify in view of the serious consequences of the disease reported in single case studies (Table 3). During follow-up of NBS, however, we observed a high incidence of mild MCCD phenotypes among family members, who show the natural course of this condition. We identified nine mothers and four elder siblings, all of whom were either asymptomatic or had mild unspecific symptoms (two mothers). This suggests that the share of MCCD conditions with clinically unapparent outcome may indeed be high.

A review of the literature (Table 3) on 37 individuals indicates that only 27% developed normally and stayed completely asymptomatic. Approximately 30% were reported to suffer from muscular hypotonia and psychomotor retardation, respectively, and almost half suffer from various other neurological symptoms. Even a lethality of 11% was observed. Such retrospective analysis of clinical case studies most likely reflects bias of selection, since only a rather small subgroup of MCCD individuals may develop overt clinical signs leading to diagnostic workup.

During the 20 years since the detection of MCCD in 1982, about 170,000,000 children were born in the United States and Europe, assuming a mean birth prevalence of over 8,500,000. Based on the literature (Table 3), during the same time span, four individuals died, reportedly but not proven related to MCCD. In previous studies, we observed that recognition of inherited diseases is about three times higher by expanded NBS than by clinical diagnosis [Hoffmann et al., 2004]. Even when assuming a five-fold higher rate (20 deaths) due to nonreporting or nonrecognition, the risk of death might at maximum be only 1:8,500,000. A total of 27 individuals became apparent by some, albeit minor, clinical symptoms. Again, when assuming a five-fold higher rate (135), the risk for expression of some clinical symptoms would be about 1:1,200,000. Compared with our mean birth prevalence seen in NBS (1:84,000), we hypothesize that the share of individuals that might develop minor symptoms is clearly below 10% and only less than 1 to 2% might have a risk for severe adverse outcome. Under such assumptions, had NBS been implemented 20 years ago it might have identified more than 2,000 MCCD individuals with a potentially clinically unapparent outcome. Another 50,000 families would have been afflicted by false-positive findings assuming our rate of 0.03%.

Can information on the genetic underpinnings of the MCCD condition contribute to better risk estimation? We unraveled a striking genetic heterogeneity, with mutations being equally distributed on both genes, MCCCA and MCCCB. A heterogeneous range of mutations may in theory be used to describe subpopulations of the condition with differing susceptibility for biochemical or clinical expression [Liu et al., 2004]. However, we observed no obvious correlations. There was no clear relation between the impaired MCC enzyme activity and the concentrations of accumulated metabolites in both blood and urine. Nevertheless, all MCCD individuals analyzed showed clear biochemical alterations, indicating that the mutations we describe are indeed causing the biochemical phenotype. Biochemical manifestation of a disease, however, does not necessarily imply that it will result in significant clinical symptoms. This phenomenon is well known for other inborn errors of metabolism detectable by NBS, like mild hyperphenylalaninemia in phenylketonuria screening, isovaleric acidemia (IVA), or medium-chain acyl-CoA dehydrogenase deficiency (MCADD) [Weglage et al., 2001; Ensenaue et al., 2004; Maier et al., 2005]. Novel mutations were identified in asymptomatic individuals with IVA and MCADD, which have not been found so far in any symptomatic individuals, thus indicating the frequent occurrence of mild and potentially asymptomatic phenotypes.

The analysis of this study and the overall available mutational data on MCCD do not allow specific mutations to be related to subgroups with mild or severe clinical phenotypes [Baumgartner et al., 2001; Gallardo et al., 2001; Holzinger et al., 2001; Baykal et al., 2005; Dantas et al., 2005]. In the group of reported individuals (Table 3), we compared the extremes in outcome measures such as death (with variations like E99Q and c.1574+1G>T in MCCB) vs. asymptomatic MCCD individuals (with variations like A289V and Q421RfsX9 in MCCA and R155Q, D172DfsX25, P310R, and I437V in MCCB). In addition, we compared both groups with mutations identified in this study and were equally not able to define mild or severe allelic variations. As an example, the variation E99Q was identified in both a Kurdish boy who died at the age of 11 months [Bannwart et al., 1992] and in an Amish/Mennonite adult woman with mild symptoms who was diagnosed by abnormal NBS of her healthy child [Gibson et al., 1998]. The mutation C509X (MCCA) was detected in both a Turkish child with severe clinical outcome [Dantas et al., 2005] and in an asymptomatic neonate of Kurdish origin (this study).

Finally, the so far most frequent variant R385S (MCCA) was found in a individual with a severe clinical phenotype [Steen et al., 1999], in two biotin responsive individuals (one severe, one asymptomatic) [Baumgartner et al., 2004], and in an asymptomatic neonate (this study). Consequently, genotyping appears to be of no help in predicting the biochemical phenotype or clinical outcome of MCCD individuals detected by NBS.

Why is it so difficult to delineate predictive markers? The leucine catabolic pathway (Fig. 1) apparently shows a high “functional reserve.” Only defects in the first enzyme of the metabolic cascade (branched chain 2-oxo acid dehydrogenase) show severe clinical symptoms in the majority of cases (maple syrup urine disease, MSUD) [Chuang and Shih, 2001]. However,
in this condition the breakdown of two additional amino acids, isoleucine and valine, is also compromised. Interestingly, deficiencies of leucine catabolic enzymes prior to the MCC step (isovaleryl-CoA dehydrogenase) or after the MCC step (3-methylglutaconyl-CoA hydratase) can also show unapparent clinical expression, as we observe in MCCD [Ly et al., 2003; Ensennauer et al., 2004].

Under normal (anabolic) conditions, MCC enzyme deficiency could functionally be compensated by alternative catabolic steps and sufficient mechanisms to eliminate potentially toxic metabolites. Under such premises, the observed lack of obvious correlations between underlying genotypes and biochemical or clinical MCCD phenotypes would not be surprising and fits well into already long established models of metabolic homeostasis [Kacser and Burns, 1981]. In this sense, MCCD appears to be a (monogenetic) risk factor or a susceptibility locus, rather than a disease with obligatory expression of clinical symptoms early in childhood.

Compared to other organic acidemias, MCCD also appears to have a significantly higher tolerance toward metabolic stress. Even complete absence of MCC seems to cause clinical manifestations in association with environmental triggering factors only in a small subgroup of both treated and nontreated individuals. Studies on in vivo leucine tolerance may potentially help to clarify whether MCCD individuals are at risk when exposed to environmental stressors.

The available literature evidence and the results of our study revealed that a potential risk to develop clinical symptoms cannot be predicted from any data available in NBS. A disproportionate high share of maternal MCCD conditions was noted during follow-up of their healthy newborns. This unwanted surplus information can potentially create problems. As an example, one afflicted but completely asymptomatic MCCD mother raised concerns that her own life would have been adversely affected by stigmatization, had her (benign) MCCD condition been detected when she was a newborn. In addition, she stated that it is very easy to analyze a largely healthy population and perform a systematic survey “disguised” as a preventive medical checkup. Such anecdotal experience illustrates the potential burden of stigmatization and unnecessary treatment generated by NBS. However, this burden needs to be weighed against the potential benefit to prevent adverse outcome in a few individuals. After 6 years of pilot screening for MCCD, the overall benefit-to-harm ratio of NBS within the population was judged unfavorable.

These considerations were instrumental for German federal authorities to exclude MCCD from NBS panels recommended as standard care procedures that are financed by public health care [Hess, 2005]. However, this proposition is equally not fully evidence-based. At the present stage of knowledge, continuation of NBS for MCCD would only rarely be preventive and would create considerable difficulties in counseling affected families in the absence of a rationale for individual risk prediction or any proven necessity for preventive measures.

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