

## 3-Methylcrotonyl-CoA carboxylase deficiency

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### Abstract

*Isolated 3-methylcrotonyl-CoA carboxylase (MCC) deficiency is an autosomal recessive disorder of leucine catabolism. MCC is a heteromeric mitochondrial enzyme comprised of biotin-containing alpha-subunits and smaller beta-subunits. The recent introduction of neonatal screening programs based on tandem mass spectrometry has revealed an unexpectedly high frequency of this disorder, which appears to be the most common organic aciduria in some populations. The phenotype is variable, ranging from neonatal onset with severe neurological involvement to asymptomatic adults. Most symptomatic patients have normal growth and development until presenting with an acute metabolic crisis between 2 and 33 months of age. Such an episode usually follows a minor infection or introduction of a protein-rich diet. Symptoms include vomiting, opisthotonus, involuntary movements, seizures, coma and apnoea, and are often accompanied by severe hypoglycemia, ketoacidosis and mild hyperammonemia. The major abnormal metabolites are 3-methylcrotonylglycine and 3-hydroxyisovaleric acid in urine, and 3-hydroxyisovalerylcarnitine in blood. The patients usually respond to intravenous fluids and cessation of protein feeding and are asymptomatic between acute episodes. Some children have been placed on a leucine-restricted diet supplemented with oral L-carnitine, but the efficacy of this approach is unproven. Recent studies provide evidence that the missense mutation MCCA-R385S in the presence of the wild type allele has a dominant negative effect that may lead to biochemical and clinical abnormalities in heterozygous individuals. Moreover, in such subjects biotin therapy appears to counteract the dominant negative effect in vivo.*

### Keywords

Leucine catabolism, organic aciduria, acute metabolic crisis, 3-methylcrotonylglycine, 3-hydroxyisovaleric acid, biotin, L-carnitine supplementation

### Disease name and synonyms

Isolated methylcrotonyl-CoA carboxylase deficiency (MIM 210200 and 210210) is generally not responsive to biotin and must be distinguished from MCC deficiency caused by

the [biotin-responsive multiple carboxylase deficiencies](#). Therefore the specific term "isolated MCC deficiency" is preferable to MCC deficiency. The following terms are synonyms for isolated MCC deficiency

**Synonyms**

3-Methylcrotonylglycinuria  
 Isolated MCC deficiency  
 Isolated biotin-resistant MCC deficiency  
 MCC deficiency  
 MCCC1 = MCC alpha-subunit = MCCA = MIM  
[210200](#)  
 MCCC2 = MCC beta-subunit = MCCB = MIM  
[210210](#)

**Definition / Diagnostic criteria**

3-Methylcrotonyl-CoA carboxylase (MCC, EC 6.4.1.4) is responsible for the carboxylation of 3-methylcrotonyl-CoA, the fourth step in leucine catabolism [1]. Hence leucine catabolism is disturbed in isolated MCC deficiency.

Patients typically present with acute metabolic acidosis following intercurrent illness. The possibility of isolated MCC deficiency should be considered in patients with typical signs of an organic aciduria and especially those with hypoglycemia or Reye-like syndrome. It should also be considered in patients with hypotonia, seizures, or developmental delay. After the recent introduction of tandem Mass Spectrometry (MS) newborn screening in an increasing number of screening centres, the range of clinical symptoms has become even wider ranging from neonatal onset with poor outcome to asymptomatic adults (see clinical description). Therefore, clinical signs alone do not allow diagnosis of isolated MCC deficiency.

Urinary organic acid analysis usually enables the physician to make the diagnosis of isolated MCC deficiency. There is a characteristic organic aciduria with massive excretion of 3-hydroxyisovaleric acid (3-HIVA) and 3-methylcrotonylglycine (3-MCG), usually in combination with a severe secondary carnitine deficiency (see diagnostic methods). In addition, accumulated acyl-CoA compounds are trans-esterified to acylcarnitine esters. The major abnormal metabolite, 3-hydroxyisovaleryl-carnitine, is found in blood and urine (see diagnostic methods). It should be noted that modest elevations of 3-HIVA and 3-hydroxyisovalerylcarnitine are not specific for isolated MCC deficiency.

Enzymatic assay of MCC and at least one other mitochondrial carboxylase to exclude multiple carboxylase deficiencies is essential for confirmation of isolated MCC deficiency. If a patient is clinically or biochemically suspected to have isolated MCC deficiency, confirmation by direct enzyme assay is highly recommended (see diagnostic methods).

**Prevalence**

Until recently isolated MCC deficiency has been considered to be a rare inborn error of metabolism. Tandem MS, recently introduced to newborn screening in an increasing number of screening centers, allows for the first time detection of a large variety of organic acidurias including isolated MCC deficiency [2]. First results show that isolated MCC deficiency appears to be the most frequent organic aciduria detected in tandem MS-based screening programs in North America [3-5], Europe [6,7] and Australia [8] with an overall frequency of approximately 1 in 40,000 to 1 in 50,000.

**Clinical description**

At least 50 cases of isolated biotin-resistant MCC deficiency have been described [4,9-34]. The clinical phenotype is highly variable: some patients present in the neonatal period with seizures and muscular hypotonia and a poor outcome despite treatment [15,16,32]; others are asymptomatic newborns or adult women identified only by detection of abnormal metabolites in the neonatal screening samples of their healthy babies [4,31]. Most symptomatic patients however have normal growth and development until presenting with an acute metabolic crisis between 2 and 33 months of age. Such an episode usually follows a minor infection or introduction of a protein-rich diet. Symptoms include vomiting, opisthotonus, involuntary movements, seizures, coma and apnoea, and are often accompanied by severe hypoglycemia, ketoacidosis and mild hyperammonemia (< 250 mmol/l). Among about 50 reported cases, 4 patients died: one from cerebral edema during acute metabolic crisis [12], one from respiratory failure [22], one from cardiocirculatory crisis after a prolonged epileptic attack [15] and one from disseminated encephalomalacia [32].

Additional manifestations include failure to thrive in the neonatal period [21,29], developmental delay [18], familial hypotonia [23] or even hypertension [21]. A number of affected sibs of symptomatic patients have been clinically normal [11,13,14], suggesting that the genotype at the MCC locus is not the sole determining factor.

The unexpectedly high incidence of isolated MCC deficiency in apparently asymptomatic newborns implies that many of the "patients" with this disorder do not present with pathologic features and usually remain undetected. However, carnitine deficiency has been found in asymptomatic "patients" [33] and even in heterozygous parents of MCC deficient subjects [34] and thus may be prevalent among asymptomatic "patients". Even though

asymptomatic, these individuals are at risk of developing acute episodes with potentially life threatening metabolic crisis or permanent neurological damage.

### Differential diagnosis

#### *From the clinical point of view*

Differential diagnosis includes all the organic acidurias causing metabolic crisis. Since in many MCC deficient patients metabolic crisis are accompanied by severe hypoglycemia and mild hyperammonemia, fatty acid oxidation disorders and other causes of Reye syndrome have to be considered too.

#### *From the biochemical point of view (similarity in organic acid profile)*

Isolated MCC deficiency, which is generally not responsive to treatment with biotin, must be distinguished from biotin-responsive multiple carboxylase deficiencies that are due to a deficiency of biotinidase or holocarboxylase synthetase and affect all four biotin-dependent carboxylases [35]. In all these disorders, the major abnormal metabolites are 3-MCG and 3-HIVA due to MCC deficiency. In the multiple carboxylase deficiencies additional metabolites which are characteristic of pyruvate carboxylase and propionyl-CoA carboxylase deficiencies, e.g. lactic acid, 3-hydroxypropionate and small amounts of methylcitrate, are also elevated. Therefore, differential diagnosis of isolated MCC deficiency depends on careful quantitative analysis of urinary organic acid profiles, and on demonstration of MCC deficiency together with normal activity of at least one other carboxylase by enzyme assay in cultured fibroblasts or lymphocytes.

Modest elevations of 3-HIVA is seen in patients with severe ketosis of any cause. Finally, patients with 3-hydroxy-3-methylglutaryl-CoA lyase deficiency also have elevated 3-hydroxyisovalerylcarnitine, but this is accompanied by elevated 3-methylglutaryl carnitine.

### Management including treatment

Treatment of acute episodes with intravenous glucose, carnitine and cessation of protein feeding has usually been effective. Most patients are asymptomatic between acute episodes. Some children have been placed on a leucine-restricted diet (0.75-2 g/kg/d of total protein) supplemented with oral L-carnitine, but the efficacy of this approach is unproven.

Because patients with isolated MCC deficiency have a secondary carnitine deficiency, treatment with L-carnitine (75-100 mg/kg/d) is important

[19,20,27,28]. To increase detoxification of 3-methylcrotonyl-CoA, a limited number of patients have been treated with glycine to increase excretion of 3-MCG [27,28]. Treatment with glycine increased 3-MCG excretion more than twofold, with the maximum effect at 175 mg glycine/kg/day [28]. Although combined therapy with glycine and carnitine was not tried in these studies, increasing excretion of glycine and carnitine conjugates are complementary rather than competitive means of detoxification and thus may be the most effective treatment for acute metabolic crisis. Whether combined therapy is best (or necessary) for chronic treatment is unknown.

### Etiology

MCC catalyzes the fourth step of the leucine catabolic pathway by carboxylating 3-methylcrotonyl-CoA at the 4-carbon to form 3-methylglutaconyl-CoA [1]. Hence, 3-methylcrotonyl-CoA accumulates in MCC deficiency and is converted to 3-MCG and 3-HIVA, the characteristic compounds elevated in MCC deficiency. Since the enzymatic step upstream to MCC (isovaleryl-CoA dehydrogenase) is irreversible, there is no accumulation of isovaleric acid or its derivatives. Similar to other organic acidurias accumulation of the compounds prior to the enzymatic block is presumed to be the cause of the primary pathologic effects, that is, toxicity of the accumulated substrates and/or endogenous compensatory mechanisms, such as increased fatty acid oxidation and ketogenesis to provide fuel replacement for a blocked gluconeogenic pathway. Furthermore, accumulation of acyl-CoAs inhibits the urea cycle, resulting in hyperammonemia, and alters the availability of free CoA, thus affecting the CoA-carnitine exchange mechanism across the mitochondrial membrane and catabolism of other acyl moieties from fatty acid and amino acid oxidation.

MCC is a member of the family of biotin-dependent carboxylases, a group of enzymes with diverse metabolic functions but common structural features [35,36]. Members of this family have three structurally conserved functional domains: the biotin carboxyl carrier domain, which carries the biotin prosthetic group; the biotin carboxylation domain, which catalyzes the carboxylation of biotin; and the carboxyltransferase domain, which catalyzes the transfer of a carboxyl group from carboxybiotin to the organic substrate specific for each carboxylase [36,37]. These carboxylation reactions require ATP and utilize bicarbonate as the source of the carboxyl group [1,35,36]. Bovine MCC has an approximate size of 835

kDa and appears to be comprised of six heterodimers ( $\alpha\beta$ )<sub>6</sub> [38]. Like propionyl-CoA carboxylase, MCC has a larger  $\alpha$  subunit, which covalently binds biotin, and a smaller  $\beta$  subunit [1]. MCC is predominantly localized to the inner membrane of mitochondria and is highly expressed in kidney and liver [1]. Three groups reported cloning of *MCCA* and *MCCB* cDNAs and the organization and chromosomal mapping of their structural genes [9,30,31]. In a series of 14 MCC-deficient probands, two complementation groups, CG1 and CG2 were defined (CGB and CGA in the terminology of Gallardo *et al.*[30]), resulting from mutations in *MCCB* and *MCCA*, respectively [9].

Recent studies provide evidence that the missense mutation *MCCA*-R385S in the presence of the wild type allele has dominant negative effect that may lead to biochemical and clinical abnormalities in heterozygous individuals. Moreover, in such subjects biotin therapy appears to counteract the dominant negative effect in vivo [39].

## Diagnostic methods

### Metabolites

#### Urinary organic acid analysis

There is a characteristic organic aciduria with massive excretion of 3-HIVA (500-7000 mmol/mol creatinine) and 3-MCG (50-4000 mmol/mol creatinine), usually in combination with a severe secondary carnitine deficiency [11,19,20,23-25,27]. These elevations occur without elevation of isovalerylglycine or of the distal metabolites of the leucine catabolic pathway, e.g. 3-methylglutaconic acid or 3-hydroxy-3-methylglutaric acid, and without the modest elevation of 3-hydroxypropionic, methylcitric and lactic acids seen in multiple carboxylase deficiencies. Moderate ketosis with secondary elevation of dicarboxylic acids has been reported [20,24]. It should be noted that modest elevation of 3-HIVA ranging from 5 - 200 mmol/mol creatinine can be seen in patients with severe ketosis of any cause.

#### Acylcarnitine analysis

Tandem MS analysis of plasma or dried blood spots for acylcarnitines reveals a large elevation of 3-hydroxyisovalerylcarnitine in isolated MCC deficiency [40,41]. 3-methylcrotonylcarnitine may or may not be present. Patients with 3-hydroxy-3-methylglutaryl-CoA lyase deficiency also have elevated 3-hydroxyisovalerylcarnitine, but this is accompanied by elevated 3-methylglutaryl-carnitine. Patients with multiple carboxylase deficiency may also have elevated

3-hydroxyisovalerylcarnitine, but this is usually accompanied by elevated propionylcarnitine.

### Enzyme assay

The activities of MCC and the other two mitochondrial carboxylases propionyl-CoA carboxylase and pyruvate carboxylase are usually assayed in fibroblast homogenates of lymphocytes or cultured skin fibroblasts by determining incorporation of <sup>14</sup>C-bicarbonate into acid-non-volatile products [1,42]. For definitive diagnosis of isolated MCC deficiency and exclusion of multiple carboxylase deficiency, deficient MCC activity with normal activity of at least one other biotin-dependent carboxylase should be shown in lymphocytes or cultured skin fibroblasts, regardless of treatment with biotin or concentration of biotin in the culture medium, respectively. MCC activity in homogenates of cultured fibroblasts of patients is usually less than 2% of the mean control value [9,22]. No correlation between the level of residual enzyme activity and clinical presentation has been observed.

Heterozygotes for isolated MCC deficiency cannot be reliably diagnosed by assay of the enzyme in cultured fibroblasts or lymphocytes because its activity is usually within the normal range [11,19,27].

### Mutation analysis

To date, a total of 9 mutant *MCCA* alleles and 13 mutant *MCCB* alleles have been reported [9,30,31,43]. Only 2 *MCCA* [R385S, (Q421fs(+1))] and 2 *MCCB* [E99Q, S173fs(+1)] alleles were identified in more than one proband, indicating that most patients carry private mutations. So far, the data do not enable the determination of a genotype-phenotype correlation. Mutation analysis is carried out only by few laboratories on research basis and should not be the first diagnostic approach.

### Genetic counseling and prenatal diagnosis

Isolated MCC deficiency is inherited as an autosomal recessive trait. Given the wide phenotypic range and the increasing number of asymptomatic patients and even asymptomatic affected siblings of symptomatic patients, prenatal diagnosis according to my opinion is only warranted in siblings of patients with severe disease. In such cases prenatal diagnosis is possible either by stable isotope dilution analysis for elevated 3-HIVA in amniotic fluid, as shown for multiple carboxylase deficiency [44], or by assay of carboxylase activities in chorionic villi (CV) biopsy or cultured CV cells or amniocytes [45]. In families with an index case with known genotype, mutation analysis is possible.

## Unresolved questions

### **Phenotypic heterogeneity**

Most symptomatic patients develop normally until presenting with an acute metabolic crisis following an infection. However, some patients develop severe symptoms in the neonatal period, before the first crisis; others never have a crisis, but show developmental delay or hypotonia.

Why do some patients develop symptoms while others, even in the same family, remain asymptomatic?

What are the variables (environment, modifying genes) influencing the phenotypic consequences of isolated MCC deficiency?

### **Asymptomatic newborns**

Should we treat and if yes, how should we treat these individuals?

Which individuals benefit from therapy?

Should we include isolated MCC deficiency in tandem MS-based newborn screening?

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