## Recessive congenital methemoglobinemia in immediate generations

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We report herein on our observation of recessive congenital methemoglobinemia (type I), an autosomal recessive disorder, in immediate generations (in a mother and her daughter). Molecular analysis revealed a mechanism of inheritance not reported previously, despite the high probability of occurrence in autosomal recessive disorders. This report is also the first publication describing an extremely rare mutation (Arg50Gln) causing this disorder in the Turkish population.

Key words: recessive congenital methemoglobinemia, immediate generations, Arg50Gln mutation, Turkish patient.

Recessive congenital methemoglobinemia (RCM) is an autosomal recessive (AR) disorder due to NADH-cytochromeb5 reductase (cytb5r) deficiency<sup>1</sup>. This enzyme exists in both soluble and membrane-bound forms. The soluble erythrocytic isoenzyme is involved in methemoglobin reduction, while the membrane-bound enzyme participates in fatty acid desaturation and drug metabolism. All isoforms of human cytb5r are synthesized by a single gene, CYB5R3<sup>2</sup>. This disorder occurs as a result of mutations in this gene and has two clinical phenotypes: type I, benign form with mild cyanosis, and type II, severe form with cyanosis and neurological impairment. In type I RCM, cytb5r deficiency is restricted to erythrocytes, while in type II RCM, a profound enzyme deficiency in all tissues is present. To date, 50 different mutations in CYB5R3 leading to RCM have been described<sup>3</sup>. As it is AR in character, the condition is only manifested in individuals having two copies of the mutant allele. A child born to two parents carrying one copy each of the mutant allele becomes symptomatic, with a classical risk of 25%. Accordingly, the RCM patients reported thus far are individuals born to heterozygous and asymptomatic parents. We herein present our

observation of an AR disorder uncommonly derived in immediate generations.

## Case Report

The proband is a 51-year-old woman of Turkish origin, who was found incidentally to have an elevated methemoglobin level. Her history revealed perioral cyanosis from childhood. She was investigated for this symptom for the first time one month ago when her daughter was admitted to our hospital for diarrhea. During the assessment of diarrhea, arterial blood gas test was ordered for the daughter and an elevated level of methemoglobin was detected (12.7%, normal range 0-1.5%). Subsequent parental study showed that the mother also had a high level of methemoglobin (16.2%). They were both consulted to Hematology Department.

The parents of the proband were first-degree relatives and she had three healthy siblings. Cyanosis had been considered a sequela of measles in early childhood from which she had recovered. Physical examination revealed cyanosis of perioral skin and oral mucosa. Her husband was 55 years old and healthy without cyanosis. They were not related but were from the same region. The daughter was 17 years

| Family<br>member | Age<br>(years) | Clinical presentation | MetHb<br>(%) | Mutation                     | Exon | Change   | Disease<br>status | Clinical phenotype |
|------------------|----------------|-----------------------|--------------|------------------------------|------|----------|-------------------|--------------------|
| Mother           | 51             | Mild<br>cyanosis      | 16.2         | CGG to<br>CAG at<br>codon 50 | 2    | Arg50Gln | Homozygous        | Туре І             |
| Father           | 55             | None                  | 0.5          | CGG to<br>CAG at<br>codon 50 | 2    | Arg50Gln | Heterozygous      | -                  |
| Daughter         | 17             | Mild<br>cyanosis      | 12.7         | CGG to<br>CAG at<br>codon 50 | 2    | Arg50Gln | Homozygous        | Туре І             |

Table I. Some Clinical and Laboratory Findings of Family V.

CGG: Cytosine-Guanine-Guanine, CAG: Cytosine-Adenine-Guanine, Arg50Gln: Arginine50Glycine

old and healthy except for mild perioral and buccal cyanosis that has been incidentally observed only recently. No cardiac, pulmonary or intestinal disorder was present, and no environmental toxicity was determined.

Based on the clinical and laboratory findings, hereditary methemoglobinemia was considered. Hemoglobin analysis by high performance liquid chromatography (HPLC) showed normal results. DNA was isolated by standard protocols, after obtaining informed consent, and polymerase chain reaction (PCR)-direct sequencing of the CYB5R3 gene was performed as previously described<sup>4</sup>. Molecular analysis showed that the family had the same mutation, as CGG to CAG at codon 50 in exon 2, resulting in Arg50Gln. The father was heterozygous and both mother and daughter were homozygous. Family tree, sequence analysis and clinical laboratory data of the core family are shown in Figures 1A and B, and Table I, respectively. Ascorbic acid was initiated for cosmetic purposes and the mild cyanosis disappeared.

## Discussion

Cyanosis may develop due to reduced oxygen supply to the tissues resulting from the production of non-functional hemoglobin containing oxidized Fe<sup>+3</sup>, namely methemoglobin. Methemoglobinemia can develop by three distinct mechanisms: genetic mutation resulting in the presence of abnormal hemoglobin, a deficiency of methemoglobin reductase enzyme, and toxin-induced oxidation of hemoglobin<sup>4</sup>.

In the absence of environmental toxins, the presence of cyanosis suggests a hereditary

cause. In case of cyanosis present in immediate generations, probable causes are limited to autosomal dominantly inherited disorders, such as "hemoglobin M disease" (HbM disease). Hemoglobin M disease results from amino acid substitutions in the heme binding pocket of globin chains<sup>4</sup>. Such structural alteration can lead to spontaneous oxidation of the Fe<sup>+2</sup> ion embedded in heme and cyanosis may develop



**Fig.1.** (A) Family tree indicating the position of the mother and daughter with cyanosis due to recessive congenital methemoglobinemia (RCM).

(B) The sequences with the homozygous mutation found in the patient and daugther (as compared to wild type), and heterozygous mutation identified in the husband. from birth. The diagnosis depends on the determination of HbM variants or on molecular analysis. In this family, the lack of abnormal hemoglobins with HPLC excluded HbM disease.

Recessive congenital methemoglobinemia is the most frequent cause of hereditary cyanosis. Only homozygous individuals (more correctly, individuals having two copies of the mutant allele) are cyanotic. How could both a mother and her daughter be homozygous for an AR disorder? The situation might be explained by de novo RCM, as the rarest cause. Similar to the other genetic hemoglobin disorders<sup>5</sup>, RCM might also develop de novo. While the development of a de novo disease in immediate generations is extremely rare, it cannot be excluded. In this family, however, the clinical laboratory features are not suggestive of de novo development of RCM. The other explanation might be uniparental disomia (UPD). This disorder should be suspected in an individual manifesting a recessive disorder, where only one parent is symptomatic. Interestingly, UPD has been described in RCM6, and we have experienced this situation firsthand (Percy MJ, unpublished observation). In this family, both parents had the same mutation as that affecting the child and further evaluation for UPD is unnecessary. The most likely situation is the fact that one parent has homozygous and the other has heterozygous RCM. This is hypothetically possible and widely expected in AR disorders, especially among consanguineous populations; since one parent has two mutant alleles and the other parent has one, the risk of a child born with two mutant alleles is 50%. rather than the usual 25% in recessive diseases. However, and surprisingly, it has not been reported previously in RCM. This basic pattern of Mendelian inheritance should be borne in mind, especially in populations with a high rate of consanguinity, and for those disorders that are mildly symptomatic even in homozygous state, such as RCM type I. This condition should be ruled out first, before investigating more sophisticated rare conditions.

This mutation was first described in 2001<sup>7</sup>. In that Spanish family, the father was heterozygote for this mutation and the mother was heterozygote for a different mutation. Two of their children were compound heterozygotes. One child had a methemoglobin level of 6%,

reduced enzyme activity, and cyanosis. The other was noncyanotic with a methemoglobin level of 2% and a low enzyme activity. Our patients were in homozygous state, and in this regard, they are the first such cases. Based on the clinical laboratory features of both previous cases and our patients, we can conclude that this mutation may lead to a mild phenotype, which might have contributed to the delayed diagnosis in our family.

Although specific mutations in the CYB5R3 gene may not be exclusive to each type<sup>8</sup>, molecular diagnosis of RCM is important, particularly for type II disease. For genetic counseling, mutational patterns of different populations are required. In this regard, this report also contributes to the current knowledge on the molecular basis of Turkish RCM cases.

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