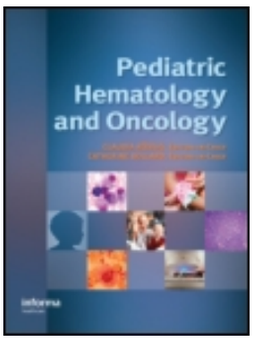

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Pediatric Hematology and Oncology

ISSN: 0888-0018 (Print) 1521-0669 (Online) Journal homepage: http://www.tandfonline.com/loi/ipho20

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To cite this article: Tugba Arıkoglu MD, Nese Yarali MD, Abdurrahman Kara MD, Ali Bay MD, Ikbal O. Bozkaya MD, Bahattin Tunc MD & Melanie J. Percy PhD (2009) A NOVEL L218P MUTATION IN NADH-CYTOCHROME B5 REDUCTASE ASSOCIATED WITH TYPE I RECESSIVE CONGENITAL METHEMOGLOBINEMIA, Pediatric Hematology and Oncology, 26:5, 381-385

To link to this article: http://dx.doi.org/10.1080/08880010902979233

Published online: 13 Aug 2009.

Pediatric Hematology and Oncology, 26:381–385, 2009

ISSN: 0888-0018 print / 1521-0669 online
DOI: 10.1080/08880010902979233

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healthcare

A NOVEL L218P MUTATION IN NADH-CYTOCHROME B5 REDUCTASE ASSOCIATED WITH TYPE I RECESSIVE CONGENITAL METHEMOGLOBINEMIA

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□ *The presence of central cyanosis that is unrelated to cardiopulmonary causes alerts clinicians to a possible diagnosis of methemoglobinemia. Congenital methemoglobinemia due to deficiency of nicotinamide-adenine dinucleotide (NADH)-cytochrome b5 reductase (cb5r) is an autosomal recessive disorder characterized by life long cyanosis. Here we report a six-year old boy who presented with central cyanosis and upon examination revealed a methemoglobin level of 19.0%. Sequencing the* CYB5R3 *gene identified a homozygous T→C transition at base c.653, which changed codon 218 from leucine to proline (L218P) in cb5r protein. Treatment with ascorbic acid relieved the cyanosis and returned methemoglobin levels to normal.*

Keywords congenital methemoglobinemia, cyanosis, cytochrome b5 reductase deficiency

Methemoglobinemia is a rare condition characterized by cyanosis and results from elevated methemoglobin levels. Three distinct mechanisms may induce increased methemoglobin levels in the body: toxin-induced oxidation of hemoglobin, genetic mutations causing the production of the M hemoglobins, and deficiency of NADH-cytochrome b5 reducase (cb_5r) [1–3]. Deficiency of cb_5r is known as recessive congenital methemoglobinemia (RCM) and there are two different clinical forms, types I and II. Both forms are characterized by cyanosis from birth; type I is benign, whereas type II is associated with severe neurological impairment [2].

Here we present a case of a young cyanotic boy showing normal neurological development with a diagnosis of RCM type I. Analysis of the *CYB5R3*,

Received 16 October 2008; Accepted 18 March 2009.
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which encodes cb_5r, revealed a novel homozygous mutation, which supports the diagnosis of RCM.

CASE REPORT

A 6-year old boy was admitted to Dr Sami Ulus Children's Hospital with a long history of bluish discoloration of nails and lips. He had no history of cough, dispnea, syncope, or weight loss. He was the second child of a consanguineous marriage. The family history was unremarkable and his parents and older sibling were healthy. He did not have any history of drug or toxic substance usage.

On admission he was found to be well but had mild central cyanosis. His pulse rate was 90 beats and regular, arterial pressure was 100/70 mmHg. The auscultation of chest was clear, with normal heart sounds. Neurological examination was unremarkable. Oxygen saturation by pulse oximetry was 89% in room air and remained low even on 100% oxygen. Arterial blood gas analysis revealed pH 7.41, pCO_2 36 mmHg, P_aO_2 74 mmHg and HCO_3 23 mEq and the color of his blood was chocolate brown. His hemoglobin was 14.6 g/dL, mean corpuscular volume was 88 fL, and erythrocyte count was $4.53 \times 10^6/\mu$L. Chest X-ray and echocardiography were normal. Congenital methemoglobinemia was considered and his methemoglobin level was measured at 19.5% (Normal range: 0–1%). Methemoglobin levels of his parents and brother were in the normal range. Treatment with ascorbic acid 500 mg/day orally resulted in improved oxygen saturation and his cyanosis disappeared after 4 days.

Sequencing the *CYB5R3* gene revealed a novel homozygous mutation of T→C in exon 8 at base c.653, changing codon 218 from Leu to Pro (L218P) (Figure 1). Measurement of the child's cb_5r enzyme activity was not performed due to technical difficulties. On follow-up, he had no obvious

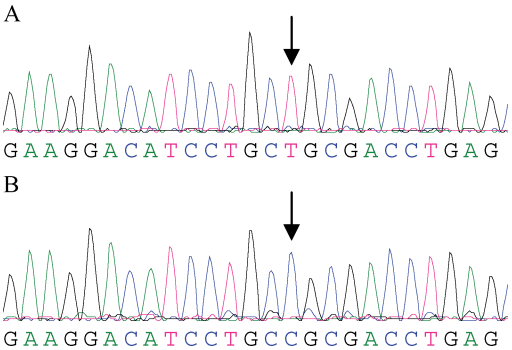

FIGURE 1 Sequencing chromatogram of exon 8 of the *CYB5R3* gene showing bases c.639 to c.663. Comparison of the normal sequence (A) with that of the child with RCM (B) revealed a homozygous T to C change at base c.653, causing leucine to proline change at amino acid 218.

cyanosis and his methemoglobin level after 6 doses of ascorbic acid was reduced to 1%.

DISCUSSION

Cyanosis is the characteristic blue color of the skin due to lack of oxygen in the blood. Sepsis, congenital heart disease with right to left shunt, congestive heart failure, pulmonary disorders, sulfhemoglobinemia, and hemoglobin variants such as the M group of hemoglobins should be considered in differential diagnosis of central cyanosis [1]. Some oxidizing drugs and toxic chemicals, such as lidocaine and nitrites, can enhance the normal rate of production of methemoglobin by direct or indirect oxidation of the iron. Also neonates have low cb5r activity until the age of 2–3 months and they are at risk of developing methemoglobinemia by oxidizing agents. The structural alteration of the heme, which cause spontaneous oxidation of the Fe^{+2}, results methemoglobinemia. The Hb M variants are inherited in an autosomal dominant pattern, contrary to RCM. Therefore, family history is usually helpful in differentiating RCM from hemoglobin M disease. In addition, the presence of Hb M can be established by electrophoresis and DNA sequencing [2].

Rare genetic disorders, such as RCM, often manifest in families where the parents are first-degree relatives. In this case a diagnosis of RCM was considered when the child's blood was noted to be chocolate brown due to the presence of methemoglobin. Treatment with ascorbic acid alleviated the cyanosis and RCM was confirmed by the presence of a homozygous L218P mutation in cb_5r. Recently, a similar leucine to proline change was reported at the adjacent amino acid of 217 in an Indian patient with type I RCM [4]. Neither P217 nor P218 is located in the NADH binding site but both amino acids are present in a region conserved in a wide range of species. Although the enzyme activity of the P217L variant was minimally affected by the mutation, the thermostability of the protein was greatly reduced [4].

Clinical RCM exists in two forms, with type I being benign with cyanosis alone. In contrast, type II disease the cyanosis is accompanied with progressive neurological and cognitive impairment, encephalopathy, mental retardation, spasticity, microcephaly, and growth retardation [1, 5, 6]. In general, mutations that reduce the activity and thermostability of cb_5r but do not abolish the function of the protein result in type I disease. Mutations that cause loss of function are often, but not always, associated with type II disease. Thus, it appears that when the activity of cb_5r falls below a critical threshold, type II disease develops [2].

The cb_5r enzyme exists in two distinct forms, each with a different function. Membrane-bound cb_5r in somatic cells participates in the destruction

and elongation of fatty acids, cholesterol biosynthesis, and drug metabolism. Soluble cb_5r is present in erythrocytes and catalyzes the reduction of methemoglobin to hemoglobin. Mature erythrocytes have lost the ability to synthesize new protein and consequently any reduction in the half-life of cb_5r will manifest in erythrocytes. Methemoglobin spontaneously arises during oxygen delivery but cannot carry oxygen and causes leftward shift of the O_2 dissociation curve. Cyanosis becomes apparent when the mean capillary concentration of reduced hemoglobin exceeds 5 g/dL [5]. The clinical manifestations of elevated blood methemoglobin levels vary from mild headache, dyspnea, fatigue, tachycardia, and dizziness to coma or death [1, 2].

The course of recessive congenital methemoglobinemia type I is benign. Generally, most patients tolerate their condition well with weakness and dyspnea being major symptom [1, 2]. Ascorbic acid provides non-enzymatic reduction of methemoglobin, but the rate of reaction is slow. Another treatment option is methylene blue, which is recommended for symptomatic patients with methemoglobin levels greater than 20%. Methylene blue accelerates the enzymatic reduction of methemoglobin by methemoglobin reductase [2, 7]. However, it should not be administered to a patient with combined glucose-6-phosphate dehydrogenase deficiency. Hyperbaric oxygen and exchange transfusion should be considered for patients who do not respond to methylene blue [8]. Although our patient's methemoglobin level was 19%, he had cyanosis with no other clinical symptoms; thus, ascorbic acid therapy was tried and he responded well.

In conclusion, methemoglobinemia presenting with central cyanosis causes a diagnostic dilemma. Cyanosis that is unrelated to cardiopulmonary causes and absence of improvement after the administration of oxygen must alert the clinicians to the possibility of diagnosis of RCM, with type I being considered in the absence of neurological impairment.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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