Familial idiopathic methemoglobinemia revisited: original cases reveal 2 novel mutations in NADH-cytochrome b5 reductase

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In 1943, the first description of familial idiopathic methemoglobinemia in the United Kingdom was reported in 2 members of one family. Five years later, Quentin Gibson (then of Queen’s University, Belfast, Ireland) correctly identified the pathway involved in the reduction of methemoglobin in the family, thereby describing the first hereditary trait involving a specific enzyme deficiency. Recessive congenital methemoglobinemia (RCM) is caused by a deficiency of reduced nicotinamide adenine dinucleotide (NADH)-cytochrome b5 reductase. One of the original propositi with the type 1 disorder has now been traced. He was found to be a compound heterozygote harboring 2 previously undescribed mutations in exon 9, a point mutation Gly873Ala predicting a Gly291Asp substitution, and a 3-bp in-frame deletion of codon 255 (GAG), predicting loss of glutamic acid. A brother and a surviving sister are heterozygous; each bears one of the mutations. Thirty-three different mutations have now been recorded for RCM. The original authors’ optimism that RCM would provide material for future genetic studies has been amply justified.

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Introduction

In an era dominated by functional genomics, it is salutary to reflect that the first hereditary disorder involving an enzyme deficiency was discovered just over half a century ago by Quentin Gibson. In 1943, Dr James Deeny, a general practitioner, described 2 brothers, Russell and Fred Martin from Banbridge in Northern Ireland, who had a blue appearance. When Russell was treated with vitamin C, he turned pink, and Dr Deeny assumed that he had corrected an underlying heart condition. However, the cardiologists in Belfast were more skeptical and were unable to find any abnormality in either brother. The conundrum attracted the attention of the physiologist Henry Barcroft, who carried out a detailed study of Dr Deeny’s cases during treatment and found raised levels of methemoglobin in 2 members of the Martin family. Gibson correctly defined the pathway involved in the reduction of methemoglobin in the family, and, in so doing, he described the first hereditary trait involving a specific enzyme deficiency.

The disorder recessive congenital methemoglobinemia (RCM; McKusick no. 250 800) is caused by a deficiency of reduced nicotinamide adenine dinucleotide (NADH)-cytochrome b5 reductase (cytb5r: E.C.1.6.2.2). Two forms of cytb5r are known, a soluble form and a membrane-bound form, and are localized in different cellular compartments. The soluble form is present mainly in red cells and is involved in the reduction of methemoglobin. The membrane-bound form is found mainly in the endoplasmic reticulum and outer mitochondrial membrane, where it participates in the desaturation and elongation of fatty acids and in the biosynthesis of cholesterol and P-450-mediated drug metabolism. The cytb5r gene is 31-kb long, contains 9 exons, and has been localized to chromosome 22q 13-qter. Both forms of the enzyme are generated from tissue-specific alternative transcripts (Figure 1), which give rise to the 275-amino acid soluble form and the 300-amino acid membrane-bound form. They have an identical hydrophilic catalytic domain but differ at the N-termini, where the membrane-bound form has 25 additional hydrophobic amino acids. There are 2 distinct clinical forms of cytb5r deficiency. Type 1 is characterized clinically by a single symptom, cyanosis, and biochemically by a deficiency of the red cell–soluble form of the enzyme. In type 2, cyanosis is accompanied by severe mental retardation and neurologic impairment involving the soluble and the membrane-bound forms of the enzyme.

We were curious to know the actual mutations involved in Gibson’s landmark discovery, and eventually we had the opportunity to analyze blood from one of the propositi, Fred, who had emigrated to Australia in 1968, and from 2 surviving siblings.

Patients, materials, and methods

Case history

The pedigree for the family originally reported by Deeny et al and Barcroft et al2,3 is shown in Figure 2. Three surviving family members were available for study—one of the original propositi (#6, FM) who has RCM type 2, a brother (#3, BM), and a sister (#5, EH). Unfortunately, during the course of this study, both Fred Martin and his brother BM died.

Polymerase chain reaction amplification of genomic DNA

Genomic DNA was isolated from buffy coats by the Nucleon BACC 1 DNA extraction kit (Nucleon Biosciences, Manchester, United Kingdom). Nucleotide sequences of the 9 exons were obtained from the GenBank database...
Results and discussion

The cytR5r activity, measured using the NADH-ferricyanide method, was less than 0.1 IU/g hemoglobin in FM, 6.37 IU/g hemoglobin in BM, and 7.75 IU/g hemoglobin in EH (normal range, 11.51-29.9 IU/g hemoglobin). These results suggested that BM and EH were heterozygote carriers of a cytR5r mutation. DNA sequencing of PCR products of the 9 exons revealed 2 mutations in exon 9 of FM, one in each allele. The first was a point mutation of G to A at codon 291, which would cause a change of glycine to aspartic acid. The second was a 3-bp in-frame deletion resulting in the loss of codon 255 and the deletion of glutamic acid. Each sibling carries one mutation—G to A at codon 291 in BM and the 3-bp deletion causing loss of codon 255 in EH. This was confirmed by cloning and sequencing cDNA from all 3 subjects. Thus, enzymic activity analysis and DNA sequencing indicate that FM is a compound heterozygote and that his siblings BM and EH are heterozygotes for cytR5r mutations.

In the late 1940s, when Gibson was investigating methemoglobinemia, Warburg and his colleagues had just shown that sugar is oxidized to pyruvate as methemoglobin is reduced to hemoglobin in intact red cells. It was also known that methylene blue accelerates the rate of reduction of methemoglobin. Gibson showed that methylene blue could reduce methemoglobin in the patients’ red cells, but incubation of the cells with sugar alone had little effect. He postulated that these patients had an enzymatic defect. Later he showed that isocitric acid, an inhibitor of glycerol-3-phosphate dehydrogenase, inhibited the reduction of methemoglobin when sugar, but not lactate, was added as substrate, and he deduced that the proposed enzyme defect affected the reaction between Coenzyme I (nicotinamide adenine dinucleotide, NAD) and methemoglobin. This deduction was confirmed in many subsequent investigations. An intriguing account of this early work has been recorded by Gibson.

These 2 hitherto unidentified mutations in the family originally studied by Gibson bring to 33 the number of different mutations described in patients with RCM (Figure 3). Previously, 15 mutations had been associated with the type 1 disorder and 16 with type 2 RCM. Mutations have been found in all exons but 1 and 18. Mutations that cause exon skipping and those predicted to give truncated proteins with severe impairment of function have been described only in type 2, except in one child in whom neurologic abnormalities were not observed at the age of 1 year.

Because the structure of cytR5r has recently been resolved by X-ray crystallography, it is possible to interpret the effects of mutations on enzyme function. Although the 2 novel exon 9 mutations are located outside the FAD and NADH binding sites,
each would be expected to have a deleterious effect on enzyme activity (Figure 4). Loss of E255 would affect the packed hydrophobic environment formed by I177, L206, A208, T237, M256, M278, and C283, and the presence of aspartic acid at codon 291 instead of aspartic acid at codon 291 instead of proline would also lead to perturbation of the secondary structure.

In 1943, Deeny and O’Riordan reported the first case of RCM in the United Kingdom, and it was hoped that the mode of inheritance would be defined and the genetic basis of the disorder established. More than 40 years later the cytb5r gene was cloned, thereby allowing the genetic basis of the disorder to be defined. It took another 20 years for the mutations in the original family to be identified.

Acknowledgments

We thank Dr. Quentin Gibson for making available his original notebooks and the propositi’s sister, EH, for providing family histories.

References