

A Missense Mutation in the Mitochondrial Cytochrome *b* Gene in a Revisited Case with Histiocytoid Cardiomyopathy

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ABSTRACT

We describe a pathogenic mutation in the mitochondrial cytochrome *b* gene in a patient with a multisystem disorder presenting as histiocytoid cardiomyopathy in whom a defect of ubiquinol cytochrome *c* oxidoreductase of the electron transport chain had been documented biochemically. The mutation, a G to A transition at nucleotide 15498, results in the substitution of glycine with aspartic acid at amino acid position 251. The mutation, which is heteroplasmic and fulfills all accepted criteria for pathogenicity, is likely to impair the function of the holoenzyme as deduced from its effects on the crystal structure of ubiquinol

cytochrome *c* oxidoreductase. This is the first molecular defect associated with histiocytoid cardiomyopathy. (*Pediatr Res* 48: 311–314, 2000)

Abbreviations

complex III, ubiquinol cytochrome *c* oxidoreductase
cyt *b*, cytochrome *b*
mtDNA, mitochondrial DNA
RFLP, restriction length polymorphism
nt, nucleotide

Histiocytoid cardiomyopathy, initially described in 1962 by Voth (1), has received various names including infantile xanthomatous cardiomyopathy (2), focal lipid cardiomyopathy (3), oncocytic cardiomyopathy (4), infantile cardiomyopathy with histiocytoid change (5), and foamy transformation of infancy (6), although the term histiocytoid cardiomyopathy is now generally accepted (12). This is a rare but distinctive disorder of infancy and childhood characterized by the presence of characteristic pale granular foamy histiocyte-like cells within the myocardium. It usually affects children younger than 2 y of age, with a clear predominance of females over males. These infants present with dysrhythmia or cardiac arrest, and the clinical course is usually fulminant, sometimes simulating sudden infant death syndrome.

Approximately 61 cases of histiocytoid cardiomyopathy have been reported (1–31), but the etiology of this disorder is still unknown. It has been suggested that histiocytoid cardio-

myopathy may be due to an abnormality of cardiac development (31) or to a developmental disorder of the atrioventricular conduction system and of the Purkinje cells (12, 16, 18).

In 1984, we reported an infant girl who died of cardiac arrest at age 4 wk who had typical clinical and pathologic features of histiocytoid cardiomyopathy but who also showed involvement of other organs including liver (hepatic steatosis) and kidney (acute tubular necrosis). Biochemical studies of cardiac muscle showed isolated complex III deficiency and marked defect of reducible cyt *b*, the only mtDNA-encoded subunit of complex III of the respiratory chain, suggesting that the disease was due to an inborn error of the electron transport chain (13). Our studies of this patient preceded the era of “mitochondrial genetics” that opened in 1988 (32), but, even after that date, we did not consider the possibility of an mtDNA defect because there was no evidence of maternal inheritance, which is one of the hallmarks of disorders associated with point mutations in mtDNA. In 1999, however, we noted that mutations in the cyt *b* gene are often sporadic and can arise during embryogenesis, affecting a limited number of cells and resulting in tissue-specific phenotypes (33, 34).

These findings prompted us to revisit our case of histiocytoid cardiomyopathy and complex III deficiency. Although heart

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tissue was no longer available, we could analyze kidney and liver obtained at autopsy. Molecular genetic studies revealed a pathogenic mutation in the *cyt b* gene of mtDNA that fulfills all accepted criteria for pathogenicity. This is the first molecular defect associated with histiocytoid cardiomyopathy.

METHODS

Total DNA was isolated by standard methods from liver and kidney obtained at autopsy. To sequence the *cyt b* gene, a PCR fragment overlapping the entire *cyt b* gene was obtained using the following set of primers: forward, nt 14680–14699, and reverse, 15991–15973 [numbers according to the Cambridge reference sequence (35)]. Direct sequencing of the PCR product was performed using eight internal primers (310 Automatic Sequencer, Perkin Elmer, CA, U.S.A.) according to the strategy we previously reported (33, 34, 37). Because in the presence of a mismatched primer, the mutation creates a new restriction site for *TaqI*, this enzyme can be used in RFLP analysis for detection and quantitation of the mutation. A 92-bp fragment was amplified by PCR using the following set of primers: a mismatched 37-mer forward (nt 15461–15497, containing a C at nt 15496) and 20-mer reverse (nt 15552–15533). The PCR conditions were 30 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 45 s, and extension at 72°C for 1 min. $\alpha^{32}\text{P}$ -dATP was added during the last PCR cycle to avoid the underestimation of the mutated mtDNA due to heteroduplex formation. PCR products were digested with *TaqI*, electrophoresed through a 12% nondenaturing polyacrylamide gel, and subjected to autoradiography.

RESULTS

Sequence analysis revealed four nt substitutions in the patient's *cyt b* gene compared with the reference sequence. Three of them (T14766C, A15326G, and A15758G) were considered neutral polymorphisms based on previous reports (36, 37). The fourth mutation, G15498A, resulted in the substitution of an encoded glycine (GGC) with an aspartic acid (GAC) at position 251 of the amino acid sequence (Fig. 1A). RFLP analysis (Fig. 1B) showed that the mutation was heteroplasmic in both liver (90%) and kidney (82%).

DISCUSSION

The diagnosis of histiocytoid cardiomyopathy is based on the presence of histiocyte-like cells with foamy or granular cytoplasm in the myocardium. These pathognomonic cells are found mainly in the ventricles, although similar alterations have been described in the pericardium, endocardium, valves, and conduction system (1, 4, 5, 7, 8, 10–12, 18, 20, 23). The muscular origin of the histiocytoid cells has been well established, as they show strong immunohistochemical reactivity for actin, desmin, and myoglobin, whereas they do not stain for markers of genuine histiocytes (CD68 and lysozyme) (9, 21, 22, 30).

In most reported cases, electron microscopy showed intracellular aggregates of enlarged mitochondria with disoriented cristae, suggesting that the disorder may have a mitochondrial

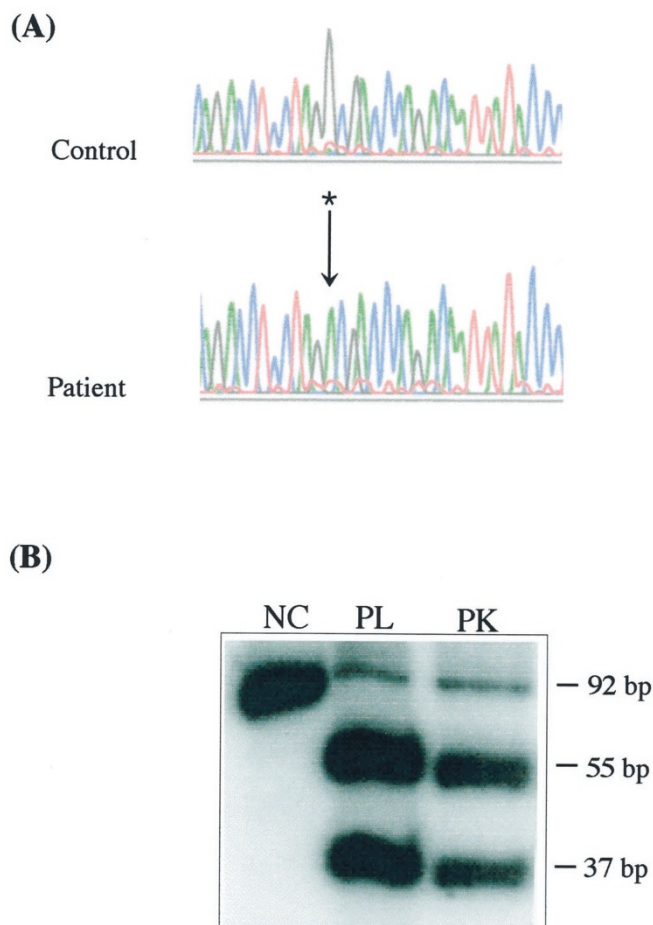


Figure 1. (A) Electropherogram of the *cyt b* gene showing the sequence of liver DNA in a control and in the patient. Sequencing was performed using a forward primer and shows (*) the G15498A transition. (B) RFLP analysis. *TaqI* cuts the original fragment (92 bp) into two fragments of 55 and 37 bp. NC indicates normal control; PL, patient's liver; PK, patient's kidney.

origin (2–7, 9, 13, 16, 18, 23, 25). A few reports offered even more convincing evidence of a mitochondrial etiology. Otani *et al.* (30) described a 15-mo-old girl with histiocytoid cardiomyopathy who also had hypotonia, lactic acidosis, and cytochrome *c* oxidase-deficient fibers in the muscle biopsy. Although the authors did suggest an mtDNA origin for the disease, molecular genetic studies were not performed. In 1984, we described complex III deficiency and decreased reducible *cyt b* in the patient revisited here, but, at that time, defects of mtDNA were not yet known. Until recently, point mutations in mtDNA were expected to be generalized and maternally inherited. This concept has now been revised because, in the past few years, several pathogenic mutations in protein-coding genes have broken the canonical laws of mitochondrial genetics. In particular, we and others have observed many mutations in the *cyt b* gene that are neither generalized (they are only present in differentiated skeletal-muscle) nor maternally inherited (all cases were sporadic) (33, 34, 39). Studies in cell culture suggest that these mutations arose as spontaneous events during embryogenesis. It seems reasonable to assume that mutations in the *cyt b* gene can also arise in stem cell populations other than myoblasts, and this probably occurred in the present patient.

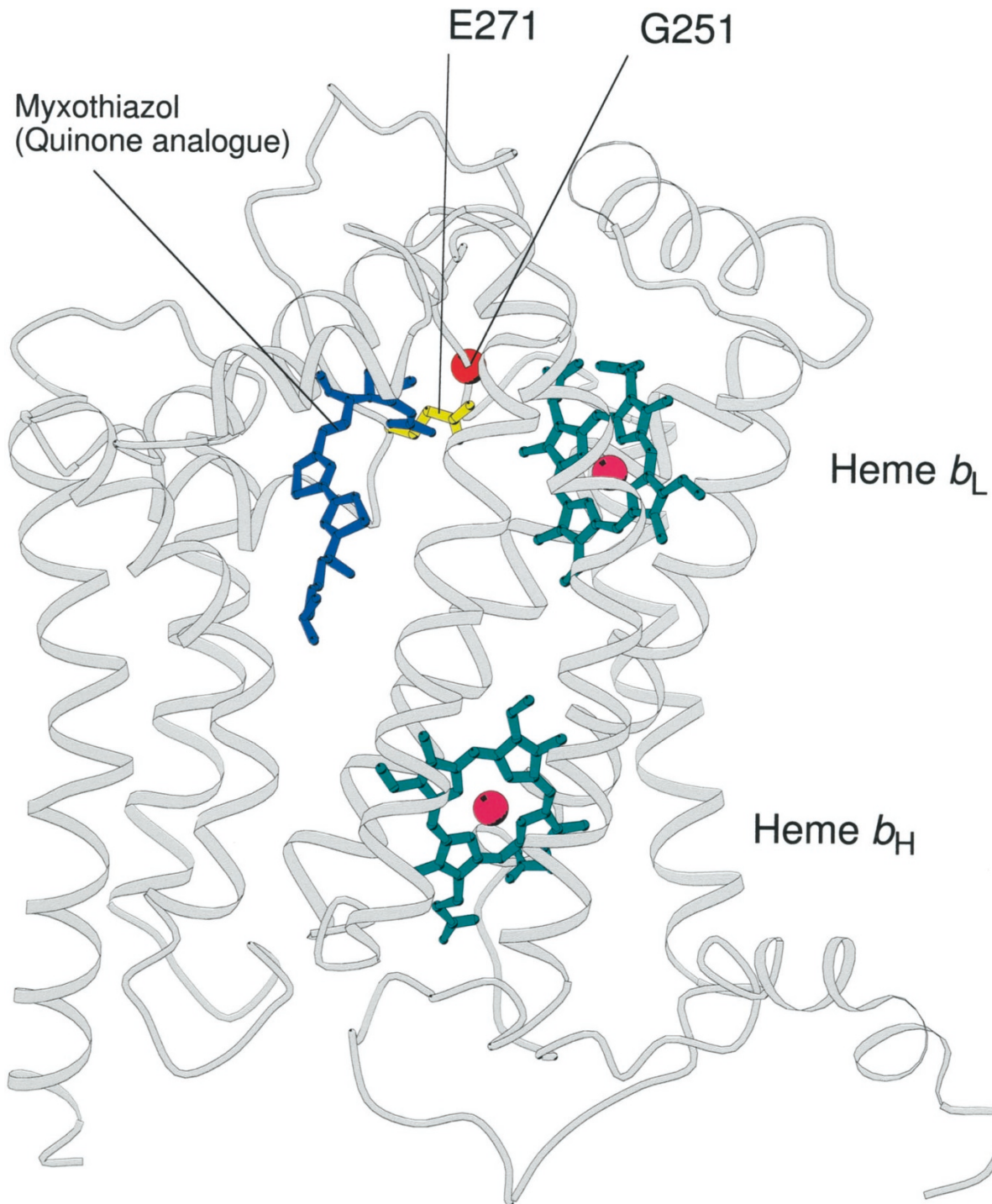


Figure 2. Crystal structure of the *cyt b* protein. Hemes b_L and b_H are shown (green). Amino acid G251 (which is mutated in the present patient) is in close contact with E271 (yellow), which is an amino acid in the domain 2 of the Qp site (ubiquinone-oxidation site) as determined by binding of quinone analogues (myxothiazol, dark blue). The mutation G251D would change the structure of the Qp site, impairing proton transfer.

Table 1. Evolutionary conservation of the *cyt b* protein

Patient	PDLL D DPDNY
Homo sapiens	PDLLGDPDNY
Beef	PDLLGDPDNY
Rabbit	PDLLGDPDNY
Chicken	PNLLGDPENF
Carp	PNLLGDPENF
Fly	PNLLGDPDNF
Yeast	PDTLGHDPNY

Although heart tissue was not available for molecular genetic analysis, the mutation was found in liver and kidney, which were clinically and pathologically involved in this patient. We consider the G15498A mutation pathogenic for the following reasons.

1. This mutation was never reported as a neutral polymorphism despite extensive sequencing of the *cyt b* gene in normal individuals from different ethnic backgrounds (36, 37).

2. The mutation is consistent with the biochemical defect because the patient had both complex III deficiency and decreased amount of reducible cyt *b* in heart tissue (13).
3. The mutation was heteroplasmic in both tissues analyzed, and heteroplasmy is a common feature of pathogenic mtDNA mutations.
4. The genetic defect was identified in liver and kidney, and both organs were affected (*i.e.* hepatic steatosis and acute tubular necrosis).
5. The mutation results in the replacement of a neutral amino acid (glycine) with an acidic one (aspartic acid) (G251D), a drastic change because glycine-251 is highly conserved throughout evolution (Table 1).
6. The effect of the replacement (G251D) on the crystal structure of complex III, which was described in 1998 (40), bolsters the pathogenic role of the G251D replacement. This residue is in close contact with the Qp site (one of the ubiquinone-binding sites of complex III) close to heme bL (Fig. 2). The presence of D instead of G should cause charge repulsion with E271, a residue at the Qp site. This, in turn, would change the structure of the Qp site and impair hydroquinone binding. As this site must be functional for proton transfer to occur, the mutation is likely to prevent proton transfer and to result in a partial block of the respiratory chain at complex III.

The G15498A mtDNA mutation is the first genetic abnormality identified in a patient with histiocytoid cardiomyopathy. Although this syndrome may be due to diverse etiologic factors, the presence of abnormal mitochondria in many reported cases and our present findings suggest that defects in mtDNA should be considered in patients with this disorder.

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