Letters to the Editor

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Maternally Inherited Cardiomyopathy: An Atypical Presentation of the mtDNA 12S rRNA Gene A1555G Mutation

To the Editor:

Human mitochondrial disorders comprise a heterogeneous group of multisystem diseases, characterized by morphological, biochemical, or genetic abnormalities of mitochondria. Mutations in mtDNA have been described predominantly in a variety of rare encephalomyopathies but are also emerging in association with more common disorders, such as sensorineural hearing loss (SNHL) and cardiomyopathies (DiMauro and Bonilla 1997). Most of the identified mtDNA mutations are associated with specific clinical phenotypes (DiMauro and Bonilla 1997). In a recent issue of the *Journal*, Estivill et al. (1998) reported that the A1555G mutation in the mitochondrial 12S rRNA is responsible for a significant number of cases of maternally inherited nonsyndromic hearing loss and that its pathogenic role is enhanced by treatment with aminoglycosides.

Idiopathic cardiomyopathies are an important cause of morbidity and mortality throughout the world, both in children and adults, with an annual incidence of 2–8/100,000 in the United States and Europe (Manolio et al. 1992). The application of molecular genetic techniques has started to delineate the molecular bases of these syndromes through the demonstration of alterations of myocardial contractile and structural proteins, such as the cardiac β-myosin heavy-chain (MYH7) gene, which accounts for ~75% of the familial cases of hypertrophic cardiomyopathies (Geisterfer-Lowrance et al. 1990). There is growing evidence that mtDNA mutations can cause cardiac disease, including cardiomyopathies and cardiac conduction block. In addition, cardiomyopathy may result from bioenergetic defects caused by mutations in nuclear-encoded subunits of the respiratory chain or in nuclear genes controlling the integrity, replication, and expression of mtDNA (Cortopassi et al. 1992; Kelly and Strauss 1994; DiMauro and Bonilla 1997).

We report here a 35-year-old woman who was evaluated because of heart failure. At age 23–24 years, during her first pregnancy, the patient noted easy fatigability, shortness of breath, and palpitations. Chest x-ray revealed cardiomegaly with prominent left-atrial enlargement. Cardiological evaluation suggested a restrictive cardiomyopathy. Episodes of atrial fibrillation and flutter required cardioversion on several occasions. Four years later, her clinical condition worsened during a second pregnancy. While in sinus rhythm, she was a New York Heart Association class I-II patient but when in atrial arrhythmia she worsened to class III. A clinical and metabolic work-up for heart transplantation was performed. Family history was remarkable for the mother and maternal grandmother, who had both died of unspecified heart diseases in their late 30s. A 25-year-old brother had a childhood heart murmur but was free of cardiac symptoms. The proposita's two daughters are asymptomatic at ages 11 and 7 years, but the older daughter had had a cardiac murmur in infancy (fig. 1*A*).

At age 35 years, physical examination, including neurological and otolaryngeal evaluations, showed a short and thin woman without other symptoms and signs commonly found in patients with mitochondrial encephalomyopathy, including ptosis, external ophthalmoparesis, pigmentary retinopathy, hearing loss, or diabetes mellitus. A two-dimensional echocardiogram revealed severe restrictive cardiomyopathy, with moderate interventricular septum hypertrophy (1.3 mm; normal <1.0 mm). An endomyocardial biopsy showed normal myofibrillar array, minimal hypertrophy of cardiomyocytes, and absence of inflammation. There were no abnormal deposits of glycogen, iron, or amyloid. A diagnosis of idiopathic restrictive cardiomyopathy was made.

After the subjects gave informed consent, we performed our studies under our institutional review board's protocol. Skeletal muscle biopsy of the quadriceps did not reveal typical mitochondrial abnormalities, such as ragged red fibers or cytochrome *c* oxidase (COX)–negative fibers. The most prominent histochemical abnormalities were central or paracentral minicores, which were easily identified, in many muscle fibers, as regions of decreased COX stain. Minicores were also detected by light microscopy in NADH-stained skeletal muscle sections (fig. 2). Electron microscopy revealed

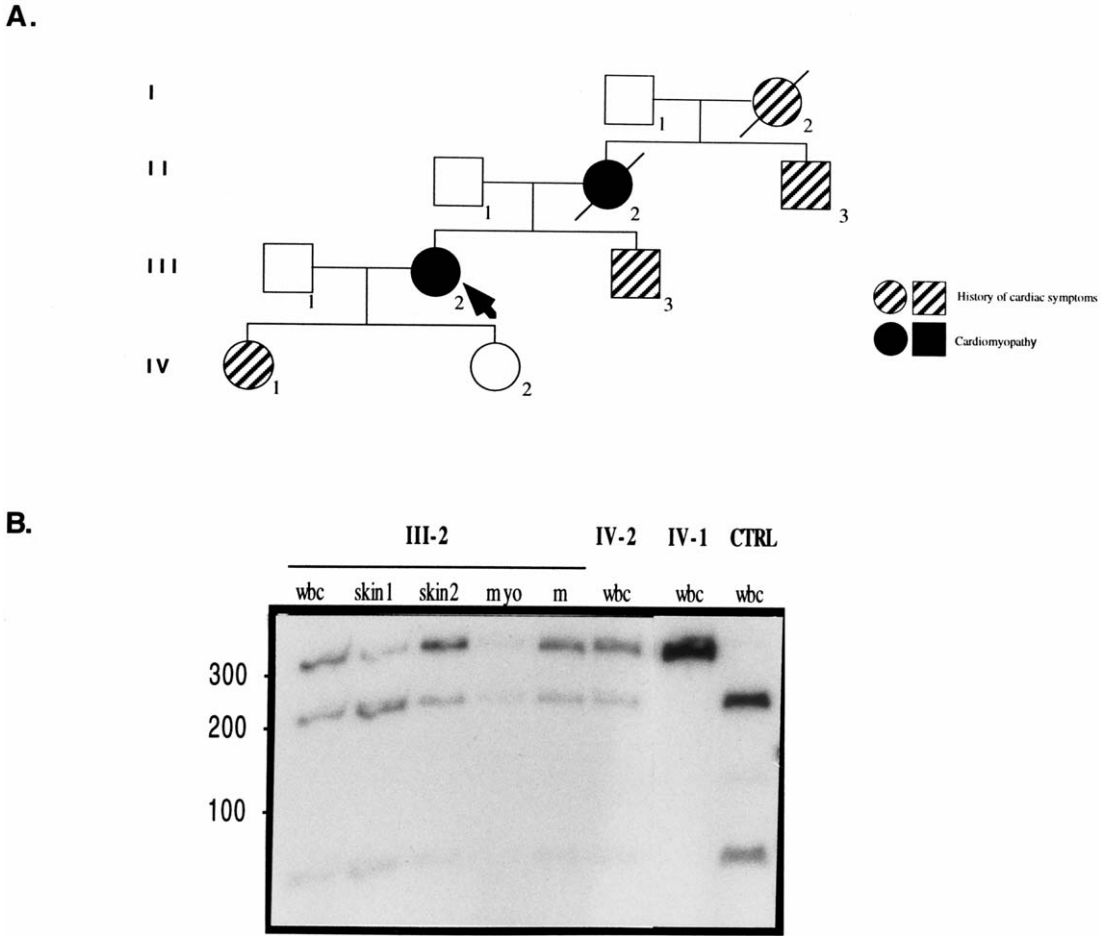

Figure 1 *A*, Pedigree of family harboring the A1555G mutation. Arrowhead indicates proband. *B*, Autoradiogram of the restriction-length–polymorphism analysis used to quantitate mutant mtDNA. The normal 316-bp PCR-amplified fragment is cut by the endonuclease *Alw*26I into two fragments (219 and 97 bp). The A1555G mutation abolishes the *Alw*26I site. Individuals are as shown in figure 1*A*. "CTRL" is a normal control; wbc = white blood cells; skin1 = cultured skin fibroblasts, first passage; skin2 = cultured skin fibroblasts, second passage; myo = cultured myoblasts; m = skeletal muscle.

foci of myofibers with prominent smearing of Z-lines and absence of mitochondria. Biochemical studies in muscle homogenate and skin fibroblasts showed slightly decreased activities of multiple complexes of the respiratory chain when values were normalized to activity of citrate synthase, a mitochondrial matrix enzyme reflecting total mitochondrial content. Specifically, the residual activities of NADH-dehydrogenase and COX were 50% and 39%, respectively, of the mean values measured in control muscles. PCR followed by SSCP and sequence analyses of our proband's muscle mtDNA identified one possible pathogenic base change, an A→G transition at nt 1555. By PCR, we amplified all 22 mtDNA-encoded tRNA genes and did not identify any additional point mutations by direct sequencing of both strands of the PCR products. The A1555G mutation was heteroplasmic, accounting for 55% of total muscle mtDNA, and similar proportions were detected in blood and primary skin fibroblasts cultures (57% and 60%, respectively). Higher levels of mutated mtDNAs were found in paraffin sections of an endomyocardial biopsy (89%). The mutation was present in high percentages in blood from the two daughters (95% in individual IV-1 and 50% in individual IV-2), the only tissues available for study (fig. 1*B*). No additional maternal members of this family were available for genetic testing.

When we examined the effects of the A1555G mutation on the translational capacity of cultured skin fibroblasts in the presence or absence of aminoglycoside (gentamicin, 0.5 mg/ml), we found moderate protein-synthesis defects under both conditions. The relative labeling ratios and electrophoretic mobility of mitochondrial translation products in cell lines harboring 60% mutated mtDNA did not differ significantly from those

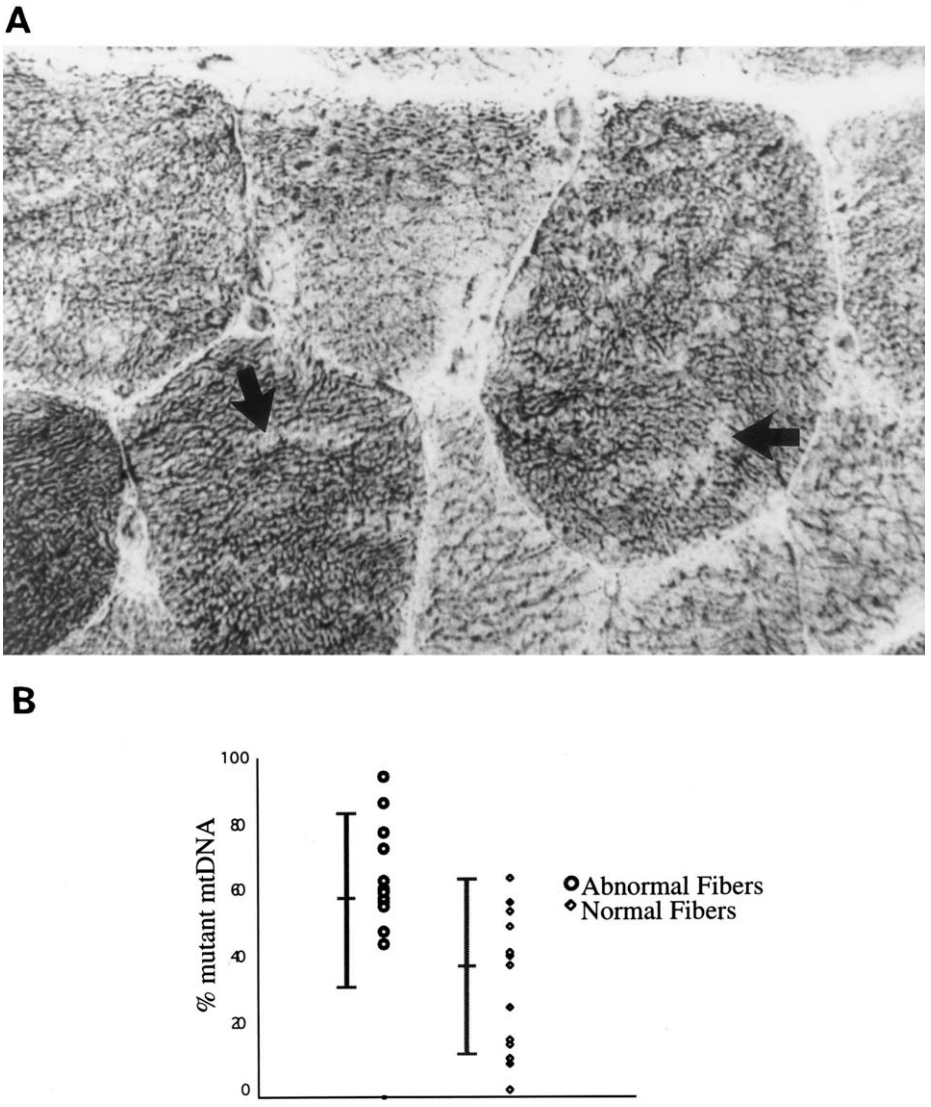

Figure 2 *A*, NADH-TR stain of the skeletal muscle biopsy. Arrows indicate minicores. *B*, Abundance of mutated mitochondrial genomes in normal and abnormal (minicore) fibers, by single-muscle-fiber PCR analysis. The difference is statistically significant ($P < .05$).

observed in controls in three independent measurements. However, there was an overall decrease in the rate of protein labeling, with an average decrease of 35%, which became more apparent (40%) when aminoglycoside was added at concentrations used routinely, in animal cell cultures, to eliminate contaminating microorganisms (fig. 3).

Clinically, our patient suffered from a restrictive cardiomyopathy from early adulthood, with a family history suggesting maternal transmission, whereas her brother and one of her daughters had transient valvular heart disease in early childhood. However, the daughters remain at risk for cardiomyopathy, because cardiac symptoms in our proposita did not start until she was in her early 20s and worsened considerably over the course of the next 10 years. Likewise, both her mother and the maternal grandmother died suddenly in their 30s of cardiac failure. We have identified the A1555G mutation, which we deem responsible for her symptoms, on the basis of the following considerations. First, the A1555G mutation was heteroplasmic, both in the patient and in her maternal relatives. Heteroplasmy, the coexistence of wild-type and mutated mtDNA molecules in the same individual, is regarded as an indicator of pathogenicity, and the abundance of mutated genomes usually correlates with the severity of the phenotype. Although this same base change has been associated with either aminoglycoside-induced deafness (AID) or nonsyndromic hearing loss in several Asian, African, and Middle Eastern pedigrees, usually in a homoplasmic

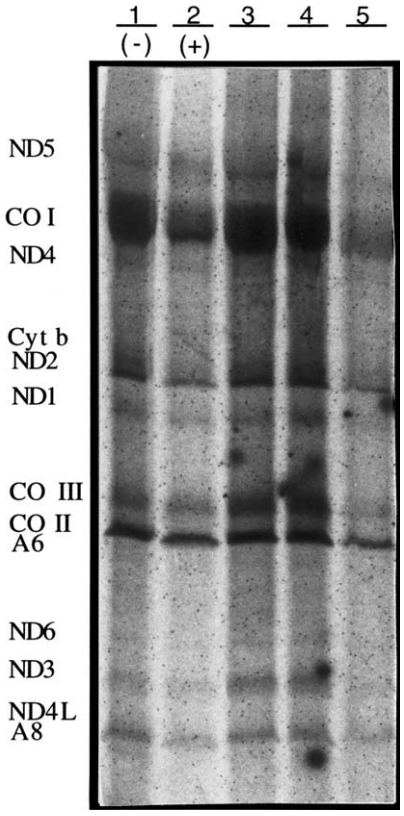

Figure 3 Electrophoretic mobility of the mitochondrial translation products. Lane 1, III-1 skin fibroblasts (aminoglycoside minus). Lane 2, III-2 skin fibroblasts (aminoglycoside plus). Lanes 3 and 4, skin fibroblasts from normal controls. Lane 5, skin fibroblasts from a disease control harboring a high percentage of the G8363A mtDNA mutation (Family A, individual III-4, in Santorelli et al. 1996).

state (Prezant et al. 1993), there is evidence of phenotypic heterogeneity. Shoffner et al. (1996) have described a Caucasian family harboring the A1555G mutation, in association with both SNHL and Parkinson disease, with onset in middle age. Complex I activity was decreased in muscle samples of several members of that family, and the degree of the biochemical defect correlated with the severity of the clinical phenotype. It is possible that additional phenotypes will be associated with this mutation. Second, the clinical expression appears consistent across three generations of this family, with variations in age at onset and disease progression, possibly resulting from differences in proportions and tissue distributions of mutant genomes. Because the symptoms do not manifest fully until adulthood, individuals IV-1 and IV-2 are at high risk of developing cardiomyopathy. Third, we noted a statistically significant correlation between the abundance of mutated mtDNAs in single muscle fibers and the presence of "minicores" in our proband ($P < .05$; fig. 2). Whereas minicores and corelike formations have been reported in other myopathies, including nemaline myopathy (Afifi et al. 1965) and limb-girdle dystrophy (Engel et al. 1971), these structural alterations usually are not found in mitochondrial encephalomyopathies. Although the finding of minicores in our proband's skeletal muscle may be nonspecific, the statistically significant association between their presence and the number of mutated genomes in single fibers suggests a causal relationship. To the best of our knowledge, no morphological studies in skeletal muscle of patients harboring the A1555G mutations have been reported. It is also noteworthy that Fananapazir et al. (1993) reported similar morphological changes in soleus muscle biopsies taken from patients with hypertrophic cardiomyopathy as a result of mutations in the MYH7 gene. In those cases, a simultaneous mitochondrial defect was hypothesized but not examined at molecular genetic or biochemical levels. Last, our data suggest that the pathogenetic mechanism of the A1555G mutation involves a primary mitochondrial translation defect, resulting from the base change in the decoding site of the small ribosome. Cells harboring the A1555G mutation showed a decreased rate of mitochondrial protein synthesis when compared with controls, even in the absence of aminoglycoside in the culture medium. Decreased synthesis of the subunits of respiratory complexes is likely to impair ATP production, with deleterious effects on cell functions and ultimately resulting in cell death. If this occurs in cardiomyocytes, it could result in heart failure, especially during periods of higher metabolic demand, such as pregnancy, as in our patient.

An intriguing issue raised by our report regards the phenotypic consequences of the A1555G mutation. Phenotypic heterogeneity is a common feature of diseases associated with mtDNA defects and is thought to result from differential tissue distribution of the mutated genomes. For example, the A3243G base change in the $tRNA^{Leu(UUR)}$ gene, although primarily associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (MELAS), is also responsible for other syndromes, such as diabetes mellitus with deafness, progressive external ophthalmoparesis, and Leigh syndrome (Shoffner and Wallace 1995). The A1555G mutation had been long considered an exception to this rule because it seemed to cause ototoxicity invariably and exclusively, occurring either spontaneously or after exposure to aminoglycosides. However, both this report and the family described by Shoffner et al. (1996) broaden the clinical spectrum for the A1555G mutation. The vulnerability of the auditory system has been attributed to the fact that the mutation affects a 12S rRNA–gene region that is homologous to the aminoglycoside-binding site of the small rRNA in bacteria. Moreover, the mutation lies within a conserved domain that in the *Escherichia coli* 6S rRNA gene forms an essential

part of the decoding site of the ribosome. This region is crucial for RNA-protein association, RNA-RNA interaction, or both; therefore, the mutation could enhance sensitivity to aminoglycosides in the hairy cells of Corti's organ, through defective protein synthesis. Deafness is not present in our family; however, restrictive cardiomyopathy is the sole clinical feature, a finding never before reported.

The fact that many patients with the A1555G mutation have been asymptomatic prior to aminoglycoside therapy suggests that the mutation alone is functionally mild (Hutchin et al. 1993). Prezant et al. (1993) hypothesized a "two-hit" model; the 12S rRNA mutation apparently alters the aminoglycoside-binding site, thus causing greater susceptibility to the toxic effects of the drug. In addition, other genetic alterations, perhaps in nDNA, modify the phenotypic expression of this mtDNA mutation. Therefore, we cannot exclude the possibility that, in addition to the abundant mutated mtDNAs detected in the proband's heart biopsy, a second genetic "hit" may have caused the cardiomyopathy in our pedigree with the A1555G mutation. By contrasting the properties of transmitochondrial cybrids harboring different percentages of the A1555G mutation from AID patients and from our cardiomyopathy patient, we may be able to detect biochemical differences, which could be attributed to a second mtDNA alteration. As an alternative, nuclear DNA factors—for example, alterations of a nuclear DNA–encoded mitochondrial ribosomal protein—could modify the phenotypic expression of the A1555G mutation.

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