

# Maternally Inherited Cardiomyopathy: Clinical and Molecular Characterization of a Large Kindred Harboring the A4300G Point Mutation in Mitochondrial Deoxyribonucleic Acid

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- OBJECTIVES** The purpose of this study was to describe the clinical and molecular features of a large family with maternally inherited cardiomyopathy (MICM).
- BACKGROUND** Recently, several mitochondrial deoxyribonucleic acid (mtDNA) point mutations have been associated with MICM. However, the distinctive clinical and morphologic features of MICM are not fully appreciated. This is partially due to the small size of the reported pedigrees, often lacking detailed clinical and laboratory information.
- METHODS** Clinical and genetic analysis of the family was carried out.
- RESULTS** Echocardiography showed mostly symmetrical hypertrophic cardiomyopathy in 10 family members. The illness had an unfavorable course. Progressive heart failure occurred in three subjects, who eventually died; one individual underwent heart transplantation. Electrocardiographic or echocardiographic signs of cardiac hypertrophy in the absence of significant clinical complaints were observed in five subjects. Neurologic examination was normal. The mutation was detected in blood from all available subjects. Abundance of mutated molecules ranged between 13% and 100% of total mtDNA genomes. The severity of the disease could not be foreseen by the proportion of mutation in blood.
- CONCLUSIONS** This report contributes a better description of the clinical aspects of MICM and provides important clues to distinguish it from hypertrophic cardiomyopathy. We suggest that mtDNA mutations, particularly in the transfer ribonucleic acid for isoleucine, should be systematically searched in patients with MICM. The identification of an underlying maternally inherited mitochondrial DNA defect in familial cases of cardiomyopathy may considerably influence the management and genetic counseling of affected patients. (J Am Coll Cardiol 1999;33:1584–9) © 1999 by the American College of Cardiology

Inherited cardiomyopathies are a heterogeneous group of disorders affecting primarily the cardiac muscle. In the last few years, several mitochondrial deoxyribonucleic acid (mtDNA) point mutations have been identified in patients with cardiomyopathies (1–12). Since mtDNA is passed to the offspring only through the mother, mtDNA-associated disorders exhibit maternal inheritance. Therefore inherited cardiomyopathies associated with mtDNA mutations are collectively known as maternally inherited cardiomyopathies (MICMs). Unfortunately, mtDNA mutations have mostly been reported in singleton cases or small pedigrees, often

lacking detailed clinical investigation. As a consequence, the clinical and morphologic spectrum of MICM in large families is not yet clearly understood.

We previously reported a novel point mutation in mtDNA, an A-to-G substitution at nucleotide position 4300, in the transfer ribonucleic acid for isoleucine (tRNA<sup>Ile</sup>) gene, in a patient with a hypertrophic form of MICM (13). We now describe the clinical and genetic findings of the extended family to better define the clinical phenotype and attempt phenotype–genotype correlations.

## METHODS

Thirty-one family members (21 women, 10 men; age range: 5 to 83 years) were studied. All patients gave their informed consent for participation in the study. Detailed clinical history was obtained from each individual. We gave partic-

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#### Abbreviations and Acronyms

|                     |  |
|---------------------|--|
| HCM                 | = hypertrophic cardiomyopathy              |
| MICM                | = maternally inherited cardiomyopathy      |
| mtDNA               | = mitochondrial deoxyribonucleic acid      |
| PCR                 | = polymerase chain reaction                |
| tRNA <sup>Ile</sup> | = transfer ribonucleic acid for isoleucine |

ular attention to cardiovascular risk factors, such as hypertension and coronary artery disease, as well as to clinical features suggestive of mitochondrial disease, including matrilineal history of neurological disease, diabetes, hearing loss and skeletal muscle disease. Both cardiologic and neurologic evaluations were carried out. The disease status of living members was determined on the basis of history, physical examination, 12-lead electrocardiogram and/or two-dimensional and Doppler echocardiogram. Electrocardiograms were interpreted according to standard criteria (14). Echocardiographic measurements of wall thickness and cavity dimensions were carried out according to the American Society of Echocardiography criteria (15). Hypertrophic cardiomyopathy was defined as the echocardiographic demonstration of a hypertrophied, nondilated left ventricle in the absence of other cardiovascular or systemic disease that could lead to left ventricular hypertrophy (16). The extent and distribution of left ventricular hypertrophy was evaluated in each patient. Left ventricular outflow obstruction was diagnosed on the basis of prolonged anterior mitral leaflet-septal contact or the presence of pressure gradient  $\geq 30$  mm Hg at Doppler examination (17). End-stage dilation in hypertrophic cardiomyopathy (HCM) was interpreted as a disease status characterized by cavity enlargement with impaired systolic function and/or left ventricular wall thinning, as the result of serial assessments (18). Deceased family members were considered affected on the basis of clinical records showing a history of progressive heart disease and cardiac death. Heart and skeletal muscle samples were obtained from the proband (IV-01). Histologic stains were carried out with hematoxylin-eosin and Masson trichrome on paraffin sections; a modified Gomori trichrome stain was performed on frozen sections according to standard methodologies.

**Genetic analysis.** Total genomic DNA was extracted from peripheral leukocytes of all available family members and from transplanted heart and skeletal muscle of the proband (IV-01). A combined polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis was used to detect the A4300G mutation. The following primers were used for PCR amplification of the mtDNA region encompassing the tRNA<sup>Ile</sup> gene: a mismatch sense primer (5'-3') 4281-4298 AGAGTTACTTTGATAGGG (underlined nucleotide is mismatch), and a reverse primer (5'-3') 4544-4523, according to the published Cambridge sequence (19). In combination with the A4300G mutation,

the mismatch creates a new restriction site for the endonuclease *Hph*I (GGTGAN<sub>8</sub>/N<sub>7</sub>). A last "hot-PCR" cycle was performed as described. Polymerase chain reaction products were digested overnight with *Hph*I and electrophoresed through a 12% nondenaturing polyacrylamide gel. Contrary to wild-type molecules, the 263-bp PCR-amplified fragments carrying the mutations are cleaved into two fragments of 235 and 28 base pairs, respectively. The relative abundance of the mutant genomes was quantitated using a Phosphorimager (Biorad, Hercules, California).

## RESULTS

Clinical, electrocardiographic, echocardiographic and genetic data of the 31 family members are listed in Table 1.

From a cardiologic standpoint, 13 family members were affected by cardiomyopathy. Diagnosis was based on the echocardiographic examination in 10 individuals (including the proband), and a history of progressive heart disease and cardiac death in three deceased members (II-07, III-02, III-16). In five cases (III-01, III-03, III-04, III-05, III-23) electrocardiography was abnormal, but echocardiography was not available. Thus, cardiomyopathy was considered only suspected.

### Clinical Profile

**Proband.** The proband (IV-01), a 37-year-old man, was referred at age 34 years for heart transplantation. His complaints of exertional dyspnea dated back to his early 20s. At that time nonobstructive hypertrophic cardiomyopathy had been diagnosed by means of echocardiography (Table 2). The patient had been treated with beta-adrenergic blocking agents and remained mildly symptomatic for the following 7 years. Thereafter, symptoms progressively worsened and the patient developed congestive heart failure; ventricular dilation was diagnosed and digoxin and vasodilators were added to therapy. Notwithstanding, the patient entered New York Heart Association class IV. On first examination, the electrocardiogram showed sinus rhythm, with extreme QRS left axial deviation, and deep Q waves in leads I, aVL and V<sub>6</sub>. The echocardiogram showed a hypertrophied left ventricle with cavity dilation and a depressed systolic function (Fig. 1). At the age of 34 years, he underwent heart transplantation. He is alive and in good health condition three years after surgery. Gross examination of the explanted heart showed left ventricular hypertrophy with mild dilation of the trabecular portion. Histologic examination revealed severe myocyte hypertrophy with diffuse sarcoplasmic vacuolization and mild interstitial fibrosis. Myofiber disarray was only focal and inconspicuous (<5%) on multiple ventricular samplings. No vascular changes were observed. Histologic examination of skeletal muscle showed ragged-red fibers, a hallmark of mitochondrial dysfunction. This prompted us to carry out mtDNA analysis, which eventually led us to identify a novel mtDNA mutation (13).

**Table 1.** Clinical Characteristics of Family Members

| Subject | Age (yr)/<br>Gender | Clinical<br>Symptoms | ECG                         | 2D<br>Echocardiogram | % mtDNA<br>Mutation B# |
|---------|---------------------|----------------------|-----------------------------|----------------------|------------------------|
| II-01   | 83/F                | None                 | Normal                      | n.a.                 | 62                     |
| II-07   | 41/F                | CD                   | n.a.                        | n.a.                 | n.a.                   |
| III-01  | 56/F                | None                 | T                           | n.a.                 | 88                     |
| III-02  | 54/F                | CD                   | n.a.                        | n.a.                 | n.a.                   |
| III-03  | 54/F                | Dyspnea              | LVH,T                       | n.a.                 | 95                     |
| III-04  | 51/F                | None                 | T                           | n.a.                 | 40                     |
| III-05  | 56/M                | Dyspnea              | LVH,T                       | n.a.                 | 82                     |
| III-13  | 45/F                | None                 | Normal                      | LVH                  | 100                    |
| III-14  | 43/F                | None                 | Normal                      | n.a.                 | 100                    |
| III-15  | 42/F                | Dyspnea, CP          | LVH,T                       | LVH                  | 92                     |
| III-16  | 33/F                | CD                   | n.a.                        | n.a.                 | n.a.                   |
| III-23  | 44/M                | LC                   | LVH                         | n.a.                 | n.a.                   |
| IV-01   | 37/M                | NYHA IV              | LVH                         | LVH and dilation     | 95                     |
|         |                     |                      |                             |                      | 99(H) 99(M)            |
| IV-02   | 29/F                | None                 | Normal                      | Normal               | 87                     |
| IV-03   | 36/F                | Dyspnea              | LVH,T, short<br>PR interval | LVH                  | 95                     |
| IV-05   | 33/M                | CP                   | LVH                         | LVH                  | n.a.                   |
| IV-07   | 23/F                | None                 | Normal                      | Normal               | 92                     |
| IV-08   | 19/M                | None                 | LVH                         | LVH                  | n.a.                   |
| IV-09   | 28/F                | Dyspnea              | LVH,T                       | LVH                  | 13                     |
| IV-20   | 25/M                | None                 | LVH,T                       | LVH                  | 76                     |
| IV-22   | 24/F                | None                 | Normal                      | Normal               | 94                     |
| IV-23   | 23/M                | None                 | LVH                         | LVH                  | 100                    |
| IV-24   | 24/F                | None                 | Normal                      | n.a.                 | 90                     |
| IV-25   | 25/F                | None                 | Normal                      | Normal               | 93                     |
| IV-26   | 16/F                | None                 | Normal                      | Normal               | 95                     |
| IV-27   | 20/M                | None                 | Normal                      | n.a.                 | n.a.                   |
| V-01    | 10/M                | None                 | Normal                      | n.a.                 | n.a.                   |
| V-02    | 5/M                 | None                 | Normal                      | n.a.                 | n.a.                   |
| V-05    | 10/F                | None                 | Normal                      | Normal               | 88                     |
| V-06    | 12/F                | None                 | Normal                      | Normal               | 93                     |
| V-14    | 7/F                 | None                 | LVH                         | LVH                  | n.a.                   |

2D = two-dimensional; CD = cardiac death; CP = chest pain; ECG = electrocardiogram; H = heart; LC = lung cancer; LVH = left ventricular hypertrophy; M = muscle; mtDNA = mitochondrial deoxyribonucleic acid; n.a. = not available; NYHA = New York Heart Association; T = T wave inversion.

**Family members.** Family history revealed many relatives suffering from “cardiac disease.” An extensive survey disclosed 12 affected and 5 suspected individuals on the maternal side of the family (Table 1, Fig. 2).

Reportedly, three family members (II-07, III-02, III-16) had died of cardiac death at the age of 41, 54 and 33 years, respectively. Available clinical information confirmed the diagnosis of cardiac disease, with onset in early adult life and a progressive course leading to refractory heart failure, similar to that observed in the proband. Of the nine living affected members, two (IV-03, IV-09) complained of dyspnea on exertion, one (IV-05) of chest pain and one of both (III-15); the others (III-13, IV-08, IV-20, IV-23, V-14) were asymptomatic. In two symptomatic patients beta-blocker therapy was started with some benefits. None of the family members had a history of systemic hypertension or blood pressure higher than 140/90 mm Hg at rest or other causes of myocardial hypertrophy.

The clinical profile of the five subjects with cardiomyopathy suspected on the basis of electrocardiographic abnormalities was characterized by dyspnea on exertion in two cases (III-03, III-05) and absence of cardiac symptoms in three (III-01, III-04, III-23).

Neurologic examination was normal in all living subjects. In particular, no signs of skeletal or extraocular muscle involvement were disclosed.

#### Cardiac Findings

Echocardiographic features of the 10 affected members are reported in Table 2. Ten individuals showed a hypertrophic form of cardiomyopathy. In nine, both the left ventricular septum and the free wall, including the posterior wall (Fig. 3), were involved. None had complete systolic anterior motion of the mitral valve or evidence of left ventricular gradient on Doppler ultrasonography. Nine patients with HMC had abnormal electrocardiograms with a pattern of

**Table 2.** Echocardiographic Features of Affected Family Members

| Subject | Age (yr)/<br>Gender | 2D Echocardiogram |            |    |               |               |            |
|---------|---------------------|-------------------|------------|----|---------------|---------------|------------|
|         |                     | LVWT              |            |    | LVEDD<br>(mm) | LVESD<br>(mm) | LA<br>(mm) |
|         |                     | VS<br>(mm)        | PW<br>(mm) | LA |               |               |            |
| III-13  | 45/F                | 13                | 12         | 38 | 25            | 32            |            |
| III-15  | 42/F                | 22                | 16         | 49 | 36            | 33            |            |
| IV-01   | 20/M                | 17                | 13         | 47 | 30            | n.a.          |            |
|         | 34                  | 18                | 11         | 69 | 60            | 37            |            |
| IV-03   | 36/F                | 14                | 15         | 48 | 32            | 18            |            |
| IV-05   | 32/M                | 18                | 23         | 47 | 33            | 30            |            |
| IV-08   | 19/M                | 27                | 25         | 45 | 25            | 37            |            |
| IV-09   | 28/F                | 17                | 18         | 40 | 26            | 31            |            |
| IV-20   | 25/M                | 18                | 17         | 49 | 32            | 33            |            |
| IV-23   | 23/M                | 13                | 16         | 46 | 30            | 46            |            |
| V-14    | 7/F                 | 12                | 15         | 45 | 32            | 31            |            |

2D = two-dimensional; LA = left atrium; LVEDD = left ventricular end-diastolic diameter; LVESD = left ventricular end-systolic diameter; LVWT = left ventricular wall thickness; n.a. = not available; PW = posterior wall; VS = ventricular septum.

left ventricular hypertrophy and T wave inversion; in one case (IV-03) a short PR interval (0.10 ms) was also measured. The remaining patient with HCM on echocardiographic examination had a normal electrocardiogram. In five cases (III-01, III-03, III-04, III-05, III-23) HCM was suspected based on the electrocardiographic pattern of left ventricular hypertrophy (voltage criteria) and strain or T wave inversion in  $\geq 2$  leads.

**Genetic Analysis**

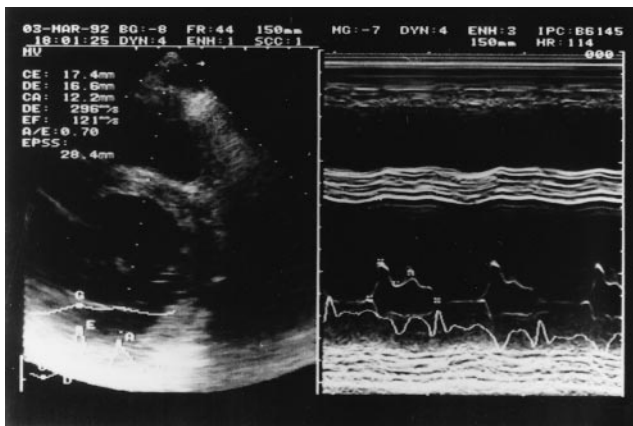
The presence and relative abundance of the A4300G mutation was investigated in peripheral leukocytes from 21 available family members. The results are reported in Table 1. Coexistence of mutated and wild-type mtDNA genomes (heteroplasmy) was detected in all but three subjects (III-13, III-14, IV-23), with mutated mtDNAs in blood ranging widely between 13% and 95%. Since no patient had overt

skeletal myopathy requiring muscle biopsy and endomyocardial biopsy was not indicated, the abundance of mutated mtDNAs in tissues other than blood could be assessed only in the proband (IV-01). Very high percentages were found in all tissues, although they were slightly higher in the heart and skeletal muscle than in blood.

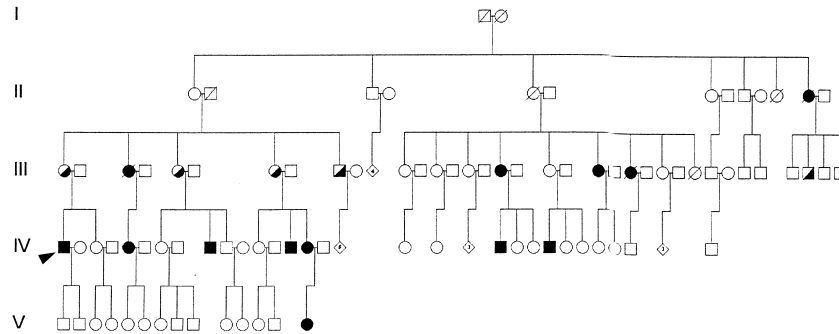
**DISCUSSION**

More than 50 different mtDNA point mutations have been associated with a wide variety of human diseases (20). These disorders are clinically, histologically and biochemically diverse, but they all preferentially affect tissues with high metabolic requirements such as brain, skeletal muscle and the heart. The latter is often affected in the frame of a multisystem disorder. As an example, about 20% of patients with the syndrome of mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes have a cardiomyopathy, mostly hypertrophic (21). On the other hand, mtDNA mutations have been reported in patients with cardiomyopathy as the sole or dominant clinical feature, a condition known as MICM (13). However, the clinical description of MICM is often incomplete. Its specific features are poorly recognized and it is unclear how it can be differentiated from other forms of familial cardiomyopathy. Our observations in a large family with MICM provide the opportunity of further defining the clinical and laboratory characteristics of this disease and comparing it to other well defined forms of inherited cardiomyopathy such as HCM.

**Clinical features of the affected members.** All affected members were found to be maternally related over five generations. Despite the considerable number of family members, no case of male-to-male transmission was observed. On the other hand, only mothers, either affected or healthy, transmitted the disease to their male and female



**Figure 1.** M-mode and two-dimensional echocardiogram of the proband showing left ventricular wall hypertrophy, left ventricular dilation and diffuse hypokinesis.



**Figure 2.** Family tree. Arrowhead indicates the proband. Filled symbols represent affected individuals.

offspring. These data on the extended pedigree strongly support a maternal mode of inheritance.

**Cardiac findings.** All affected patients presented with a pure hypertrophic form of cardiomyopathy without symptoms of other system dysfunction, including the central nervous system.

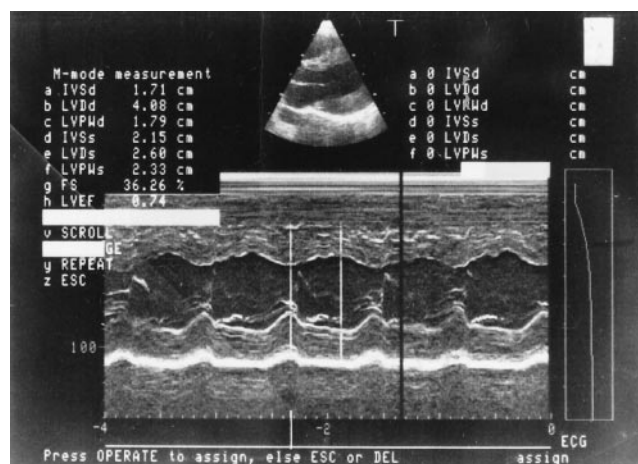
Echocardiography disclosed left ventricular hypertrophy, without outflow obstruction, in all affected members. Left ventricular hypertrophy was more often symmetrical than asymmetrical; moreover, almost all patients showed an increased wall thickening of the posterior wall, which was clearly predominant in two cases (IV-05, IV-23). In HCM, left ventricular hypertrophy is characteristically asymmetric; a concentric hypertrophic pattern accounts for only 1% of the cases (22). The anterior ventricular septum is the predominant region of hypertrophy in the majority of cases (83%); hypertrophy mainly involving the left ventricular posterior wall is rare (2%). In addition, significant myofiber disarray, which is the histologic hallmark of HCM, was not observed. In the proband, serial echocardiograms demonstrated an evolution from a hypertrophied, nondilated left

ventricle to left ventricular cavity enlargement and impaired systolic function, which is considered rare in HCM (18).

The illness often had an adverse clinical course, with three deceased members (II-07, III-02, III-16), and a fourth individual received a transplant (IV-01). Only five subjects (III-13, IV-08, IV-20, IV-23, V-14) showed electrocardiographic or echocardiographic signs of hypertrophy, while asymptomatic. Notably V-14 is a seven-year-old girl. These findings suggest a more severe form than HCM with a relatively common rapidly progressive course. Interestingly, neither atrial fibrillation nor sudden death were relevant features. Whether this clinical presentation is related to this specific mtDNA mutation or represents a common feature of MICM remains to be established. The latter hypothesis seems to be supported by other reports (2,4,6,8,12); however, more clinical and follow-up observations are needed.

**Genotype-phenotype correlations.** All studied subjects harbored the A4300G mutation in the tRNA<sup>Ile</sup> gene. However, no obvious correlation was found between disease status or severity and abundance of mutated genomes in peripheral leukocytes. In fact, a very high proportion (>95%) of mutated mtDNAs was found both in affected members (IV-01, IV-23) and healthy subjects (IV-26). This lack of correlation is not infrequent in other disorders associated with mtDNA mutations (23). Possible explanations include the widely variable degree of heteroplasmy as well as differences in the threshold for clinical expression among different tissues. Moreover, the fate and expression of a given mtDNA mutation in different cultured tissues appear to be strongly influenced by tissue-specific factors encoded in the nucleus, which could be different even within the same family (24).

**Conclusions.** The true frequency of MICM within the familial forms of cardiomyopathy has not been clearly established. Because many cardiologists are relatively unfamiliar with mitochondrial disorders, a matrilinear lineage of cardiomyopathy may be confused with an autosomal dominant one. We suggest that mtDNA analysis be included in the working-up of familial as well as sporadic cases of



**Figure 3.** M-mode echocardiogram of an affected family member showing symmetrical left ventricular wall hypertrophy with normal diastolic and systolic diameters.

HCM, especially when hypertrophy is symmetrical or when the family history is compatible with maternal inheritance. The tRNA<sup>Ile</sup> gene is probably a mutational "hot spot" for MICM and should be preferentially studied (1,6,12,13). Our original description of the A4300G mutation was, indeed, the successful result of such an approach in a series of familial cases of unexplained HCM. The same strategy has recently yielded the identification of two novel mutations in the tRNA<sup>Ile</sup> gene (24).

From a more practical point of view, it should be kept in mind that detecting mtDNA mutations associated with cardiomyopathy in a given family might have considerable impact on management and genetic counseling. The offspring of male patients can be safely excluded as potential affected subjects, whereas all the children of an affected mother should be considered at risk.

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