A Case of Cardiomyopathy Showing Progression From the Hypertrophic to the Dilated Form

— Association of Mt8348A→G Mutation in the Mitochondrial tRNA\textsubscript{\text{Lys}} Gene With Severe Ultrastructural Alterations of Mitochondria in Cardiomyocytes —

Fumio Terasaki, MD; Masashi Tanaka, MD*; Keishiro Kawamura, MD; Yumiko Kanazaki, MD; Makoto Okabe, MD; Tetsuya Hayashi, MD; Hiroaki Shimomura, MD; Takahide Ito, MD; Michihiro Suwa, MD; Jian-Sheng Gong, MD*; Jin Zhang, MD*; Yasushi Kitaura, MD

This report describes a case of cardiomyopathy with a novel point mutation of mitochondrial DNA coding lysine tRNA in association with severe ultrastructural alterations of the mitochondria in the cardiomyocytes. Abnormalities of energy production and/or abnormal protein synthesis because of the mutation of mitochondrial DNA may have played an important role in the pathogenesis of this case, which showed severe cardiomyocyte degeneration and deterioration from hypertrophic cardiomyopathy to severe dilated cardiomyopathy. (Jpn Circ J 2001; 65: 691–694)

Key Words: Cardiomyopathy; Endomyocardial biopsy; Mitochondrial DNA; Ultrastructure

Various point mutations in the mitochondrial genome have been reported in patients with mitochondrial cardiomyopathy,\textsuperscript{1,2} and in the patient presented here, a new mutation of mitochondrial DNA associated with severe ultrastructural changes of the mitochondria has been identified.

Case Report

A 46-year-old Japanese man has been followed up at Osaka Medical College Hospital for 26 years under the clinical diagnosis of hypertrophic cardiomyopathy (HCM) progressing to the dilated phase (d-HCM)\textsuperscript{3–7} with chronic congestive heart failure.

In 1974, at the age of 20 years, cardiomegaly was suspected on a physical checkup and he was admitted to hospital for further evaluation. Left ventricular hypertrophy was noted on the ECG (SV1 + RV5 = 13.1 mV, inverted T wave in II, III, aVF, V4–6) and chest X-ray (cardiothoracic ratio = 63%). The echocardiogram revealed marked concentric hypertrophy of the left ventricle (LV) with normal wall motion. The end-diastolic wall thickness was 22 mm in the left ventricular posterior wall. Left ventricular end-diastolic and end-systolic dimensions were 50 mm and 30 mm, respectively. The fractional shortening of the LV was 40%. Because of the absence of systemic hypertension and any heart diseases of known cause, he was clinically diagnosed as HCM.

Thereafter, cavity dilatation, wall thinning, and diffuse hypokinesis of the LV progressed, and the signs and symptoms of congestive heart failure also gradually worsened, although the patient was treated with digitalis, diuretics, angiotensin-converting enzyme inhibitors, and positive inotropic agents. He had also taken \(\beta\)-adrenoreceptor blockers in the last few years. Frequent ventricular arrhythmias have been observed despite treatment with anti-arrhythmic agents. In April 1997, he underwent partial left ventriculotomy (ie, Batista operation)\textsuperscript{8} and currently (October 2000) is in a relatively stable condition with an improvement in his quality of life. Table 1 shows the progression in the echocardiographic data and the New York Heart Association (NYHA) cardiovascular function classification.

Genetic Analysis

Cardiac Contractile Proteins The reported gene mutations (\(\beta\)-myosin heavy chain, troponin T, \(\beta\)-tropomyosin, myosin binding protein-C and titin) were not detected by genetic analysis.

Mitochondrial DNA Total DNA was extracted from peripheral leukocytes and myocardium, and the sequencing analysis of the entire mitochondrial genome was conducted as described previously. Among several mutations observed, an A→G transition at position 8348 of mitochondrial lysine tRNA was a novel mutation (Fig 1).

Confirmation of the Mt8348A→G Mutation by Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP)

In order to detect the mutation at nucleotide position...
(np) 8348, a 193-bp mtDNA fragment [np 8206–8398] was amplified with the forward primer mtL8206 (5'-GCC CAT CGT CCT AGA ATT AA-3', corresponding to np 8206–8227) and the mismatch reverse primer mtH8349 (5'-GGT GGG CCA TAC GGT AGT ATT TAG TTG GGG CAT TTC ACT GTA AAG AGC TG-3', corresponding to np 8398–8349) by PCR, and the PCR product was digested with the restriction enzyme BbvI. In the presence of Mt8348G, the PCR product was cleaved into 2 fragments (155 bp and 38 bp), but in its absence the 193-bp fragment was not cleaved.

The study protocol, including DNA analysis, was approved by the institutional Committee for Human Research and written informed consent was obtained from the patient.

**Results**

The Mt8348A→G transition was detected in both the patient’s heart tissue (lane 1, Fig 2) and blood (lane 2), as well as in the blood from the patient’s brother (lane 3). The PCR product from the normal control was not digested by BbvI (lane 4). The coexistence of both the wild-type and the mutant mtDNA suggests that this transition is a new mutation.

Echocardiography of the patient’s brother showed that he also had mild concentric hypertrophy of the LV, but further cardiac evaluation has not been performed because he does not have any cardiac symptoms. Their mother is alive at the age of 83 years and has not been suffered from cardiac symptoms throughout her life. She would not consent to analysis of her DNA.

**Histopathological Findings**

**Endomyocardial Biopsy** During the 26-year follow-up, endomyocardial biopsies were conducted twice, in 1974 and 1990. Hypertrophy and severe vacuolization of cardiomyocytes were observed in both biopsy specimens by light microscopy (Fig 3). Electron microscopy revealed various abnormalities of the mitochondrial structure, most commonly pleomorphic mitochondriosis with a lucent matrix and disorganized cristae. The most distinctive feature was large mitochondria with concentric cristae or stacked parallel cristae looking like finger prints (Fig 4). Additionally, some mitochondria contained glycogen granules and very electron-dense small inclusion bodies. These ultrastructural

---

**Table 1 Progression of Echocardiographic Data and NYHA Class**

<table>
<thead>
<tr>
<th>Date</th>
<th>7/74</th>
<th>8/80</th>
<th>8/84</th>
<th>5/91</th>
<th>11/93</th>
<th>5/95</th>
<th>3/97</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVSd (mm)</td>
<td>--</td>
<td>15</td>
<td>16</td>
<td>13</td>
<td>13</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>PWd (mm)</td>
<td>22</td>
<td>15</td>
<td>14</td>
<td>12</td>
<td>9</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>LVDd (mm)</td>
<td>30</td>
<td>33</td>
<td>46</td>
<td>61</td>
<td>68</td>
<td>70</td>
<td>73</td>
</tr>
<tr>
<td>FS (%)</td>
<td>40</td>
<td>32</td>
<td>17</td>
<td>15</td>
<td>13</td>
<td>11</td>
<td>10</td>
</tr>
</tbody>
</table>

IVSd, end-diastolic thickness (ET) of interventricular septum; PWd, ET of left ventricular posterior wall; Dd, end-diastolic dimension of left ventricle (LV); Ds, end-systolic dimension of LV; FS, fractional shortening; NYHA, functional classification of cardiovascular disability by New York Heart Association.
alterations are similar to those found in the cardiac biopsy of patients with Kearns-Sayre syndrome, which is a distinct and sporadic form of mitochondrial myopathy.

Resected Myocardium The histopathological changes were more severe than those observed in the previous biopsy specimens. Cardiomyocytes showed vacuolar degeneration associated with severe fibro-fatty replacement. Electron microscopy revealed a large number of abnormal mitochondria, one form of which was giant mitochondria with concentric cristae (Fig 5A,B) that appeared to result from fusion of several small mitochondria (Fig 5B).

Discussion

Progression of HCM to left ventricular dilatation and dysfunction (ie, dilated cardiomyopathy, DCM), occurs in upward of 10% of patients resulting, at least in part, from the wall thinning and scar formation consequent to myocardial ischemia caused by small vessel coronary artery disease. Recent developments in molecular biology suggest that there may be a relation between the genotype abnormalities of contractile proteins and the d-HCM phenotype, but the genesis of the progression to the dilated phase is still unclear. In the present case, the reported gene mutations of cardiac contractile proteins (α-myosin heavy
chain, troponin T, \( \delta \text{-tropomyosin, myosin binding protein-} \ C \text{ and titin) were not detected by genetic analysis, but several mutations of mitochondrial DNA were documented, including a novel point mutation of mitochondrial DNA coding lysine tRNA (M8344A→G transition), in association with severe ultrastructural alterations of mitochondria. Abnormalities of energy production and/or abnormal protein synthesis because of the mutation of mitochondrial DNA may have played an important role in the pathogenesis of this case, but it is still unclear whether the M8344A→G transition is absolutely pathogenetic or not, because in other primates (pygmy chimpanzee, gorilla, and orangutan) G is present at the site in the TPC loop of rRNA-Lys.

Mutant mtDNA was dominant in the heart tissue of the patient, whereas wild-type mtDNA was detected in his blood samples. In patients with mitochondrial disorders, the percentage of mutant mtDNA is often lower in the blood cells than in the skeletal muscles or cardiac tissues. The cells with a lower ratio of mutant mtDNA may be selected during proliferation of hemopoietic cells. In contrast, cardiomyocytes do not proliferate under normal conditions, so it is unlikely that there is functional selection for wild-type mtDNA at the cellular level. Thus the high ratio of mutant mtDNA in the heart would result mainly from the random distribution of mutant mtDNA during embryogenesis or subsequent development.

The ratio of mutant mtDNA in the blood from the patient’s brother was higher than his own, whereas the brother’s cardiac symptoms were milder. One explanation is that the ratio of mutant mtDNA in the heart of the brother was lower than that of the patient. These findings and possibilities are probably the result of random distribution of mutant mtDNA among oocytes and then among the tissues of each individual.

Mutations within the Leu(UUR)-tRNA gene (eg, 3243A→G, 3260X→Y, and 3270X→Y), as well as within the ATP6 gene (eg, 8993T→G and 8993T→C) have been reported in patients with cardiomyopathy. The 8348A→G mutation appears to be more benign than the 3243A→G mutation, because the present patient developed cardiac symptoms in adulthood, whereas most patients with a high ratio of the 3243A→G mutation develop cardiac symptoms in childhood. The full effect of the 8348A→G mutation on the function of the Lys-tRNA is still to be examined.

A point mutation in the transfer RNA gene for lysine (M8344A→G) has been identified as a cause of myoclonic epilepsy and ragged red fibers (MERRF).\(^{14}\) Interestingly, the present patient does not have the neurological and skeletal muscle abnormalities observed in mitochondrial encephalomyopathies, including MERRF. The clinical features are restricted to cardiac muscle, which might partly be due to the difference in the mutation site and heteroplasmy in the involved organs and is why we prefer the term ‘mitochondrial cardiomyopathy’.

It has been reported recently that a cardiomyopathy with mitochondrial involvement should be included with the ‘unclassified cardiomyopathies’.\(^ {15}\) However, we consider that mitochondrial cardiomyopathy should be classified as a specific cardiomyopathy because several point mutations\(^ {1,2,16,17}\) have been demonstrated in the heart mitochondrial DNA of these patients, associated with severe morphological changes of the mitochondria.

Acknowledgments

The authors are deeply grateful to Professor Akinori Kimura, Medical Research Institute, Tokyo Medical and Dental University, for performing the genetic analysis of cardiac contractile proteins, and to Drs Hisayoshi Suma, Tadashii Isomura and Yasuhiko Horii, The Department of Cardiovascular Surgery, Hayama Heart Center, for performing the partial left ventriculectomy. Thanks are also extended to Yoshiro Kitagani, Chieko Ota, Akiyo Saito, Sadao Uchida and Tomomi Sakagawa for their valuable technical assistance.

This study was supported in part by a grant-in-aid for scientific research (No. 10670862) from the Ministry of Education, Science and Culture of Japan; and by a research grant for intractable diseases from the Ministry of Health and Welfare of Japan.

References