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Patients with idiopathic cardiomyopathy belong to the same mitochondrial DNA gene family of Parkinson's disease and mitochondrial encephalomyopathy

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Summary: **Comparison of total mitochondrial DNA sequences of patients with idiopathic (delated or hypertrophic) cardiomyopathy with those of patients with Parkinson's disease and mitochondrial encephalomyopathies revealed distinct clustering of point mutations among patients. Furthermore, an inverse relation was found between the total number of base-substitution and life span of the patients. Among point mutations found in each patient, sequentially diverged six clusters consisting of 14, 10, 7, 1, 2, and 3 mutations, respectively, were detected. Five sub-clusters consisting of 2, 2, 11, 1, and 1 mutations, respectively, were detected. From each cluster, the patient's unique mutations were diverged with three types of the mutations specific for the disease. The divergence allowed construction of a phylogenetic tree which clearly indicated that patients with idiopathic cardiomyopathy belong to the same mitochondrial DNA gene family of Parkinson's disease and mitochondrial encephalomyopathies.**

In the previous paper (1), we have reported that the total sequence data for mitochondrial DNA (mtDNA) revealed distinct clustering of point mutations (*pms*) in mtDNA among patients with myoclonus epilepsy with ragged-red fibers (MERRF), with Parkinson's disease (PD), with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), and with fatal infantile cardiomyopathy (FICM). Among *pms* found in each of patients, sequentially diverged five clusters of pms were detected and a phylogenetic tree of mitochondrial encephalomyopathies (ME) was constructed. The tree clearly indicated that the ME and PD patients are members of the same gene family. From the characteristic features of each patient's unique *pms*, the types of the mutations specific for the disease were classified as *mit* $^-$ (base substitutions in protein subunit gene) + *syn* $^-$ (base substitutions in transfer RNA gene) for MERRF, *mit* $^-$ + ρ^- (deletions) for PD, and *syn* $^-$ + *mit* $^-$ for MELAS. Recent findings (2) of a 4,977-base pair (bp) deletion in mitochondrial DNA (mtDNA) in the striatum of the patients with Parkinson's disease (PD) indicated that PD could be classified as a type of mitochondrial encephalomyopathy (ME). The deletion in PD patients can also be detected in a young normal

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control, and the amount of deleted mtDNA increases with age (3). Ozawa *et al.* (4) reported the existence of multiple mtDNA deletions among the patients with idiopathic dilated or hypertrophic cardiomyopathy (DCM or HCM). The amount of the 7,436 bp deletions between the ATPase6 gene and the D-loop region was found to increase with age as a contributory factor to "presbycardia" (5). Yeast *mit* $^-$ mutations which probably lead to instability of mtDNA are almost always associated with a significant rise in the rate of ρ^- type mutation, as mentioned previously (6). Thus, in an analogous fashion, it is considered that pre-existing *mit* $^-$ mutations in human result in ρ^- type mutations. Consequently, the total or near total sequences for four mtDNAs of patients with DCM or HCM were determined to detect existence and phylogeny of *mit* $^-$ mutations and to identify the type of mutation specific for the disease.

Materials and Methods

The total sequence data for the mtDNA of the patients with MERRF, PD, MELAS, and FICM were reported in the previous paper (1).

Frozen autopsied heart muscles of the DCM patient-1 were used for the sequencing. A 74-year-old male was admitted to a hospital (Nagoya Red Cross Hospital) because of dyspnea on exertion for a month. The chest X ray showed cardiomegaly and bilateral pleural effusion. The echo-cardiogram showed diffuse wall thinness and hypokinesis in wall motion. The ejection fraction was 25%. No abnormality in coronary arteries was found by coronary angiography. Heart failure ameliorated with the use of diuretics and digitalis. One year after the admission, he died of heart failure.

Frozen autopsied heart muscles of the DCM patient-2 were used for the sequencing. The patient, 40-year-old male, with five years history of DCM was admitted to a hospital (Nagasaki Univ.) for detailed cardiovascular examination. His two younger brothers had been diagnosed as DCM. His electrocardiogram (ECG) showed complete left bundle branch block, and cardiomegaly was observed by chest X-ray (cardio-thoracic ratio, 67%). Pulmonary congestion was also observed. Echo-cardiographic findings indicated enlargement of left ventricular chamber, and hypokinetic movement of left ventricular wall. Paroxysmal atrial fibrillation and flutter occurred often, and ventricular arrhythmias were frequently developed. After 5 years from his first admission, he died of heart failure and renal failure.

Biopsied heart muscles of the HCM patient-1 were used for the sequencing. A 21-year-old male was admitted to a hospital (Osaka Medical College) because of palpitation attack and dyspnea on exertion for a month. ECG showed the findings of Wolff-Parkinson-White syndrome. The echocardiogram showed marked left ventricular wall thickness and mild diffuse hypokinesis in wall motion. No abnormality in coronary arteries was found by coronary angiography. Endomyocardial biopsy at right ventricle was performed. Microscopy showed slight hypertropy and vacuolation of cardiomyocytes and mild fibrosis. Electron microscopy showed the accumulation of glycogen granules around the nucleus. Laboratory data showed the increases in creatine phosphokinase, lactate and pyruvate at rest. Short stature, slight mental retardation, and perceptive deafness were also found.

Frozen autopsied heart muscles of the HCM patient-2 were used for the sequencing. The patient, 43-year-old male, with 20 year history of cardiomegaly was admitted to a hospital (Komaki Municipal Hospital) because of general malaise and dyspnea on exertion. The chest X-ray showed cardiomegaly with 68 % cardio-thoracic ratio. He was diagnosed as DCM from the echo-cardiogram and cardiac catheterization showing left ventricular enlargement and diffuse hypokinesis in wall motion. The the ejection fraction was 20%. The endomyocardial biopsy samples showed myofiber hypertrophy and marked disarray, which are characteristic of HCM, although the hemodynamic features resembled DCM. Family history showed that his younger sister also had HCM. Two years after the first admission, he died of heart failure and renal failure.

The total mtDNAs from the patients were sequenced by using a fluorescence-based automated direct sequencing technique (7) using an Applied Biosystems Model 373A DNA Sequencer.

Results

Fig. 1 summarizes point mutations (*pms*) found in the mtDNAs from the patients as compared with the human mtDNA sequence reported by Anderson *et al.* (8) (Cambridge sequence). Nucleotide substitutions at nucleotide position (nt) 8860, 9559, and 13702 are possible errors in the Cambridge sequence, as reported in the previous paper(1). Thus, these three are eliminated from the total number of *pms* and from consideration of the patients' phylogeny. Distinct clustering of *pms* among the patients was detected and subsequently diverged clusters are designated as C-1 to C-6. The phylogeny from C-1 to C-5 illustrated by bold lines is the same as

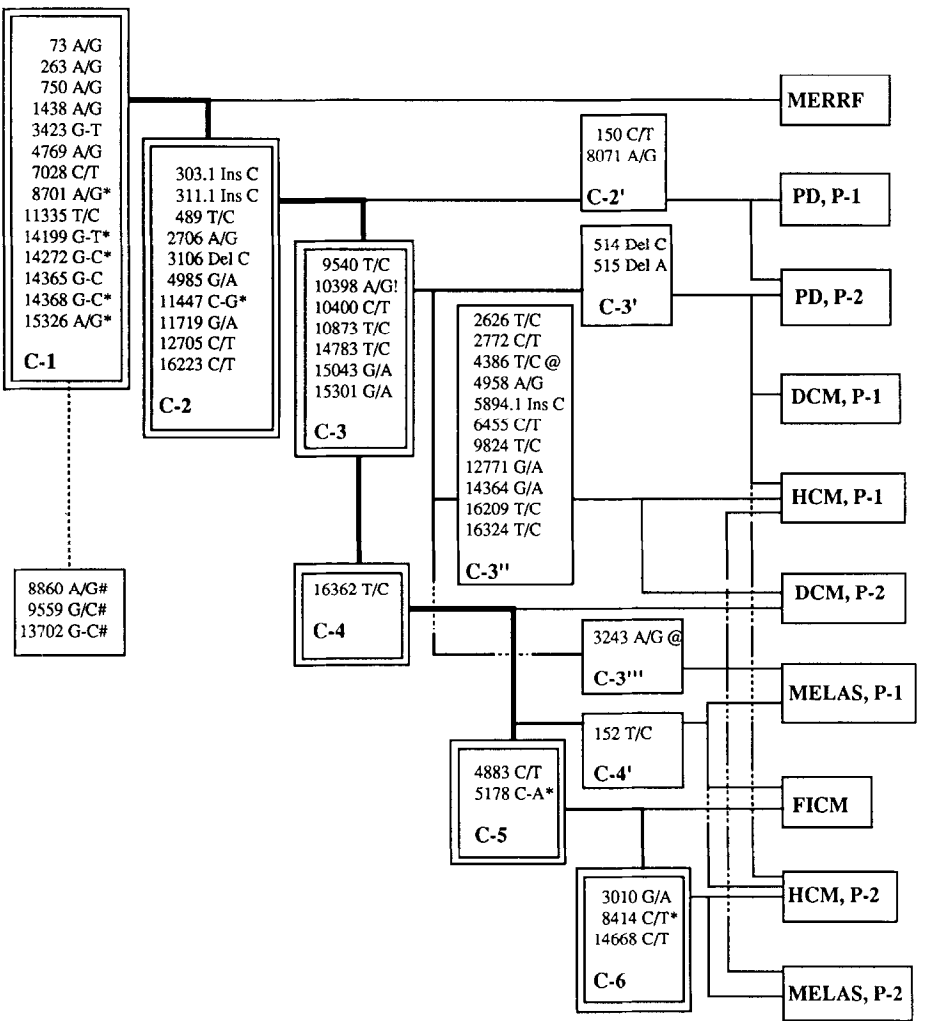


Fig. 1. Clustering of point mutations in mtDNA among the idiopathic cardiomyopathy, PD, and ME patients.

C-1 to C-5 are reported in the previous paper and connected with bold lines.

Abbreviations: #, possible error of the Camb; /, transition mutation; –, transversion mutation; *, mutation which changes amino acid residue; !, mutation which changes evolutionary conserved amino acid residue; @, mutation in the tRNA gene; ^, reverted mutation; P, patient.

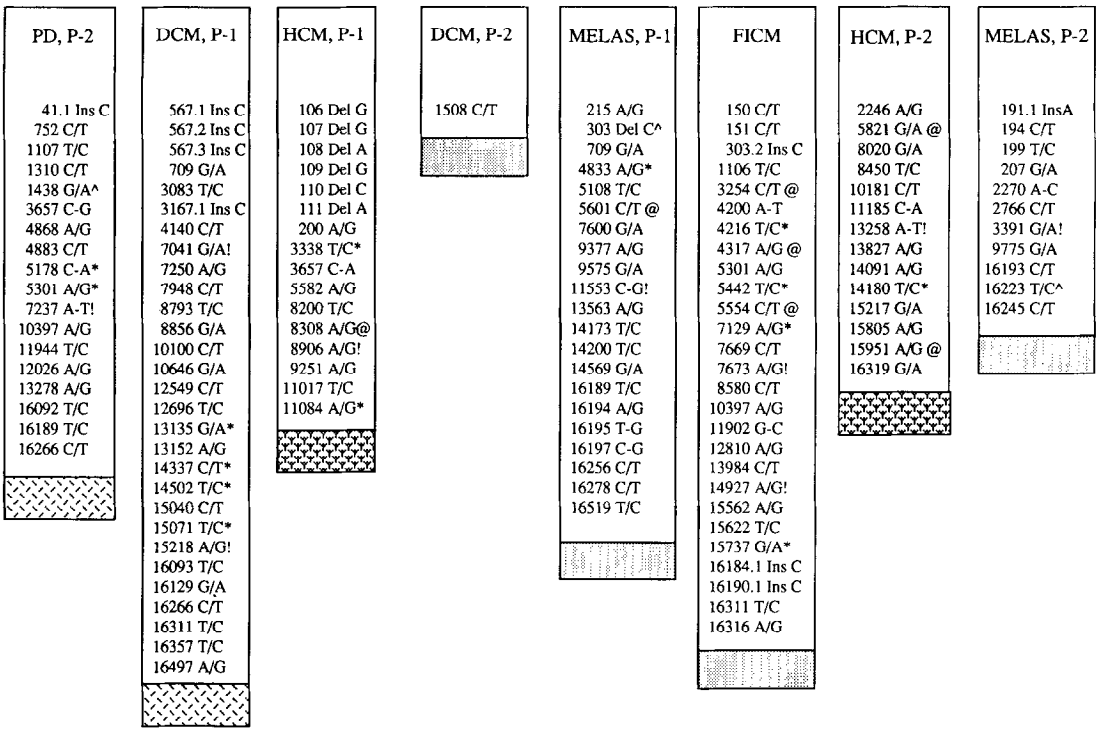


Fig. 2. The unique mutation of the patients.

Abbreviations are the same as in Fig. 1. The types of mutations as below are illustrated.

$=syn^- + mit^-$, $= mit^- + \rho^-$, $= syn^- + mit^- + \rho^-$

reported in the previous paper (1). In addition, five sub-clusters are identified. The patient's unique *pms* specific for the disease are diverged from each cluster, as shown in Fig. 2. The divergence allows construction of a precise phylogenetic tree of idiopathic cardiomyopathy. The tree clearly indicates that the cardiomyopathy patients belong to the same gene family of PD and ME.

Among the sub-cluster of *pms*, the C-3', petite deletions at nt 514 and 515, are commonly shared by the PD patients-2, the DCM patients-1, and the HCM patient-1 and -2. A *syn*$^-$ mutation, a T-to-C transition at nt4386 in the $tRNA^{Gln}$ gene (the DHU loop), exists in the C-3" which is shared by the DCM patient-2 and the HCM patient-1. Another *syn*$^-$ mutation, an A-to-G transition at nt3243 in the $tRNA^{Leu(UUR)}$ gene (the DHU loop) reported as specific for the MELAS patient in the previous papers (7, 9), is designated as the C-3'", as it is shared by the HCM patient-1 as well as the MELAS patient-1 and -2. The T-to-C transition at nt152, which could alter the secondary structure of the 5' end of the D-loop region located near the conserved sequence block I (10, 11) of the D-loop (Fig. 3) is designated as the C-4', as it is shared by the MELAS patient-1, the FICM patient, and the HCM patient-2.

Among unique *pms* of the DCM patient-1 (Fig. 2), there exists two *mit*$^-$ mutations changing conserved amino acid residues: namely, a G-to-A transition at nt7041 in the CO1 gene alters valine to isoleucine and that at nt15218 in the Cyt*b* gene threonine to alanine. Four *mit*$^-$

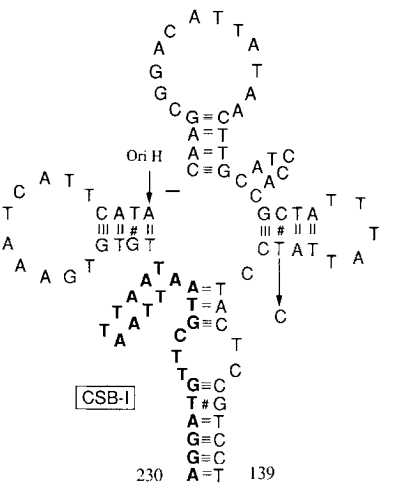


Fig. 3. The transition mutation (nt152) on the secondary structure of 5'-end of the D-loop region in mtDNA illustrated by an arrow with the conserved sequence block I (CBS-I) by the bold letters.
The original figure of the secondary structure of 5'-end of the D-loop region presented by Brown at al. (11) is modified with illustration of the origin of the H-strand replication (Ori H) by an arrow.

mutations at nt13135, 14337, 14502, and 15071 in the ND5, ND6, ND6, and Cyt*b* genes, respectively, change alanine to threonine, valine to methionine, isoleucine to valine, and tyrosine to histidine, respectively. There exists no *syn* $^-$ mutation, but a 7,436 bp deletion which is common among the patients with the idiopathic cardiomyopathy as reported previously (11). Thus, the type of mutation specific for the DCM patient-1 could be designated as *mit* $^-$ + ρ^-.

Among the unique *pm*s in the HCM patient-1, a 6 bp deletion at nt106 to 111 and a *syn* $^-$ mutation, an A-to-G transition at nt 8308 in the $tRNA^{Lys}$ gene (the DHU loop) are noticed. One of three *mit* $^-$ mutations at nt8906 in the ATPase6 gene alters the conserved histidine to arginine. Thus, the type of mutation specific for the HCM patient-1 could be designated as *syn* $^-$+ *mit* $^-$ + ρ^-

The DCM patient-2 (Fig. 2) has one unique *pm* and no detectable deletion surveyed by polymerase chain reaction (PCR). Thus, the type of mutation specific for the DCM patient-2 could be designated as *mit* $^-$ + *syn* $^-$.

Among the unique *pm*s in the HCM patient-2, two *syn* $^-$ mutations are found in the tRNA genes; namely, a G-to-A transition at nt5821 in the $tRNA^{Cys}$ gene at the aminoacyl acceptor (AA) stem and an A-to-G transition at nt15951 in the $tRNA^{Thr}$ gene (the AA stem). Both transitions would decrease the stability of the AA stems. The *mit* $^-$ mutation at nt13258 in the ND5 gene changes the conserved serine residue to cysteine, and that at nt14180 (ND6) alters tyrosine residue to cysteine. Previously reported multiple deletions common among three patients with the idiopathic cardiomyopathy (11) are also exist in the mtDNA of the HCM patient-2. Thus, the type of mutation specific for hypertrophic cardiomyopathy could be designated as *syn* $^-$+ *mit* $^-$+ ρ^-.

An inverse relation with a correlation coefficient of 0.864 is obtained between the life span of the DCM patient-2, the MELAS and FICM patients who have the *mit* $^-$ + *syn* $^-$ type of mutation and the total number of base-substitution in their mtDNA (Fig. 4).

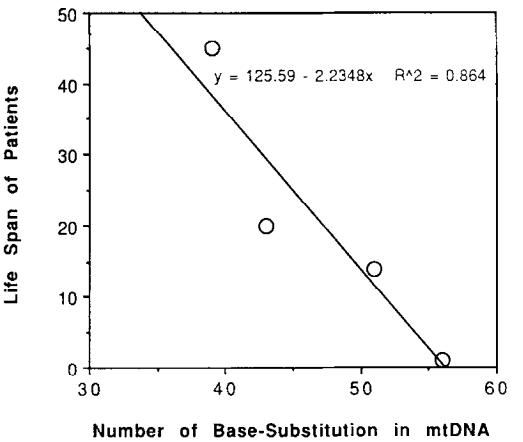
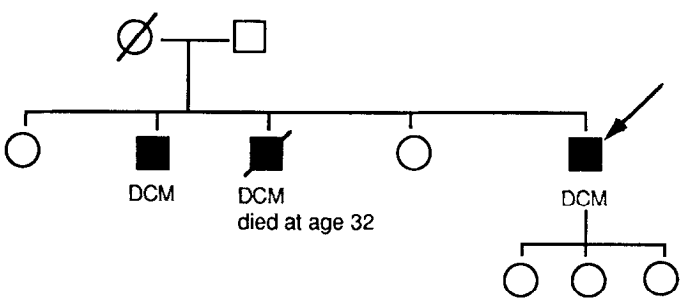


Fig. 4. An inverse relation between the life span of the DCM patient-2, MELAS, and FICM patients and the total numbers of base-substitutions.
The life span of each patient is in the text, and the total numbers of base-substitutions are illustrated in Fig. 1 and 2.

Discussion

Fig. 1 clearly indicates that, although these patients are resident in different districts and not related, they belong to the same mitochondrial DNA gene family, diverged from a common ancestor. The molecular genetic lineage could be nominated as "mtDNA disease". From the family history of the DCM patient-2 as shown below, he was admitted to a hospital under suspicion of familial cardiomyopathy caused by nuclear gene mutation.



However, the phylogeny of his mtDNA mutations presents an unequivocal evidence that he belongs to the lineage of "mtDNA disease". This tree also explains the partly overlapped clinical symptoms and therapies among the patients: for example, the HCM patient-1 had lactic acidosis, short stature, slight mental retardation, and perceptive deafness which are characteristic symptoms of the MELAS, and conversly the MELAS patient-1 had cardiac failure and the MELAS patient-2 had HCM and died from heart failure. Dopamine therapy is common to the end stage of the patients with PD and DCM.

As the deletion of mtDNA found in the PD patients (2) or one of the multiple deletions in the idiopathic cardiomyopathy patients (4) accumulates with age even in the normal controls, we

consider PD as premature ageing of the brain (2) and the deletion as a contributory factor to "presbycardia" (5). However, relative amount of the deleted mtDNA to the normal mtDNA in the PD patients was at least ten times larger than that in the normal (3). As in the case of yeast (6), *mit* $^-$ mutations in the patients would very likely accelerate ρ^- mutations. In the D-loop region of mtDNA, the patients examined had no mutations among recently reported *cis*-elements (12) which are essential for coordinate expression of mitochondrial and nuclear genes. However, the two insertions in the conserved sequence block II (10) in the D-loop at nt311.1 and 303.1 in the C-2 and one transition in the C-4' at nt152 near the conserved sequence block I (10) may also contribute to deletions during the replication of mtDNA by altering the secondary structure of the D-loop region (Fig. 3), leading to the premature ageing of parkinsonian neurons or cardiomyopathic myocardium as compared with the normal individual without the *pms*.

The increase in the total number of the base-substitution which leads to instability of mtDNA seems to have an inverse relation to the patients' life span. An inverse relation with a high correlation coefficient is obtained between the life span of the DCM patient-2, the MELAS and FICM patients having the *mit* $^-$ + *syn* $^-$ type of mutation and the total number of the base-substitution in their mtDNA (Fig. 4). This figure indicates that a man could survive up to his age of 125, like the Old Par, if he has no mutation comparing with the Cambridge sequence, namely the Hela cell's sequence (8). In the previous paper, we noticed the three *mit* $^-$ transversions in C-1 at nt14199, 14272, and at 14368 in the ND6 gene, especially an A-to-G transition at nt10398 in the ND3 gene converting the conserved threonine to alanine. Because, they could be equivalent to the inhibition of Complex I by rotenone or 1-methyl-4-phenylpyridinium ion (MPP^+) causing experimental parkinsonism and promoting the production of active oxygen (13). It is a well known fact (14) that the repetitive administration of doxorubicin, a potent promoter of the free radical formation, produces a dose related cardiomyopathy in man or rabbit (Adriamycin cardiomyopathy). With active oxygen, we have observed (15) massive conversion of guanine residue to 8-hydroxy-guanine both *in vitro* using damaged submitochondrial particles and *in vivo* by oral administration to mouse of azidothymidine (AZT). Thus, the *mit* $^-$ mutations in the "mtDNA disease" lineage shown in Fig. 1 could be considered as *built-in* toxins in mtDNA leading to the continuous production of active oxygen. The data presented here suggest that not one particular mutation, but the type and the total number of mutations of a patient is an indispensable factor for the disease. Three *mit* $^-$ mutations at nt8701, 14199, and 10398 in the reported partial sequence of the American Leber's patient with disease (one of the ME sub-group) patient also exist in C-1, C-1, and C-3, respectively, of the patients reported here. This can hardly be considered as a mere coincidence, but rather further evidence for existence of the lineage among human races.

The data reported here could provide an important clue to explain progressive and distinct but partly overlapping clinical symptoms and pathological findings among DCM, HCM, PD and ME on molecular biological basis, and to predict the patient's natural term of life. For the patient with the idiopathic cardiomyopathy having *built-in* toxins in mtDNA as reported here, the developement of gene therapy rather than heart transplantation would be a matter of urgency in near future. Also, this information presented here will provide a practical method for diagnosing

patients with symptoms as well as "at-risk" individuals by the restriction-enzyme digestion surveys of mtDNA mutations in blood cells.

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