Mitochondrial 5178A/C Genotype is Associated With Acute Myocardial Infarction

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A single nucleotide polymorphism of mitochondrial 5178A/C, causing a Met to Leu replacement within the NADH dehydrogenase subunit, is reported to be associated with longevity. The purpose of the present study was to assess the contribution of mitochondrial polymorphisms, particularly the 5178A/C genotype, to the susceptibility to acute myocardial infarction (AMI) in a Japanese study population. There were 4 groups: 150 patients with AMI, 150 with essential hypertension, 100 with diabetes mellitus, and 150 subjects matched for age and sex who served as the control group. Mitochondrial 5178A/C was detected by the polymerase chain reaction restriction fragment length polymorphism method. The allelic frequency of 5178C was significantly higher in the AMI group than in the control group, and this difference was more marked in younger patients. There were differences in allelic frequencies among the essential hypertension group, diabetes mellitus group and control group, but a higher frequency of the C allele was seen in the AMI group compared with the essential hypertension and diabetes mellitus groups. This particular polymorphism was found to be associated with development of AMI, especially in younger patients and constitutes a new risk factor for AMI. (Circ J 2003; 67: 16–20)

Key Words: Acute myocardial infarction; Coronary risk factor; Mitochondria; Polymorphism

Cardiovascular diseases are thought to result from the interaction of multiple environmental and genetic factors. The ECTIM study showed that the ACE DD genotype was more frequent in patients with myocardial infarction than in control subjects; and Tiret et al also showed that the association between the ACE DD genotype and myocardial infarction was increased in a subset of patients who also carried the angiotensin II type 1 C allele. Other studies have confirmed the association of the ACE D allele and angiotensin II type 1 receptor C allele with an increased risk of myocardial infarction; and furthermore, there have been several reports of the association of some nuclear genes with coronary heart diseases in Japanese subjects. However, few have addressed the association between polymorphisms of the mitochondria (mt) and coronary heart disease.

Human mitochondrial DNA (mtDNA) consists of 16,569 base pairs (bp) located on the circular double strand of the H- and L-chains: 13 types of proteins, 22 types of transfer RNA (tRNA) and 2 types of liposome RNAs are densely coded in a region other than the non-coding region of approximately 1 kb. The mtDNA is highly polymorphic and controls the oxidative phosphorylation system regulating the oxygen metabolism.

Tanaka et al reported a mutation specifically seen in Japanese subjects at nucleotide 5178 within the NADH dehydrogenase in the mitochondrial electron transport system Complex 1; mitochondrial 5178A/C is involved in a Met to Leu substitution. According to them, the ratio of adenine (A) is approximately half that in healthy people and Japanese centenarians mostly carry A at 5178, which is rarely seen in Europeans or non-Japanese Asians. Based on their findings, they suggested that the high ratio of 5178A may be specific to the Japanese population and possibly related to the fact that the life expectancy at birth in Japan is among the highest in the world.

Maternally transmitted mitochondrial genotypes may influence oxidative damage and 5178A/C may be relevant to common diseases. The aim of the present research was to examine the association between genetic factors and the incidence of myocardial infarction. In particular, we focused on the association between mitochondrial genotypes and coronary heart disease; in order to demonstrate a contribution of mitochondrial single nucleotide polymorphisms (SNPs) to the susceptibility to acute myocardial infarction (AMI), we investigated the association of mitochondrial 5178A/C polymorphism with the occurrence of AMI.

Methods

Study Population

Participants were randomly selected inpatients and outpatients treated at the University Hospital and affiliated hospitals in Tokyo, Japan. We divided the study subjects, all of them Japanese, into 4 groups.

(1) AMI group, which consisted of 150 patients (102 men, mean age: 64 years old) who underwent coronary angiography at our University Hospital from 1997 to 1999. AMI was diagnosed from clinical evidence of cardiac attack, electrocardiographic findings, and serum cardiac enzyme concentrations. Reliable information from medical records obtained before the admission related to AMI was evaluated for the presence of 3 conventional coronary risk factors (ie, hypertension, diabetes mellitus and hypercholesterolemia) and the patients were divided into 3 sub-
groups on the basis of the presence or absence of the risk factor: (i) hypertension, (ii) diabetes mellitus or (iii) hypercholesterolemia. Hypertension was found in 46%, diabetes mellitus in 32%, and hypercholesterolemia in 31% of these patients.

(2) The hypertension group consisted of 150 outpatients attending the University hospital, affiliated hospitals or medical examination center because of essential hypertension (102 men, mean age: 63 years old). The patients were diagnosed as having hypertension according to the JNC VI guidelines (systolic blood pressure >140 mmHg, or diastolic blood pressure >90 mmHg) and had been on antihypertensive drugs for more than 1 year. The patients had no clinical or biological signs of secondary hypertension, coronary heart disease, diabetes mellitus or hyperlipidemia.

(3) The diabetes mellitus group consisted of 100 outpatients attending the University hospital, affiliated hospitals or medical examination center because of diabetes mellitus (68 men, mean age: 63 years old). The diagnosis was established according to the World Health Organization criteria and the patients had been on oral hypoglycemic agents or the subjects were free of any abnormality. The control group did not have evidence of coronary heart disease, hypertension, hyperlipidemia or diabetes mellitus.

All subjects gave their informed consent to participate in the study, and the study protocol was approved by the University Ethics Committee.

Table 1 Matching of the Patients With Acute Myocardial Infarction (AMI) and the Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>AMI</th>
<th>Control</th>
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<tbody>
<tr>
<td>No. (M/F)</td>
<td>150 (102/48)</td>
<td>150 (102/48)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64±8</td>
<td>64±10</td>
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<tr>
<td>p value</td>
<td>NS</td>
<td></td>
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</table>

Age is expressed as mean±standard deviation, and comparison was performed by ANOVA.

Table 2 Clinical Characteristics of the Hypertension, Diabetes Mellitus and Control Group

<table>
<thead>
<tr>
<th></th>
<th>Hypertension</th>
<th>Diabetes mellitus</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (M/F)</td>
<td>150 (102/48)</td>
<td>100 (68/32)</td>
<td>150 (102/48)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63±8</td>
<td>63±8</td>
<td>64±10</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2±1.1*</td>
<td>25.5±2.2*</td>
<td>21.2±2.2</td>
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<td>SBP (mmHg)</td>
<td>148±10*</td>
<td>132±12*</td>
<td>120±8</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>92±10*</td>
<td>86±11*</td>
<td>78±8</td>
</tr>
<tr>
<td>T. Chol (mmol/L)</td>
<td>212±24*</td>
<td>210±21*</td>
<td>190±11</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>47±12*</td>
<td>42±12*</td>
<td>60±12</td>
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<tr>
<td>TG (g/L)</td>
<td>164±56*</td>
<td>165±34*</td>
<td>92±14</td>
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<tr>
<td>FPG (mmol/L)</td>
<td>108±18*</td>
<td>128±20*</td>
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<tr>
<td>UA (μmol/L)</td>
<td>6.4±1.2*</td>
<td>6.5±1.9*</td>
<td>4.9±1.3</td>
</tr>
</tbody>
</table>

Date are expressed as mean±standard deviation, and comparison was performed by ANOVA.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; T. Chol, total cholesterol; HDL-C, high density lipoprotein-cholesterol; TG, triglycerides; FPG, fasting plasma glucose; UA, uric acid.

*p<0.001 Hypertension vs Control, #p<0.001 Diabetes mellitus vs Control.

Results

The patients’ clinical background data are shown in Table 2. In samples obtained from 550 unrelated Japanese individuals, PCR-RFLP electrophoresis on 2% agarose gels disclosed 2 genotypes for mitochondrial 5178A/C polymorphism and Table 3 shows the distributions of the allelic frequency of 5178A/C polymorphism in the AMI and control groups. The allelic frequency was in Hardy-Weinberg equilibrium. The frequency of 5178C was 65% in the AMI group and 53% in the control group (odds ratio (OR) 1.69; 95% confidence interval (CI) 1.06–2.69, p=0.03). These
data indicated that the mt5178C allele was associated with the occurrence of AMI. There are reports of mitochondrial 5178A/C polymorphism and coronary heart disease in Japanese subjects (Jpn Circ J vol. 63 supplementary, 1999), and the same tendency was seen in the present AMI group. However, there were no significant differences among the groups for the distribution of the allelic frequency of 5178A/C by gender (data not shown).

Furthermore, to examine whether the mitochondrial genotype was associated with acceleration of atherosclerosis and the occurrence of AMI, we evaluated the relationship between mitochondrial genotype and the complications of AMI. Table 4 shows the distribution of the allelic frequency of 5178A/C in the AMI patients with/without the 3 conventional risk factors for AMI. The difference was not statistically significant, but 5178C was observed more frequently in AMI patients with hypertension, diabetes mellitus or hypercholesterolemia than in those without these diseases. These findings indicate that 5178/C may be associated with the development of AMI.

Table 5 shows the allelic frequency of mitochondrial 5178A/C in AMI subjects distributed by age: it was observed more frequently in AMI patients younger than 50 years old than in their older counterparts [OR 2.57 (95% CI 1.04–6.38, p=0.03)]. The age at onset of AMI was significantly lower in patients with mt5178C than in patients with mt5178A.

Table 6 shows the allelic frequency of mt 5178A/C polymorphism in the AMI, hypertension, diabetes mellitus, and control groups. The frequency of mt5178C was 65% in the AMI group, 61% in the hypertension group, 60% in the diabetes mellitus group, and 53% in the control group. The difference among the groups was not statistically significant, but there was a higher frequency of the C allele in pa-
Parkinson’s disease.24 There have been a few studies nase subunit genes have been reported in patients with Mitochondrial Polymorphism in AMI

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with hypertension or diabetes mellitus. The mitochondria have own genetic information (mtDNA) and protein synthesis system. Human mtDNA, which consists of 16,569bp and is a maternal inheritance, is an extremely small genome compared with the chromosomal DNA of the nucleus, which consists of approximately 3×10^6 bp. In the long biological evolution process, most of the components were entrusted to the nuclear DNA, and the mitochondria have only the genes encoding for 13 proteins, 2 liposome RNAs and 22 transfer RNAs (tRNA). Therefore, most of the mitochondrial proteins are synthesized by the cytosol as products of nuclear DNA that then transmigrate to the mitochondria. The mitochondria execute DNA duplication and transcription and protein synthesis in collaboration with nuclear DNA, as well as often acting independently. The enzymes involved in these processes are mostly encoded by nuclear DNA and are strongly regulated by the nucleus.

The electron transfer system operating inside the mitochondria supplies most of the biogenic energies by mediation of oxidative phosphorylation. Complex I provides the main entry point for electrons to the electron transport chain. The mtDNA is a naked circular double-stranded DNA that does not have any chromatin structure protected by histones, unlike the chromosomal DNA of the nucleus, and has an insufficient mechanism for repairing the DNA; therefore, mtDNA is characterized by a high mutation rate (5- to 10-fold that of nuclear DNA) associated with a susceptibility to damage by active oxygen species and absence of the proofreading function of a DNA polymerase in charge of duplication of mtDNA. Variations of the mtDNA cause lipid peroxidation of the oxidative phosphorylation system, which results in the facilitation of the leakage of active oxygen species in a vicious circle. There is a possibility that the genotype of the mitochondria may determine the oxidative injury of mtDNA, cellular injury and may even affect the life span of an individual.

Tawata et al reported that mtDNA mutations at nucleotides 1310, 1026, and 14577 are associated with type 2 diabetes.22,23 and several SNPs in the NADH dehydrogenase subunit genes have been reported in patients with Parkinson’s disease.24 There have been a few studies addressing the associations between mitochondrial polymorphism and diseases.25-29 but further studies are needed to clarify the role of the mitochondrial genotype in common diseases. The Framingham longevity study of coronary heart disease has indicated that longevity is more strongly associated with age of maternal death than that of paternal death.30 Among all these variations, we focused on 2 nucleotide substitutions of mt5178A/C, causing a Met-Leu replacement in the NADH dehydrogenase subunit in Complex I, showing to be significantly more frequent in centenarians. It has been previously reported that at the onset of AMI, the activity of Complex I of the electron transport system first declines and then induces irreversible ischemic cellular injury.31,32 Furthermore, Cann et al reported in their study of human evolution that only 5 Asians and 1 European of the 147 samples collected from all over the world had mt5178A.33 Another study also reported that this observation indicates that mt5178A is relatively rare in human populations around the world.34,35

We found significant differences in the allelic frequencies between the present AMI and control subjects, and the difference was more significant in younger patients in the AMI group. As shown in Table 6, although there was no statistically significant difference, 5178C tended to be present more frequently in patients with hypertension or diabetes mellitus compared with controls; our data also suggest an association of the 5178C allele with the occurrence of essential hypertension and diabetes mellitus, but the allele was more associated with AMI than hypertension or diabetes mellitus. Our results indicate that the mitochondrial genotype may be associated with the occurrence of essential hypertension and diabetes mellitus, in addition to accelerating the progression of atherosclerosis and the occurrence of AMI. The mt5178C allele may influence the age of onset of AMI through deterioration of mitochondrial function. The progression of atherogenesis should be different between individuals with different mitochondrial genotypes.

It is not clear how mt5178A/C polymorphism is related to AMI. It is possible that mitochondria harboring the 5178C allele are rather fragile and more easily damaged because the resultant amino acid change (Met to Leu) in the second portion of the NADH dehydrogenase subunit may subject the mitochondrial DNA to increased oxidative stress and new mutations therefore occur more frequently. It is also possible that the mt5178C allele is linked to another undefined mutation that confers true susceptibility to AMI or it may also interact with factors coded by nuclear DNA. Further elucidation of the interrelation between genetic polymorphism and oxidative stress should lead to an understanding of the physiological mechanism of AMI. ATP productivity or sensitivity to oxidative stress may differ between 5178A and 5178C genotypes.35 Levine et al have reported that methionine (Met) residues constitute an important antioxidant defense mechanism.36,37 and 5178A (Met) that might protect the mitochondria against oxidative damage resulting from the amino acid change (Met to Leu) related to the 5178A/C polymorphism.15,36,37 Thus, the mitochondrial genotypes associated with longevity have an anti-atherogenic effect. Mitochondrial DNA thus influences the susceptibility of individuals to various diseases.

In the mitochondria, the ratio of variant versus non-variant DNA (heterogeneity) may differ in every organ or tissue, changing under certain conditions. When the number of variant DNA exceed a certain range limit, functional disorders may be induced. For the diagnosis of mitochondrial alterations, genetic diagnosis of the organ with the functional disorder is the most conclusive method, and in the case of myocardial infarction, the cardiac muscle and coronary vessels can be used. However, this procedure, being highly invasive, is practically very difficult and can not be used for screening. Therefore, blood has to be tested, but even when a mitochondrial aberration is detected, no dysfunction will be detected if the amount of variant DNA is below the threshold. However, the presence of mitochondrial aberrations in the blood may mean possible alterations in the target organ, and because the amount of variant DNA may exceed the limit and cause dysfunction of the organ in
the future, blood screening may be meaningful.

In conclusion, 5178C may promote the occurrence of AMI, and this polymorphism appears to be a new coronary risk factor. Extensive studies on mitochondrial SNPs and their contribution to various common diseases will provide useful diagnostic or predictive information.

References


