

ORIGINAL ARTICLE

Longevity-associated mitochondrial DNA 5178 A/C polymorphism and blood pressure in the Japanese population

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It has been reported that the mitochondrial DNA 5178 adenine/cytosine (mt5178 A/C) polymorphism, also called NADH dehydrogenase subunit 2-237 methionine/leucine (ND2-237 Met/Leu) polymorphism, may be associated with longevity in Japanese individuals, and that the mt5178A genotype may have an antiatherogenic influence. To determine whether mt5178 A/C polymorphism influences blood pressure, we genotyped 412 healthy Japanese individuals and performed a cross-sectional study investigating the relationship between genotype and blood pressure. In women with mt5178A, the mean diastolic blood pressure was higher than in those with mt5178C by 3.2 mmHg ($P=0.040$). In men, no statistically significant difference in systolic or diastolic blood pressure was observed between mt5178 A/C genotypes. However, a significant correlation between

mt5178 A/C genotypes and the effects of habitual drinking on blood pressure was found. After adjustment for several factors, in men carrying mt5178C, both systolic and diastolic blood pressure were significantly higher in daily drinkers than in occasional ($P=0.002$ and 0.002 , respectively) as well as nondrinkers ($P<0.001$ and 0.001 , respectively), whereas in men carrying mt5178A, no significant differences in blood pressure were detected, irrespective of alcohol consumption. These results suggest that mt5178 A/C (=ND2-237 Met/Leu) polymorphism may influence both diastolic blood pressure in Japanese women and the blood-pressure-increasing effect of drinking in Japanese men.

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Introduction

Recent genetic epidemiological studies have shown that some mitochondrial DNA genotypes are associated with longevity in Japanese¹ and in Europeans.^{2–5} One of these is mitochondrial DNA 5178 adenine/cytosine (mt5178 A/C) polymorphism, which is also known as NADH dehydrogenase subunit 2–237 methionine/leucine (ND2-237 Met/Leu) polymorphism.¹ Tanaka *et al*¹ showed that the frequency of mt5178A was significantly higher in Japanese centenarians than in the general population. Moreover, they showed that individuals with

the mt5178C genotype were more susceptible to age-related diseases than those with mt5178A. However, the mechanisms by which mt5178 A/C polymorphism affects the longevity or occurrence of age-related diseases have yet to be elucidated. Tanaka *et al* speculated that the mt5178A genotype is likely to confer resistance to adult-onset diseases by suppressing obesity and atherosclerosis.⁶ In fact, mt5178A was reported to exert antiatherogenic effects.^{7–9} We reported that serum high-density lipoprotein cholesterol levels were significantly higher in men carrying mt5178A than in men carrying mt5178C, and that triglyceride levels were significantly lower in women carrying mt5178A than in women carrying mt5178C.⁷ Blood pressure and serum lipid levels are crucial factors in the pathology of age-related circulatory diseases. Therefore, in this study, to investigate whether mt5178 A/C (=ND2-237 Met/Leu) polymorphism is associated with blood pressure, we genotyped 412 middle-aged Japanese

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individuals and conducted a cross-sectional study using the results of medical health check-ups.

Subjects and methods

Subjects

In total, 602 volunteers were recruited from individuals visiting the Mito Red Cross Hospital for medical check-ups. The study was performed according to the provisions of the Declaration of Helsinki of 1975, and written informed consent was obtained from volunteers before participation. The study protocol was approved by the Ethical Committee of Kyorin University. In total, 79 volunteers were excluded from analysis due to lack of data, and 111 were excluded for taking antihypertensive, antihyperlipidaemic or antidiabetic medications. Therefore, subjects were 412 Japanese individuals (321 men, 91 women; mean age \pm s.d., 53.6 ± 7.8 years).

Clinical characteristics of subjects

The determination of blood chemical levels and physical data was conducted as described previously.⁷ Briefly, blood chemical levels were measured using routine methods at Mito Red Cross Hospital. For both systolic blood pressure (SBP) and diastolic blood pressure (DBP), the averages of two values consecutively measured by physicians were used for the present analyses. The body mass index (BMI) was defined as the ratio of subject weight (kg) to the square of subject height (m). A survey of drinking and smoking habits was performed by means of a questionnaire. Habitual drinking was classified based on drinking frequency (daily drinkers; occasional drinkers, who drink 'several times per week'; and non- or ex-drinkers, including those who drink 'a few times per month'). With regard to smoking, subjects were classified as non- or ex-smokers and current smokers.

Genotyping

The method used for genotyping mt5178 A/C polymorphism has been described previously.⁷ Briefly, DNA was isolated from white blood cells and polymerase chain reaction-restriction fragment length polymorphism analysis using the restriction enzyme *AluI* was performed. DNA lacking the *AluI* site was designated as mt5178A, while that possessing this restriction enzyme cutting site was designated as mt5178C.

Statistical analyses

Statistical analyses were performed using SAS statistical software, version 6.12 (1997). The interactions between mt5178 A/C genotypes and habitual drinking or smoking were assessed using analysis of covariance (ANCOVA) with mt5178 A/C polymorphism (mt5178A = 0; mt5178C = 1) habitual drinking (non or ex-drinkers = 0; occasional drinkers = 1; daily drinkers = 2), habitual smoking (non- or ex-smokers = 0; current smokers = 1), two interaction terms (mt5178 A/C \times habitual drinking, mt5178 A/C \times habitual smoking), age and BMI in the model. ANCOVA was also used to adjust SBP and DBP for age, BMI, and habitual smoking in six groups (non- or ex-drinkers/occasional drinkers/daily drinkers having mt5178 A/C genotype). *P*-values of less than or equal to 0.05 were considered to be statistically significant.

Results

The clinical profiles of the male and female subjects are summarized in Table 1. The frequency of the mt5178A genotype was 41.0% (169/412).

In men, no significant difference in SBP or DBP was found between the mt5178A and mt5178C genotypes (Table 2). However, in women with mt5178A, the mean DBP was higher than in those with mt5178C by 3.2 mmHg ($P = 0.040$; Table 2).

Table 1 Clinical features of study subjects

Characteristic	Men (n=321)	Women (n=91)	P-value
Frequency of mt5178A (%)	39.9 (128/321)	45.1 (41/91)	
Age (years)	53.01 \pm 0.43	55.82 \pm 0.81	0.002
Habitual drinkers ^a (daily drinkers) (%)	66.04 (43.61)	19.78 (10.99)	<0.001 (<0.001)
Current smokers (%)	43.61	3.30	<0.001
BMI (kg/m ²)	23.24 \pm 0.15	21.87 \pm 0.29	<0.001
SBP (mmHg)	123.26 \pm 0.80	119.00 \pm 1.50	0.013
DBP (mmHg)	72.38 \pm 0.51	68.46 \pm 0.96	<0.001
Total cholesterol (mmol/dl)	5.269 \pm 0.049	5.649 \pm 0.092	<0.001
HDL cholesterol (mmol/dl)	1.446 \pm 0.022	1.694 \pm 0.042	<0.001
Triglyceride (mmol/dl)	1.464 \pm 0.038	1.098 \pm 0.071	<0.001

For habitual drinkers (%), daily drinkers (%), and current smokers (%), *P*-values were calculated using the χ^2 test. Age, BMI, SBP, DBP, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride are given as mean \pm s.e. of mean. All *P*-Values show the differences between males and females.

^aHabitual drinkers were defined as those consuming alcohol one or more times per week.

Table 2 Blood pressure in men and women by mt5178A/C genotype

	mt5178A	mt5178C	P-value
<i>Men</i>	<i>n</i> =128	<i>n</i> =193	
SBP (mmHg)	123.41 ± 1.28	123.16 ± 1.04	0.876
DBP (mmHg)	72.61 ± 0.85	72.23 ± 0.69	0.732
Habitual drinkers ^a (daily drinkers)(%)	68.75 (46.09)	64.25 (41.97)	0.404 (0.466)
Current smokers (%)	40.63	45.60	0.379
<i>Women</i>	<i>n</i> =41	<i>n</i> =50	
SBP (mmHg)	121.00 ± 2.14	117.36 ± 1.93	0.210
DBP (mmHg)	70.24 ± 1.15	67.00 ± 1.04	0.040
Habitual drinkers ^a (daily drinkers) (%)	14.63 (7.32)	24.00 (14.00)	0.264 (0.310)
Current smokers (%)	2.44	4.00	0.678

For habitual drinkers (%), daily drinkers (%), and current smokers (%), *P*-values were calculated by using the χ^2 test. SBP and DBP are given as mean ± s.e. of mean. *P*-values show the differences between mt5178A and mt5178C.^aHabitual drinkers were defined as those consuming alcohol one or more times per week.

Table 3 Analysis of covariance for blood pressure in male subjects

Variables	Systolic blood pressure		Diastolic blood pressure	
	F-value	P-value	F-value	P-value
Mt5178A/C	0.03	0.870	0.01	0.917
Habitual drinking	7.45	<0.001	6.09	0.025
Habitual smoking	3.48	0.063	6.68	0.010
Mt5178A/C × habitual drinking ^a	3.41	0.034	1.93	0.147
Mt5178A/C × habitual smoking ^b	0.07	0.785	0.67	0.413
Age (years)	9.62	0.002	2.83	0.093
Body mass index (kg/m ²)	24.24	<0.001	37.32	<0.001
	Model <i>R</i> ² (adjusted)=0.171		Model <i>R</i> ² (adjusted)=0.184	

In analysis of covariance, some independent variables are numerically coded: mt5178A/C genotypes (mt5178A=0; mt5178C=1), habitual drinking (non- or ex-drinkers=0; occasional drinkers=1, daily drinkers=2), habitual smoking (non- or ex-smokers=0; current smokers=1).

^aMt5178A/C × drinking habit represents the interaction between mt5178A/C genotypes and habitual drinking.

^bMt5178A/C × smoking habit represents the interaction between mt5178A/C genotypes and habitual smoking.

After adjusting for age, BMI, habitual drinking, and habitual smoking, this difference remained present (*P* = 0.022) (data not shown).

ANCOVA revealed a correlation between mt5178 A/C polymorphism and the effect of habitual drinking on SBP (*P* = 0.034) (Table 3). Moreover, habitual drinking (*P* < 0.001), age (*P* = 0.002), and BMI (*P* < 0.001) significantly influenced SBP, while habitual drinking (*P* = 0.025), habitual smoking (*P* = 0.010), and BMI (*P* < 0.001) significantly influenced DBP. However, a correlation between mt5178 A/C genotype and the effect of habitual drinking on DBP was not observed.

In men, SBP was significantly higher in daily drinkers with mt5178C than in nondrinkers with mt5178C (*P* < 0.001), occasional drinkers with mt5178C (*P* = 0.002), nondrinkers with mt5178A (*P* = 0.020), and daily drinkers with mt5178A (*P* = 0.040). SBP was significantly higher in daily drinkers with mt5178A than in nondrinkers with mt5178C (*P* = 0.047; Table 4), and DBP was significantly higher in daily drinkers with mt5178C than in nondrinkers with mt5178C (*P* < 0.001), and occasional drinkers with mt5178C (*P* = 0.002). However, no significant differences in blood pressure between

drinkers and nondrinkers were observed in men with mt5178A.

Discussion

In the present study, we found that mt5178 A/C polymorphism may be associated with DBP in Japanese women, but that mt5178 A/C polymorphism is not associated with blood pressure in Japanese men (Table 2). However, the results also indicate that this polymorphism may be indirectly related to SBP and DBP through alcohol consumption in middle-aged Japanese men (Tables 3 and 4). After adjusting for several factors, SBP and DBP were found to be significantly higher in daily drinkers with mt5178C than in occasional or nondrinkers with mt5178C (Table 4), whereas in men with mt5178A, no significant difference in blood pressure between daily drinkers and occasional or nondrinkers was present (Table 4). The results of ANCOVA (Table 3) are strongly indicative of a gene-environmental interaction between mt5178 A/C polymorphism and habitual drinking, suggesting that the effects of habitual drinking on blood

Table 4 Interaction between mt5178 A/C polymorphism and drinking frequency on blood pressure in male subjects

	mt5178A			mt5178C		
	Nondrinkers ^a n=40	Occasional drinkers ^b n=29	Daily drinkers n=59	Non-drinkers ^a n=69	Occasional drinkers ^b n=43	Daily drinkers n=81
SBP (mmHg)	122.23 ± 2.13*	123.10 ± 2.49	123.54 ± 1.77* [†]	118.71 ± 1.62***	120.28 ± 2.06**	128.31 ± 1.48
DBP (mmHg)	72.92 ± 1.17	71.63 ± 1.64	71.95 ± 1.40	70.11 ± 1.07**	69.94 ± 1.36**	75.12 ± 0.98

SBP and DBP are given as least-square mean ± s.e. of mean adjusted for age, BMI, mt5178 A/C polymorphism, and habitual smoking.

^aNondrinkers were defined as those who consume alcohol less than one time per week.

^bOccasional drinkers were defined as those who consume alcohol one or more times per week, but not daily.

* $P < 0.05$ vs daily drinkers with mt5178C, ** $P < 0.005$ vs daily drinkers with mt5178C, *** $P < 0.0005$ vs daily drinkers with mt5178C, [†] $P < 0.05$ vs nondrinkers with mt5178C.

pressure may be influenced by mt5178 A/C (=ND2-237 Met/Leu) polymorphism in Japanese men.

The observed frequency of the mt5178A do not significantly differ from values reported previously,^{1,9–12} thus suggesting that there is no genetic bias in the subjects of this study.

The most important modifiable environmental risk factors for hypertension are habitual alcohol intake, high salt intake, obesity, and low physical activity.¹³ A large-scale epidemiological study in Japan demonstrated an association between alcohol consumption and high blood pressure.¹⁴ A significant relationship between habitual drinking and onset of hypertension has also been reported.^{15,16} The possible mechanism by which mt5178 A/C polymorphism influences the effects of alcohol on blood pressure in middle-aged Japanese men is unclear. The substitution of adenine with cytosine at mt5178 results in a deduced amino-acid change from methionine to leucine at residue 237 of NADH dehydrogenase subunit 2. This amino-acid change might bring about a functional change in NADH dehydrogenase, which is involved in ethanol-related production of reactive oxygen species.¹⁷ Superoxide radicals in and around vascular endothelial cells play a critical role in the pathogenesis of hypertension.¹⁸ Therefore, mt5178 A/C (=ND2-237 Met/Leu) polymorphism may affect alcohol-related elevation of blood pressure via oxyradicals. Moreover, mitochondrial function has recently been reported to influence blood pressure,^{19–21} and taken together with the present results, this demonstrates the necessity of further study into the relationship between blood pressure and mitochondrial function.

Individuals with mt5178C may be more susceptible to adult-onset diseases, such as myocardial infarction,⁹ cerebrovascular disorders,¹² and diabetes,¹¹ than those with mt5178A. In healthy middle-aged Japanese, high-density lipoprotein cholesterol levels are significantly lower in men with mt5178C than in those with mt5178A.⁷ In type-II diabetic Japanese, the intima-media thickness in the bilateral carotid arteries is significantly larger in the mt5178C group than in the mt5178A group.⁸ Moreover, Wang *et al*¹¹ reported that the mean age at onset

of diabetes was significantly lower in Japanese patients carrying mt5178C than in those carrying mt5178A. In addition to these metabolic differences, mt5178 A/C polymorphism may partially influence life expectancy by influencing the blood-pressure-elevating effects of alcohol consumption. We also reported that triglyceride concentration was significantly higher in women with mt5178C than in those with mt5178A.⁷ However, in this study, we found that DBP was significantly higher in women with mt5178A than in those with mt5178C. This finding indicates that the longevity-associated mitochondrial genotype is also associated with higher blood pressure in women. As triglyceride levels and DBP are risk factors for cardiovascular and cerebrovascular diseases, the observations suggest an apparent contradiction to previous reports regarding mt5178A/C polymorphism. Therefore, further investigation is required.

A potential limitation in the present study was the classification of drinkers/nondrinkers based on the frequency of alcohol consumption rather than by the volume consumed. Regrettably, information regarding the volume of alcohol consumption was not available. Therefore, whether the effects of mt5178 A/C are dose dependent with regard to alcohol consumption warrants further investigations. Moreover, as there were insufficient women who drank alcohol, whether there is the interaction of the mt5178 A/C genotypes and habitual drinking on blood pressure also warrants further studies. Another limitation of our study was that salt intake was not considered. The intake of sodium is reported to be a crucial risk factor for hypertension in Japanese.²² Whether mt5178 A/C polymorphism similarly influences the effects of salt on blood pressure also warrants further research.

In conclusion, our study found that mt5178 A/C (=ND2-237 Met/Leu) polymorphism may be associated with DBP in middle-aged Japanese women, and may influence the effects of habitual drinking, particularly daily drinking, on blood pressure in middle-aged Japanese men. We also found that DBP was significantly higher in women with mt5178A than in those with mt5178C. In men with mt5178C, both SBP and DBP were significantly higher in daily

drinkers with mt5178C than in occasional or non-drinkers with mt5178C. However, in men carrying mt5178A, there were no significant differences in blood pressure among daily drinkers, occasional drinkers, and nondrinkers. As the mt5178C genotype is overwhelmingly dominant all over the world,²³ our findings may contribute valuable genetic data for the international prevention and treatment of hypertension.

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