Original Article

NADH Dehydrogenase Subunit-2 237 Leu/Met Polymorphism Modifies the Effects of Alcohol Consumption on Risk for Hypertension in Middle-Aged Japanese Men

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NADH dehydrogenase subunit-2 237 leucine/methionine (ND2-237 Leu/Met) polymorphism is associated with longevity in the Japanese population, and the ND2-237Met genotype may exert antiatherogenic effects. To investigate whether ND2-237 Leu/Met polymorphism is associated with risk of hypertension, we conducted a cross-sectional study of 398 Japanese male subjects. The frequency of hypertension was significantly higher in ND2-237Leu genotypic men than in ND2-237Met genotypic men. On analysis of covariance, the interaction between ND2-237 Leu/Met polymorphism and habitual drinking was significantly associated with both systolic blood pressure and diastolic blood pressure. Multiple logistic regression analysis revealed that the ND2-237Met genotype, particularly in younger subjects (age <60 years), had a lower odds ratio for hypertension than the ND2-237Leu genotype. Moreover, the association of ND2-237 Leu/Met polymorphism with hypertension may depend on the frequency of alcohol consumption. The odds ratio for hypertension was significantly higher in daily drinkers with ND2-237Leu when compared with non- or ex-drinkers with ND2-237Leu. However, the association between the ND2-237Met genotype and hypertension may not depend on the frequency of alcohol consumption. The present results suggest that ND2-237 Leu/Met polymorphism is associated with hypertension and that modification of hypertension risk is dependent on alcohol consumption in middle-aged Japanese men. (*Hypertens Res* 2007; 30: 213–218)

***Key Words*: hypertension, NADH dehydrogenase, habitual drinking, longevity, personalized preventive medicine**

Introduction

Prevention of hypertension is a crucial factor in successful aging and longevity (*1*, *2*). Mitochondrial DNA cytosine/adenine (Mt5178 C/A) polymorphism, also known as NADH dehydrogenase subunit-2 237 leucine/methionine (ND2-237 Leu/Met) polymorphism, is associated with longevity in the Japanese population (*3*). The frequency of the ND2-237Met (Mt5178A) genotype is significantly higher in Japanese cen-

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Table 1. Clinical Characteristics of Study Subjects by ND2-237 Leu/Met Genotype

	ND2-237Leu (N=242)	ND2-237Met (N=156)	p value
Age (years)	54.4±7.8	53.2±7.8	0.137
BMI (kg/m^2)	23.3±2.8	23.5±2.6	0.452
SBP (mmHg)	125.8±15.7	125.8±14.1	0.977
DBP (mmHg)	74.0±10.5	73.8±9.1	0.854
TC (mg/dl)	203.2±33.9	201.9±31.9	0.699
HDL-C (mg/dl)	54.4±13.5	56.3±16.1	0.184
TG (mg/dl)	137.2±90.1	138.8±90.1	0.867
FPG (mg/dl)	99.6±16.2	98.2±20.2	0.399
UA (mg/dl)	5.92±1.23	5.94±1.22	0.871
Alcohol consumption (daily/occasionally/non- or ex-) (%)	46.3/35.1/18.6	48.7/37.8/13.5	0.403
Current smokers (%)	41.7	41.0	0.888
Antihypertensive (%)	19.8	13.5	0.101
Hypertension (%)	32.6	23.1	0.040

Age, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), serum total cholesterol levels (TC), serum high-density lipoprotein cholesterol levels (HDL-C), serum triglyceride levels (TG), fasting plasma glucose levels (FPG) and serum uric acid levels (UA) are given as means±SD. All p values depict significance of differences between ND2-237Leu and ND2-237Met. For alcohol consumption, current smokers, antihypertensive and hypertension, p values were calculated by χ^2 test.

tenarians than in the general population (*3*), and it is reported that Japanese individuals with ND2-237Met are more resistant to atherosclerotic diseases than those with ND2-237Leu (Mt5178C) (*4*–*6*). Moreover, ND2-237 Leu/Met polymorphism is associated with blood pressure (*7*), serum lipid levels (*8*, *9*), fasting plasma glucose levels (*10*) and serum uric acid levels (*11*), all of which are factors in atherosclerosis. In a study that excluded subjects taking antihypertensives, ND2-237 Leu/Met polymorphism was shown to modulate the effects of alcohol consumption on blood pressure (*7*). However, no evaluation of the genetic risks of ND2-237 Leu/Met polymorphism with regard to hypertension has been performed. Primary prevention of hypertension is crucial for prevention of atherosclerotic disease. Therefore, it is important to investigate whether ND2-237 Leu/Met polymorphism is a genetic risk factor for hypertension and whether these effects are modified by alcohol consumption.

The objective of this study was to investigate the relationship between ND2-237 Leu/Met polymorphism and hypertension in middle-aged Japanese male subjects.

Methods

Subjects

Participants were recruited from among individuals visiting the Mito Red Cross Hospital for regular medical check-ups between August 1999 and August 2000. This study was conducted in accordance with the Declaration of Helsinki and the Guidelines for Research on the Human Genome and Genes (Ministry of Education, Culture, Sports, Science and Technology; Ministry of Health, Labor and Welfare; and Ministry of Economy, Trade and Industry) and was approved by the Ethics Committee of Kyorin University School of Medicine. Written informed consent was obtained from 602 volunteers before participation. Among these, 406 men without diabetes, coarctation of aorta, or heart failure were enrolled in the study. Diabetic patients were excluded because the prevalence of hypertension is reportedly higher in diabetic patients than in non-diabetic patients (*12*, *13*). Eight individuals with unclear drinking frequency were excluded. Therefore, subjects comprised 398 Japanese men (age, 53.8±7.8 years [mean±SD]).

Clinical Characteristics of Subjects

Determination of blood chemical and physical data was conducted as described previously (*7*, *8*). For both systolic blood pressure (SBP) and diastolic blood pressure (DBP), the mean of two consecutive values measured by physicians was used. Hypertension was defined as SBP ≥140 mmHg and/or DBP ≥90 mmHg and/or antihypertensive drug treatment. 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 questionnaire. Habitual drinking was classified based on drinking frequency (daily drinkers; occasional drinkers, which include those who drink several times per week or per month; and non- or ex-drinkers). Habitual smokers were classified into non- or ex-smokers and current smokers.

Genotyping

Genotyping methods were as described previously (*8*).

Table 2. Analysis of Covariance for Blood Pressure

	F value	
	SBP	DBP
ND2-237 Leu/Met	1.55	1.29
Habitual drinking	3.09*	1.87
Habitual smoking	2.55	3.28
ND2-237 Leu/Met × habitual drinking	3.57*	3.84*
ND2-237 Leu/Met × habitual smoking	0.07	0.13
Age	10.7**	1.32
BMI	25.6***	35.7***
TC	0.00	0.04
HDL-C	1.50	0.36
TG	1.16	0.68
FPG	0.85	0.00
UA	0.18	1.21
Antihypertensive	32.4***	24.2***

BMI, body mass index; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; FPG, fasting plasma glucose; UA, uric acid. For ANCOVA, some independent variables were numerically coded: ND2-237 Leu/Met genotypes (Leu =0, Met =1), habitual drinking (non-/ex-alcohol drinkers =0, occasional alcohol drinker =1, daily alcohol drinkers =2), habitual smoking (non-/ex-smoker =0, current smoker =1) and antihypertensive (no treatment =0, taking medicines =1). ND2-237 Leu/Met × habitual drinking or habitual smoking represents interaction between ND2-237 Leu/Met and habitual drinking or habitual smoking. *$p<0.05$, **$p<0.01$, *** $p<0.001$.

Briefly, DNA was extracted from white blood cells. Polymerase chain reaction–restriction fragment length polymorphism using the restriction enzyme *Alu*I was performed. The absence of an *Alu*I site was designated as ND2-237Met (Mt5178A), and the presence of this restriction site was designated as ND2-237Leu (Mt5178C).

Statistical Analyses

Statistical analyses were performed using SAS statistical software, version 8.2 for Windows (SAS Institute, Inc., Cary, USA). For analysis of covariance and multiple regression analysis, ND2-237 Leu/Met genotype (ND2-237Leu =0, ND2-237Met =1), habitual smoking (non- or ex-smokers =0, current smokers =1), habitual drinking (non- or ex-drinkers =0, occasional drinkers =1, daily drinkers =2) and antihypertensive use (no drug treatment =0, taking medicine =1) were numerically coded. Multiple logistic regression analysis was used to calculate odds ratios (ORs) for hypertension. Differences with *p* values of less than 0.05 were considered statistically significant.

Results

No significant differences in any characteristics or in biophysical or biochemical data were observed between the ND2-237 Leu/Met genotypes (Table 1). However, the frequency of hypertension was significantly higher in ND2-237Leu genotypic men than in ND2-237Met genotypic men ($p=0.040$).

In analysis of covariance (Table 2), habitual drinking, age, BMI, antihypertensive drug treatment and the interaction between ND2-237 Leu/Met polymorphism and habitual drinking were significantly associated with SBP ($p=0.047$, $p=0.001$, $p<0.001$, $p<0.001$, and $p=0.029$, respectively). In addition, BMI, antihypertensive drug treatment and the interaction between ND2-237 Leu/Met polymorphism and habitual drinking were significantly associated with DBP ($p<0.001$, $p<0.001$, and $p=0.022$, respectively).

Multiple logistic regression analysis revealed that the ND2-237Met genotype had a significantly lower OR for hypertension than the ND2-237Leu genotype (OR=0.619, 95% confidence interval [CI]: 0.391–0.980, $p=0.040$) (Table 3). After adjusting for age, BMI, habitual drinking, habitual smoking, serum high density lipoprotein cholesterol levels, serum triglyceride levels and serum uric acid levels, the OR for hypertension in men with ND2-237Met remained significantly lower (OR=0.601, 95% CI: 0.365–0.988, $p=0.045$). Among younger subjects (age <60 years), ND2-237Met genotypic men had a significantly lower OR for hypertension when compared with ND2-237Met genotypic men (OR=0.503, 95% CI: 0.279–0.906, $p=0.022$). After adjusting for covariates, the OR for hypertension in men with ND2-237Met also remained significantly lower (OR=0.489, 95% CI: 0.261–0.919, $p=0.026$). However, in older subjects (age≥60 years), the association between ND2-237 Leu/Met polymorphism and hypertension was not significant.

The association of ND2-237 Leu/Met polymorphism with hypertension may depend on the frequency of alcohol consumption (Table 4). The OR for hypertension was significantly higher in daily drinkers with ND2-237Leu when compared to non- or ex-drinkers with ND2-237Leu (OR=5.432, 95% CI: 2.130–13.86, $p<0.001$). After adjustment, a significant OR was also observed (OR=4.713, 95% CI: 1.672–13.28, $p<0.001$). Moreover, among subjects not taking antihypertensive drugs, the OR for hypertension was significantly higher in daily drinkers with ND2-237Leu when compared with non- or ex-drinkers with ND2-237Leu (OR=3.196, 95% CI: 1.016–10.06, $p=0.047$). However, after adjustment, this significant OR disappeared. On the other hand, the association between the ND2-237Met genotype and hypertension may not depend on the frequency of alcohol consumption.

Discussion

In the present study, we demonstrated that ND2-237 Leu/Met polymorphism may be associated with hypertension in middle-aged Japanese men. Individuals with ND2-237Met are apparently more resistant to hypertension than those with ND2-237Leu among men aged <60 years. Moreover, the

Table 3. Odds Ratios and 95% Confidence Intervals for Hypertension by ND2-237 Leu/Met Genotype

Genotype	Genotype frequency		OR (95% CI)	Adjusted OR† (95% CI)
	Normotensive	Hypertensive		
Total (*n* (%))				
ND2-237Leu	163 (67.4)	79 (32.6)	1.0	1.0
ND2-237Met	120 (76.9)	36 (23.1)	0.619 (0.391–0.980)*	0.601 (0.365–0.988)*
Age <60 years (*n* (%))				
ND2-237Leu	131 (72.4)	50 (27.6)	1.0	1.0
ND2-237Met	99 (83.9)	19 (16.1)	0.503 (0.279–0.906)*	0.489 (0.261–0.919)*
Age ≥60 years (*n* (%))				
ND2-237Leu	32 (52.5)	29 (47.5)	1.0	1.0
ND2-237Met	21 (55.2)	17 (44.8)	0.893 (0.396–2.015)	0.903 (0.380–2.142)

OR, odds ratio; CI, confidence interval. †OR adjusted for age, body mass index, habitual drinking, habitual smoking, serum high-density lipoprotein cholesterol levels, serum triglyceride levels and serum uric acid levels. *$p<0.05$.

Table 4. Odds Ratios and 95% Confidence Intervals for Hypertension by ND2-237 Leu/Met Genotype and Drinking Habit

Genotype and drinking habit	Frequency		OR (95% CI)	Adjusted OR† (95% CI)
	Normotensive	Hypertensive		
All subjects (*n* (%))				
Non- or ex-drinker with ND2-237Leu	39 (86.7)	6 (13.3)	1.0	1.0
Occasional drinker with ND2-237Leu	63 (74.1)	22 (25.9)	2.270 (0.846–6.091)	1.921 (0.606–6.092)
Daily drinker with ND2-237Leu	61 (54.5)	51 (45.5)	5.432 (2.130–13.86)**	4.713 (1.672–13.28)**
Non- or ex-drinker with ND2-237Met	14 (66.7)	7 (33.3)	1.0	1.0
Occasional drinker with ND2-237Met	47 (79.7)	12 (20.3)	0.511 (0.169–1.545)	0.653 (0.175–2.438)
Daily drinker with ND2-237Met	59 (77.6)	17 (22.4)	0.576 (0.200–1.655)	0.786 (0.222–2.780)
Subjects not taking antihypertensive drugs (*n* (%))				
Non- or ex-drinker with ND2-237Leu	39 (90.7)	4 (9.3)	1.0	1.0
Occasional drinker with ND2-237Leu	63 (90.0)	7 (10.0)	1.083 (0.298–3.942)	1.176 (0.267–5.184)
Daily drinker with ND2-237Leu	61 (75.3)	20 (24.7)	3.196 (1.016–10.06)*	1.893 (0.502–7.134)
Non- or ex-drinker with ND2-237Met	14 (77.8)	4 (22.2)	1.0	1.0
Occasional drinker with ND2-237Met	47 (90.4)	5 (9.6)	0.372 (0.088–1.578)	0.655 (0.130–3.307)
Daily drinker with ND2-237Met	59 (90.8)	6 (9.2)	0.356 (0.088–1.433)	0.278 (0.047–1.633)

OR, odds ratio; CI, confidence interval. †OR adjusted for age, body mass index, habitual smoking, serum high-density lipoprotein cholesterol levels, serum triglyceride levels and serum uric acid levels. *$p<0.05$, **$p<0.001$.

effect of this polymorphism on the risk of hypertension may interact with the effects of habitual drinking. Particularly for ND2-237Leu genotypic men, avoidance of daily alcohol consumption may reduce the risk of hypertension.

Individuals with the longevity-associated ND2-237Met genotype may be more resistant to lifestyle-related adult-onset diseases, such as myocardial infarction (*4*, *5*), cerebrovascular diseases (*6*), and type 2 diabetes (*14*), than those with ND2-237Leu. The ND2-237Met genotype seems to exert antiatherogenic effects (*8*, *15*, *16*). These advantages of ND2-237Met may be brought about by the biophysical and biochemical properties of ND2-237Met. Methionine residues are thought to function as antioxidants (*17*). NADH dehydrogenase is involved in the production of reactive oxygen species (ROS) (*18*), the formation of which influences blood pressure (*19*, *20*), and is a target for attack by ROS.

Moreover, our findings indicate an interaction between ND2-237 Leu/Met polymorphism and habitual drinking with regard to hypertension risk. We previously reported that longevity-associated ND2-237 Leu/Met polymorphism modulates the effects of habitual alcohol drinking on blood pressure (*7*), serum triglyceride levels (*9*), serum uric acid levels (*11*) and intraocular pressure (*21*). Habitual alcohol consumption influences the production of ROS by NADH dehydrogenase (*22*), and modifies the susceptibility of mitochondrial proteins, including NADH dehydrogenase, to ROS (*23*). We hypothesized that differences in ethanol-related ROS production and/or sensitivity between ND2-237 Leu/

Met bring about the differences in blood pressure, serum triglyceride levels, serum uric acid levels, intraocular pressure and risk of hypertension. To elucidate the mechanisms responsible for the biophysical and biochemical differences between ND2-237 Leu/Met genotypes, further investigation is required.

Based on the frequency of hypertension, non-drinkers with ND2-237Leu seem to have a lower risk of hypertension. Previous studies have found that both SBP and DBP were lower in non-drinkers with ND2-237Leu than in those with ND2-237Met (*7*), and that serum uric acid levels were lower in non-obese non-daily drinkers with ND2-237Leu than in those with ND2-237Met (*11*). Only among men with ND2-237Leu, habitual drinking was reportedly and positively associated with intraocular pressure (*21*). Therefore, abstaining from alcohol consumption, or at least avoiding daily alcohol consumption, is particularly recommended for ND2-237Leu genotypic men in order to lower the risk of lifestyle-related diseases.

Much as in the case of the apolipoprotein ε4 allele (*24*), the association between ND2-237 Leu/Met polymorphism and risk of hypertension was more evident in young subjects than in elderly subjects. Blood pressure in middle age has a significant impact on life expectancy (*2*). Wang *et al*. reported that the average age at onset of diabetes was significantly lower in patients with ND2-237Leu than in those with ND2-237Met (*14*). Considering that genetic background influences age at onset of hypertension (*25*, *26*), ND2-237Leu/Met polymorphism may also be associated with both onset of diabetes and that of hypertension. The lower risk of hypertension in middle-aged ND2-237Met genotypic men is probably one of the reasons why the ND2-237Met genotype is associated with longevity.

Analysis including subjects taking antihypertensive drugs revealed significant risk for hypertension among daily drinkers with ND2-237Leu. These results were influenced by the remarkably high frequency of daily drinkers with ND2-237Leu among subjects taking antihypertensive drugs. Through the biophysical and biochemical mechanisms described above, daily drinkers with ND2-237Leu are more likely to exhibit hypertension having greater severity or earlier onset, and then take antihypertensive drugs. However, in order to confirm these hypotheses, a well-designed cohort study is required.

In addition to the small sample size and lack of information regarding the possibility of secondary hypertension or sodium intake, a limitation of this study was the evaluation of habitual drinking based on the frequency of alcohol consumption. Although we have also used this evaluation in previous reports (*7*, *9*, *10*, *21*), whether there is any interaction between ND2-237 Leu/Met polymorphism and volume of alcohol intake on risk of hypertension warrants further investigation. Recently, interactions between the angiotensinogen gene and adducing gene (*27*) and between the serotonin 2A receptor gene and endothelin-1 gene (*28*) have been reported to be associated with hypertension in Japanese. Therefore, gene-gene or gene-gene-environmental interactions on risk for hypertension should also be considered.

In conclusion, longevity-associated ND2-237 Leu/Met polymorphism may be associated with hypertension in middle-aged Japanese men. ND2-237Leu genotypic men may have a higher risk of hypertension than ND2-237Met genotypic men. However, changes in habitual drinking behavior may control the risk of hypertension and subsequent atherosclerotic diseases. As Japan becomes an increasingly "aged" society, minimizing age-related diseases will become more important. Therefore, these findings may contribute to the establishment of personalized prevention strategies for hypertension and a decrease in the incidence of atherosclerotic diseases, thereby improving longevity.

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