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Abstract

Reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 2 237 leucine/methionine (ND2-237 Leu/Met) polymorphism, is reportedly associated with longevity in the Japanese population. The ND2-237Met genotype may exert resistance to atherogenic diseases, such as myocardial infarction or cerebrovascular disorders. To investigate whether ND2-237 Leu/Met polymorphism is associated with yearly changes in serum lipid levels, we conducted a longitudinal study of 107 healthy Japanese male subjects. Analysis of covariance revealed that the interaction between the ND2-237 Leu/Met genotypes and habitual drinking was significantly associated with yearly changes in serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDLC) levels (p0.036 and p0.006, respectively). In multiple regression analysis, daily drinking was significantly and positively associated with yearly changes in serum LDLC levels in men with ND2-237Met (p0.026). After adjusting for covariates, yearly changes in serum LDLC levels were significantly lower in non-daily drinkers with ND2-237Met than in those with ND2-237Leu (p0.047). These results suggest that ND2-237Met has a beneficial impact on yearly changes in serum LDLC in non-daily drinkers but not in daily drinkers.

KEYWORDS: daily alcohol consumption, longevity, total cholesterol, low-density lipoprotein cholesterol, NADH dehydrogenase

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Original Article

Longevity-associated NADH Dehydrogenase Subunit-2 237 Leu/Met Polymorphism Modulates the Effects of Daily Alcohol Drinking on Yearly Changes in Serum Total and LDL Cholesterol in Japanese Men

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Reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 2 237 leucine/methionine (ND2-237 Leu/Met) polymorphism, is reportedly associated with longevity in the Japanese population. The ND2-237Met genotype may exert resistance to atherogenic diseases, such as myocardial infarction or cerebrovascular disorders. To investigate whether ND2-237 Leu/Met polymorphism is associated with yearly changes in serum lipid levels, we conducted a longitudinal study of 107 healthy Japanese male subjects. Analysis of covariance revealed that the interaction between the ND2-237 Leu/Met genotypes and habitual drinking was significantly associated with yearly changes in serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDLC) levels ($p = 0.036$ and $p = 0.006$, respectively). In multiple regression analysis, daily drinking was significantly and positively associated with yearly changes in serum LDLC levels in men with ND2-237Met ($p = 0.026$). After adjusting for covariates, yearly changes in serum LDLC levels were significantly lower in non-daily drinkers with ND2-237Met than in those with ND2-237Leu ($p = 0.047$). These results suggest that ND2-237Met has a beneficial impact on yearly changes in serum LDLC in non-daily drinkers but not in daily drinkers.

Key words: daily alcohol consumption, longevity, total cholesterol, low-density lipoprotein cholesterol, NADH dehydrogenase

Mitochondrial function is deeply associated with aging and longevity [1, 2]. Mitochondrial DNA cytosine/adenine (Mt5178 C/A) polymorphism, which is also known as reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit-2 237 leucine/methionine (ND2-237 Leu/Met) polymorphism, is a longevity-associated mitochondrial DNA polymorphism [3–7]. The frequency of the ND2-237Met (Mt5178A) genotype is significantly higher in Japanese centenarians than in the general population [3], and it is reported that Japanese individuals with ND2-237Leu (Mt5178C) are more susceptible to lifestyle-related adult-onset diseases than those with ND2-237Met [8–11]. In addition, the ND2-237Met genotype seems

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to exert antiatherogenic effects [12–14]. Previous reports have demonstrated that this polymorphism is associated with serum lipid levels, such as high-density lipoprotein cholesterol (HDLC) levels and triglyceride (TG) levels, in Japanese subjects [12, 15]. The ND2-237 Leu/Met polymorphism may interact with the effects of daily drinking on serum TG levels, and may also interact with the effects of habitual smoking on serum TG levels [15]. The relationship between healthy aging and lipidological properties has been epidemiologically discussed [16–18] and several vascular risk factors are reportedly associated with longevity [19]. Thus, investigating the relationship between the longevity-associated ND2-237 Leu/Met polymorphism and serum lipid levels, and especially yearly changes in serum lipid levels, is useful for gerontological understanding and for the personalized prevention of lifestyle-related atherosclerotic diseases, such as myocardial infarction and cerebrovascular diseases.

The purpose of this study was to investigate the relationship between the longevity-associated ND2-237 Leu/Met (Mt5178C/A) polymorphism and yearly changes in serum lipid levels, and to also investigate the effect of the interaction between the ND2-237 Leu/Met polymorphism and habitual drinking or habitual smoking on serum lipid levels in healthy middle-aged Japanese male subjects.

Materials and Methods

Subjects. Participants were recruited from among individuals visiting the Mito Red Cross Hospital for regular medical check-ups in 1998 and/or 1999. Among these individuals, 110 male subjects who participated in health check-ups in both 1998 and 1999 and who did not have any chronic disease or take any medication were enrolled in this study. Three individuals with unclear drinking frequency were excluded. Therefore, the subjects comprised 107 Japanese men (age, 55.0 ± 6.9 years; mean $\pm$ SD). This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Kyorin University School of Medicine. Written informed consent was obtained from all volunteers before participation.

Clinical characteristics of subjects. The determination of blood chemical and physical data was conducted as described previously [12]. Briefly, venous blood was drawn after a minimum fasting period of 12 h. Serum total cholesterol (TC) levels were measured by the Determiner L TC II (Kyowa Medex, Tokyo, Japan) [20], serum HDLC levels by the Determiner L HDL-C (Kyowa Medex) [21], and serum TG levels by the Determiner L TG II (Kyowa Medex) [22]. Serum low-density lipoprotein cholesterol (LDLC) levels were calculated according to Friedewald's formula [23]. Yearly changes in the TC, HDLC, LDLC, and TG levels (ΔTC, ΔHDLC, ΔLDLC, and ΔTG) were calculated for each subject using the data obtained in 1998 and 1999. Body mass index (BMI) was defined as the ratio of subject weight (kg) to the square of subject height (m). Yearly changes in BMI (ΔBMI) were also calculated for each subject using the data from 1998 and 1999. A survey of drinking and smoking habits was performed by means of a questionnaire in 1999. Habitual drinking was classified based on drinking frequency (daily drinkers; occasional drinkers, which include those who drink several times per week; and non- or ex-drinkers, which include those who drink a few times per month). Regarding habitual smoking, individuals were classified as non- or ex-smokers and current smokers.

Genotyping. Genotyping methods were as described previously [12]. 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, NC, USA, 1999). In the analysis of covariance and multiple regression analysis, the ND2-237 Leu/Met genotypes (ND2-237Leu = 0, ND2-237Met = 1), habitual smoking (non- or ex-smokers = 0, current smokers = 1), and habitual drinking (non-/ex-/occasional-, namely non-daily, drinkers = 0, daily-drinkers = 1) were numerically coded. Serum lipid levels (1998), age (1998), BMI (1998), and ΔBMI were included as covariates in the models. Differences with p values of less than 0.05 were considered statistically significant.

Results

Although one of the traits of inherence of mitochondrial DNA is heteroplasmy, with respect to the Mt5178C/A (ND2-237 Leu/Met) polymorphism in subjects enrolled in this study, no heteroplasmy was detected photographically.

No significant differences in serum lipid characteristics in 1998 or 1999 were observed between the ND2-237 Leu/Met genotypes (Table 1). However, serum LDLC levels in 1998 tended to be higher in men with ND2-237Leu than in men with ND2-237Met ($p=0.075$), and ΔHDLC was significantly lower in men with ND2-237Met than in men with ND2-237Leu ($p=0.005$).

In the analysis of covariance (Table 2), interactions between the ND2-237 Leu/Met genotype and habitual drinking were significantly associated with ΔTC and ΔLDLC ($p=0.036$ and $p=0.006$, respectively). Serum lipid parameters in 1998 were significantly associated with yearly change in serum lipid levels (TC: $p=0.002$, HDLC: $p<0.001$, LDLC: $p<0.001$, and TG: $p<0.001$, respectively). Habitual smoking was significantly associated with ΔTC ($p=0.012$). Habitual drinking was significantly associated with ΔHDLC and ΔTG ($p=0.013$ and $p=0.008$, respectively). Age and BMI in 1998 were significantly associated with ΔHDLC ($p=0.021$ and $p<0.001$, respectively). ΔBMI was significantly associated with ΔTC, ΔHDLC and ΔLDLC ($p<0.001$).

In multiple regression analysis for ΔTC and ΔLDLC (Table 3), daily drinking was significantly and positively associated with ΔLDLC in men with ND2-237Met ($p=0.026$). Daily drinking was positively associated with ΔTC in men with ND2-237Met ($p=0.063$). Habitual smoking was significantly and positively associated with ΔTC only in men with ND2-237Met ($p=0.009$). Serum TC levels (1998) were significantly and negatively associated with ΔTC in men with ND2-237Met ($p<0.001$). ΔBMI was significantly and positively associated with ΔTC (ND2-237 Leu: $p=0.036$, ND2-237Met: $p=0.003$, respectively) and ΔLDLC (ND2-237Leu: $p=0.011$, ND2-237Met: $p=0.005$, respectively).

After adjusting for age (1998), BMI (1998), ΔBMI, habitual smoking and serum lipid parameters (1998), ΔHDLC was significantly higher in daily drinkers with ND2-237Leu than in non-daily drinkers with ND2-237Leu ($p=0.015$: Bonferroni's multiple comparison test) (Table 4). ΔLDLC was significantly lower in non-daily drinkers with ND2-237Met than in those with ND2-237Leu ($p=0.047$: Bonferroni's multiple comparison test). In all subjects with the ND2-237Met genotype, after adjustment, ΔTC and

Table 1 Serum lipid levels in 1998 and 1999 and yearly changes

	ND2-237Leu (N = 64)	ND2-237Met (N = 43)	*P* value
Age (1998)	55.8±0.9	53.8±1.0	0.144
BMI (1998)	22.9±0.3	23.3±0.4	0.529
BMI (1999)	22.9±0.3	23.4±0.4	0.374
ΔBMI	−0.03±0.07	0.10±0.09	0.249
TC (1998)	201.7±4.1	195.3±5.0	0.330
TC (1999)	204.5±4.2	194.5±5.0	0.138
ΔTC	2.8±2.4	−0.8±3.0	0.591
HDLC (1998)	54.8±2.0	59.7±5.0	0.122
HDLC (1999)	54.5±1.7	55.4±2.2	0.697
ΔHDLC	−0.3±1.0	−4.3±1.1	0.005
LDLC (1998)	119.6±3.9	108.4±4.8	0.075
LDLC (1999)	124.4±4.0	114.3±4.7	0.109
ΔLDLC	4.8±2.5	5.9±3.1	0.792
TG (1998)	136.7±9.4	136.0±11.5	0.965
TG (1999)	128.4±7.9	132.3±9.6	0.802
ΔTG	−8.3±6.9	−3.7±8.4	0.671

Age, body mass index (BMI), serum total cholesterol (TC), serum high-density lipoprotein cholesterol (HDLC), serum low-density lipoprotein cholesterol (LDLC), serum triglyceride (TG) and yearly changes (ΔBMI, ΔTC, ΔHDLC, ΔLDLC, and ΔTG) are given as means ± S.E. All *P* values depict the significance of differences between ND2-237Leu and ND2-237Met.

Table 2 Analysis of covariance for yearly changes in serum lipid levels

	ΔTC	ΔHDLC	ΔLDLC	ΔTG
TC (1998)	9.96***			
HDLC (1998)		57.7****		
LDLC (1998)			15.95****	
TG (1998)				57.1****
ND2-237 Leu/Met genotype	1.25	3.65	0.31	0.03
Habitual smoking	6.51*	0.13	3.87	1.24
Habitual drinking	0.09	6.41*	0.22	7.26**
ND2-237 Leu/Met genotype × Habitual smoking	0.91	0.41	0.87	0.00
ND2-237 Leu/Met genotype × Habitual drinking	4.54*	3.27	8.06**	0.34
Age (1998)	0.03	5.50*	1.65	0.23
BMI (1998)	0.00	12.0****	0.38	2.21
ΔBMI	13.1****	14.0****	17.8****	1.93

Values are F values. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$. In analysis, some independent variables were numerically coded: ND2-237 Leu/Met genotypes (Leu = 0, Met = 1), habitual smoking (non- or ex-smokers = 0, current smokers = 1), and habitual drinking (non-daily drinkers = 0, daily drinkers = 1). ND2-237 Leu/Met genotype × habitual smoking represents the interaction between ND2-237 Leu/Met and habitual smoking. ND2-237 Leu/Met genotype × habitual drinking represents the interaction between ND2-237 Leu/Met and habitual drinking.

BMI, body mass index; TC, serum total cholesterol; HDLC, serum high-density lipoprotein cholesterol; LDLC, serum low-density lipoprotein cholesterol; TG, serum triglyceride; ΔBMI, yearly changes in body mass index; ΔTC, yearly change in serum total cholesterol; ΔHDLC, yearly changes in serum high-density lipoprotein cholesterol; ΔLDLC, yearly changes in serum low-density lipoprotein cholesterol; ΔTG, yearly changes in serum triglyceride.

Table 3 Multiple regression analysis for yearly changes in serum TC and LDLC levels

	ΔTC		ΔLDLC	
	ND2-237Leu	ND2-237Met	ND2-237Leu	ND2-237Met
TC (1998)	−0.113	−0.290****		
LDLC (1998)			−0.167*	−0.376***
Habitual smoking	6.437	12.52**	3.915	9.644
Habitual drinking	−6.924	8.401	−5.093	13.93*
Age (1998)	−0.053	0.203	−0.005	0.722
BMI (1998)	−0.068	0.003	−0.065	1.013
ΔBMI	11.70*	10.85***	13.06*	12.58***

Values are partial regression coefficients. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$. In multiple regression analysis, some independent variables were numerically coded: habitual smoking (non- or ex-smokers = 0, current smokers = 1), and habitual drinking (non-daily drinkers = 0, daily drinkers = 1).

BMI, body mass index; TC, serum total cholesterol; LDLC, serum low-density lipoprotein cholesterol; ΔBMI, yearly changes in body mass index; ΔTC, yearly changes in serum total cholesterol; ΔLDLC, yearly changes in serum low-density lipoprotein cholesterol.

ΔLDLC were positive; however, in non-daily drinkers with the ND2-237Met genotype, ΔTC and ΔLDLC were negative.

Discussion

The present study demonstrates the effect of interactions between the ND2-237 Leu/Met (Mt5178C/A) polymorphism and frequency of alcohol consumption on yearly changes in serum TC and LDLC levels. Overall, after adjustment, serum TC and LDLC levels increased from 1998 to 1999; however, in non-daily drinkers with the ND2-237Met (antiatherogenic) genotype, serum TC and LDLC levels decreased

Table 4 Comparisons of yearly changes in serum lipid levels between non-daily drinkers and daily drinkers by ND2-237 Leu/Met genotype (adjusted)

	ND2-237Leu			ND2-237Met		
	Non-daily drinkers (N = 20)	Daily drinkers (N = 44)	Total (N = 64)	Non-daily drinkers (N = 14)	Daily drinkers (N = 29)	Total (N = 43)
ΔTC	9.2 ± 4.0	1.6 ± 2.7	4.2 ± 2.3	−5.4 ± 4.8	3.1 ± 3.3	0.6 ± 2.9
ΔHDLC	−4.4 ± 1.3	0.5 ± 0.8*	−1.6 ± 0.8	−3.5 ± 1.5	−3.0 ± 1.1	−3.2 ± 0.9
ΔLDLC	11.4 ± 4.1	4.2 ± 2.7	6.0 ± 2.4	−5.4 ± 4.5*	9.3 ± 3.0	4.3 ± 2.8
ΔTG	7.4 ± 9.9	−13.2 ± 6.7	−2.1 ± 5.7	16.5 ± 12.0	−15.1 ± 8.3	−0.5 ± 7.0

Yearly changes in serum total cholesterol (ΔTC), yearly changes in serum high-density lipoprotein cholesterol (ΔHDLC), yearly changes in serum low-density lipoprotein cholesterol (ΔLDLC) and yearly changes in serum triglyceride (ΔTG) are given as means ± S.E. (mg/dl) adjusted for age (1998), BMI (1998), ΔBMI, habitual smoking, and serum lipid parameters (1998). Non-daily drinkers were defined as 'never or seldom', 'several times per month', or 'several times per week' drinkers. Bonferroni correction for multiple comparisons was applied. *$p < 0.05$ vs. non-daily drinkers with ND2-237Leu.

from 1998 to 1999.

Individuals with the ND2-237Leu genotype may be more susceptible to lifestyle-related adult-onset diseases, such as myocardial infarction [8, 9], cerebrovascular diseases [10], and type 2 diabetes [11], than individuals with ND2-237Met. Ultrasonography revealed that the mean intima-media thickness in the bilateral carotid arteries is significantly greater in type 2 diabetic patients with the ND2-237Leu genotype than in those with the ND2-237Met genotype [14]. In healthy men, after adjusting for age and BMI, serum HDLC levels were significantly higher in men with the ND2-237Met genotype than in those with the ND2-237Leu genotype [12]. Low levels of HDLC are a risk factor for atherosclerotic diseases [24, 25], while high levels of LDLC are epidemiologically and clinically known to be a crucial risk factor for atherosclerotic diseases [26, 27]. The ND2-237 Leu/Met polymorphism, which may interact with habitual drinking in affecting yearly changes in serum LDLC levels, appears to be a longevity-associated vascular genetic factor [19].

We previously reported that the ND2-237 Leu/Met polymorphism influences the effects of alcohol consumption on blood pressure [28, 29], serum TG levels [15], fasting plasma glucose (FPG) levels and response to a 75-g oral glucose tolerance test [30], serum uric acid levels [31] and intraocular pressure [32], as well as the effects of cigarette smoking on serum TG levels [15], red blood cell counts [33], intraocular pressure [32], pulmonary function [34], and serum protein fraction levels [13]. The amino acid change from leucine to methionine at residue 237 of NADH dehydrogenase subunit 2 may bring about a functional change in NADH dehydrogenase. NADH dehydrogenase is involved in the production of reactive oxygen species (ROS) [35]. Moreover, habitual drinking stimulates the production of ROS by NADH dehydrogenase [36], and chronic ethanol consumption increases the susceptibility of mitochondrial proteins, including NADH dehydrogenase, to ROS [37]. We believe that differences in ethanol-related ROS production and/or ROS sensitivity between ND2-237 Leu/Met result in differences in yearly changes in serum lipid levels. Based on an investigation showing that cigarette smoking attenuates the activity of NADH dehydrogenase [38], we postulated that there are unidentified differences in smoking-related ROS production and/or ROS sensitivity between ND2-237 Leu/Met [13, 15, 32, 33]. However, in the present study, no interaction between the ND2-237 Leu/Met polymorphism and habitual smoking was observed with regard to yearly changes in serum lipid levels (Table 2). In any case, to elucidate the mechanisms of the biophysical and biochemical differences between the ND2-237 Leu/Met genotypes [12, 13, 15, 28–33, 39], further investigation is required.

With regard to yearly changes in serum TC and LDLC levels (Table 3), weight gain is apparently disadvantageous for both ND2-237Leu genotypic men and ND2-237Met genotypic men. We previously reported that in men with a BMI < 22, FPG levels were significantly lower in the antiatherogenic ND2-237Met genotype than in the ND2-237Leu genotype

[30]. However, in men with a BMI ≥ 22, FPG levels were significantly higher in the ND2-237Met genotype than in the ND2-237Leu genotype. Therefore, gaining weight or becoming overweight may counteract the genetic merits of the longevity-associated ND2-237Met genotype.

Alcohol intake interacts with the effects of the apolipoprotein E gene polymorphism on serum lipid levels [40, 41]. Several genetic polymorphisms are associated with serum TC or LDLC levels [42–45]. Moreover, the effects of several gene-diet interactions on lipid metabolism have also been reported [46–48]. Therefore, further investigations of gene-gene or gene-gene-environment interactions, and their effects on serum lipid levels, are needed to elucidate the mechanisms of lipid metabolism.

Larger decreases in HDLC levels were seen in men with antiatherogenic ND2-237Met than in those with ND2-237Leu (Table 1). This may be a 'regression to the mean' effect, namely the phenomenon that a variable that is extreme on its first measurement will tend to be closer to the center of the distribution at later measurements [49]. Takashima *et al.* suggested the necessity of allowing the 'regression to the mean' effect for accurate evaluation of yearly changes in serum lipid levels [50]. In our studies, after considering the baseline data for serum lipid levels as covariates, the effects of interactions between longevity-associated ND2-237 Leu/Met polymorphism and daily alcohol drinking on yearly changes in serum TC and LDLC levels were observed.

This study had several limitations, including a small number of subjects, a lack of dietary information, and a lack of information regarding lifestyle disorders immediately prior to medical check-ups. In addition, the evaluation of habitual drinking was based on the frequency of alcohol consumption. Although we have used this type of evaluation in previous reports [15, 28–32], further investigation into the effect of interactions between Mt5178C/A polymorphism and volume of alcohol intake on yearly changes of serum lipid levels is warranted. Moreover, we examined only yearly changes in lipid levels in this study. Therefore, further investigations of longer-term changes in lipid levels between Mt5178C/A genotypes are required.

In conclusion, ND2-237Met (Mt5178A) may have a beneficial impact on yearly changes in serum LDLC levels in non-daily drinkers but not in daily drinkers. A decrease in LDLC levels was observed in non-drinkers with ND2-237Met. Non-daily drinking may exert an antiatherogenic advantage in men with the longevity-associated mitochondrial DNA genotype. These findings may contribute to the establishment of personalized primary and secondary prevention of hyperlipidemia and to lowering the incidence of myocardial infarction and cerebrovascular diseases.

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