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Metabolism Clinical and Experimental 55 (2006) 751–757

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

G-protein β3 subunit polymorphism C1429T and low-density lipoprotein receptor–related protein 5 polymorphism A1330V are risk factors for hypercholesterolemia in Japanese males—a prospective study over 5 years

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Received 2 September 2005; accepted 18 January 2006

Abstract

We examined the relationship between the C825T, C1429T, and A-350G variants in the G-protein β3 subunit (GNB3) gene, the A1330V and Q89R variants in the low-density lipoprotein receptor–related protein 5 (*LRP5*) gene, and the risk of hypercholesterolemia in a prospective study in Japanese workers. This study included observations over a 5-year period from 1997 to 2002 on 936 males and 662 females who were not hypercholesterolemic on entry. Hypercholesterolemia was defined as a serum total cholesterol level of 240 mg/dL or higher. Pooled logistic regression analyses were performed using either of the gene variants with age, body mass index, smoking, alcohol consumption, and habitual exercise as the covariates. The risk of the development of hypercholesterolemia was 2.27 times higher in males with the TT genotype of *GNB3*/C1429T than in males with the CC genotype (95% confidence interval, 1.04-4.94), after adjustment for the effects of other potential covariates. Simultaneously, the risk was 1.49 times higher in males with the AV genotype of *LRP5*/A1330V than in males with the AA genotype (95% confidence interval, 1.04-2.12) after adjustment for the effects of other potential covariates. This study indicates the *GNB3*/C1429T and *LRP5*/A1330V are independent risk factors for hypercholesterolemia in Japanese males and suggests that targeting these polymorphisms may be beneficial when attempting to prevent hypercholesterolemia in the general Japanese male population.

1. Introduction

In recent years, many genetic variations have been identified that are associated with congenital hereditary diseases and acquired chronic diseases such as hypertension and diabetes mellitus. These chronic diseases are considered to be polygenic and have multifactorial traits. For example, the onset of these diseases may be influenced by various genes that interact reciprocally with a combination of host factors such as lifestyle and environmental factors.

Variants of the G-protein β3 subunit (GNB3) gene (C825T) have attracted renewed attention in recent years. The *GNB3* 825T allele is associated with alternative splicing of the exon in which the C825T polymorphism is located and with enhanced G-protein activation [1]. Ethnic distribution of the 825T allele frequency ranged from 20% to 80% [2]. Several studies [1,3-7] have also demonstrated that the *GNB3* 825T allele is associated with hypertension or elevation of blood pressure. As the 825T allele was associated with obesity and elevated body mass index (BMI) in several ethnic groups [2,8-12], this allele may also affect serum total cholesterol level. Such an association between C825T and serum cholesterol level has been reported in an elderly Japanese population [13] and in young white men [14]. Other studies, however, have failed to demonstrate this association [15-18]. Because of this variability between studies, we consider that the association between *GNB3* polymorphisms and hypercholesterolemia has not been demonstrated conclusively. Other variants (A-350G, C1429T) in *GNB3* were reported to be in complete linkage disequilibrium with C825T in a previous study [19] and therefore are considered to be candidate pathogenic polymorphisms.

Low-density lipoprotein receptor–related protein 5 (LRP5) is a member of the low-density lipoprotein receptor family [20,21]. *LRP5* has been identified as a candidate gene

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0026-0495/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2006.01.011

controlling bone mass and bone density in humans [22-27]. In addition, the human *LRP5* gene is mapped within the region linked to the insulin-dependent diabetes mellitus locus, IDDM4, on chromosome 11q13 [21]. Therefore, the *LRP* gene itself may be related to diabetes mellitus and other diseases such as dyslipidemia or obesity. Fujino et al [28] showed that LRP5 is also required for normal cholesterol and glucose metabolism. Magoori et al [29] showed that the LRP gene is related to cholesterol metabolism and atherosclerotic lesions in mice lacking both the apolipoprotein E and LRP5 genes. Thus, LRP5 is thought to have multiple physiological functions linked to common human disorders, such as hypercholesterolemia, impaired glucose tolerance, and hypertension. Recently, 2 nonsynonymous variants (c.314A > G: Q89R and c.4037T > C: V1330A) were identified in a Japanese population [30]. Several studies have evaluated the influence of the Q89R [31] and A1330V [24,27,31] variants on bone mineral density. However, further evaluation of the human health effects of these variants in *LRP5* has not been performed.

In addition, the majority of earlier studies of genetic polymorphisms have had case-control designs. From an epidemiological point of view, to determine the influence of genetic polymorphisms on the occurrence of a specific disease, studies must be large-scale prospective ones in the general population. This requirement prompted us to undertake a follow-up study on the relationship between these variants and the onset of hypercholesterolemia in Japanese workers. We consider that a prospective study of this type could detect epidemiological cause-and-effect relationships. The study used pooled logistic regression analysis to examine whether polymorphism in the gene was associated independently with the disorder when factors such as age, BMI, and lifestyle were taken into account.

2. Materials and methods

2.1. Subjects

This prospective study included observations made over a 5-year period (1997-2002). The target subjects were 3834 males and 2591 females who worked in 1997 at a zipper and sash factory in the Hokuriku district of Japan. All workers in this company underwent a legally required health check-up that included measurement of height, weight, and blood pressure; analysis of blood samples; and a self-administered questionnaire designed to evaluate lifestyle factors and habits. The workers were asked to estimate their alcohol intake on the basis of a traditional Japanese drinking unit, the *gou*, which corresponds to 25 g of ethanol. The amount of ethanol consumption per week was calculated for all participants, who were then assigned to one of the following 5 groups: nondrinkers, drinkers less than 100 g/wk, drinkers 100 to 199 g/wk, drinkers 200 to 299 g/wk, and drinkers 300 g/wk or more. Smoking habits were classified as nonsmoker, ex-smoker, current smoker (smoking <20 cigarettes per day), or current smoker (smoking ≥20 cigarettes per day), whereas habitual exercise was classified as absent, light (light exercise at least once a week without shortness of breath or palpitations), moderate (strenuous exercise for >20 minutes once or twice a week, with shortness of breath, palpitations, and perspiration), or heavy (strenuous exercise >20 minutes more than twice a week, with shortness of breath, palpitations, and perspiration). After excluding workers with missing data or those who had not provided written informed consent for the analyses, we selected 1452 males and 1169 females as target subjects. Subjects who had health examinations with complete data within the next year (1998) were also included in the study. Subjects with hypercholesterolemia diagnosed during the entry year (1997) were then excluded, resulting in a cohort that consisted of 936 males and 662 females (Table 1). The ethics review boards of Kanazawa Medical University and the Graduate School of Medicine, Chiba University, approved the study protocol.

2.2. Laboratory and clinical measurements

Blood samples were collected randomly and were nonfasting, without any restrictions on food intake. The buffy coat was isolated from the blood samples collected from each subject in 1997. The target DNA sequences were amplified by direct polymerase chain reaction [32-34] using

Table 1
The number of subjects and person-years observed in the study

Sex		Age				
		-29	30-39	40-49	50-	Total
Male	The number of subjects examined	251	273	283	129	936
	The number of subjects who developed hypercholesterolemia (%)	13 (5.2%)	38 (13.9%)	54 (19.1%)	39 (30.2%)	144 (15.4%)
	Total person-years of observation	888	1077	902	531	3398
	Incidence rate per 1000 person-years	14.6	35.3	59.9	73.4	42.4
	Mean observed years per person	3.54	3.95	3.19	4.12	3.63
Female	The number of subjects examined	144	211	257	50	662
	The number of subjects who developed hypercholesterolemia (%)	10 (6.9%)	20 (9.5%)	52 (20.2%)	17 (34.0%)	99 (15.0%)
	Total person-years of observation	484	671	844	214	2213
	Incidence rate per 1000 person-years	20.7	29.8	61.6	79.4	44.7
	Mean observed years per person	3.36	3.18	3.28	4.28	3.34

Hypercholesterolemia was defined as serum total cholesterol 240 mg/dL or higher.

Table 2
Characteristics of subjects at entry year according to the development of hypercholesterolemia

	Males				*P*	Females				*P*
	Not developed		Developed			Not developed		Developed		
	n	%	n	%		n	%	n	%	
Smoking										
Ex-smoker	76	9.6	12	8.3	.941	6	1.1	1	1.0	.763
<20 per day	147	18.6	26	18.1		27	4.8	3	3.0	
≥20 per day	327	41.3	59	41.0		3	0.5	0	0.0	
Nonsmoker	242	30.6	47	32.6		527	93.6	95	96.0	
Alcohol consumption										
≤100 g/wk	189	23.9	23	16.0	.061	106	18.8	11	11.1	.320
≤200 g/wk	133	16.8	28	19.4		11	1.9	2	2.0	
≤300 g/wk	113	14.3	23	16.0		4	0.7	0	0.0	
>300 g/wk	131	16.5	35	24.3		2	0.4	0	0.0	
Abstainer	226	28.5	35	24.3		440	78.2	86	86.9	
Habitual exercise										
No habitual exercise	505	63.8	105	72.9	.212	437	77.6	81	81.8	.392
Light exercise	138	17.4	19	13.2		63	11.2	12	12.1	
Moderate exercise	120	15.2	16	11.1		54	9.6	6	6.1	
Heavy exercise	29	3.7	4	2.8		9	1.6	0	0.0	

Ampdirect (Shimadzu, Kyoto, Japan) buffers. The *GNB3*/C825T (refSNP: rs5443) genotype was determined by following the restriction fragment length polymorphism method as described previously [33,34]. The primers and restriction enzyme for the rest of the genotypes were as follows: *GNB3*/C1429T (refSNP: rs5446): 5′-CCCAGAGCCACTACCTTTGT-3′, 5′-CACAGATGTACCAGGGTGCT-3′, *Bsh*NI (Fermentas, Vilnius, Lithuania); *GNB3*/A-350G (refSNP: rs5441): 5′-GGGAGGTATAACCCTGCTCC-3′, 5′-CTGACAAATCACAGGCTCCA-3′, *Taq*I (Fermentas); *LRP5*/Q89R: 5′-CGCAGTGGACTTCCAGTTTT-3′, 5′-GATGCGGTTGGTCTCTGAGT-3′, *Ava*II (New England Biolabs, Mass); *LRP5*/A1330V (refSNP: rs3736228): 5′-GGGTCAGTGTGTGGACCTG-3′, 5′-GATGGGTGGAACTGCAGAGT-3′, *Eci*I (New England Biolabs, MA). These primers were picked from corresponding DNA sequences by using Primer3 [35]. The specificity of the primers and restriction enzymes was checked using BLAST (DNA Data Bank of Japan) [36] and NEBcutter [37].

Serum total cholesterol level was measured by the cholesterol oxidase-peroxidase method (Cholesterol-HR; Wako Pure Chemical Industries, Osaka, Japan) using an autoanalyzer (Hitachi 7700; Hitachi, Tokyo, Japan). All tests were carried out by comprehensive clinical testing laboratories that were authorized by official certification organizations. Because of this comprehensive quality control of clinical testing, we consider that the accuracy and reliability of the measurements were assured. Hypercholesterolemia was defined as a serum cholesterol level of 240 mg/dL or more [38] and treated as an endpoint during the period of follow-up. Indices considered as potential contributory factors for hypercholesterolemia included age, BMI, alcohol consumption, smoking habits, and habitual exercise.

2.3. Statistical analyses

In the univariate analyses, the genotypic and allelic frequencies of 5 polymorphisms, alcohol consumption, smoking habits, and habitual exercise at the entry year were compared between the subjects who developed hypercholesterolemia and those who remained normal using Fisher exact test (allele) and the χ^2 test (others). The mean values of serum total cholesterol, age, and BMI at entry were calculated. Student *t* test was then used to compare the mean values between subjects (grouped according to sex) who developed hypercholesterolemia and those who remained normal.

In the multivariate analyses, pooled logistic regression was used to evaluate the influence of the target variants on the development of hypercholesterolemia using the following factors as the covariates: alcohol consumption, smoking

Table 3
Continuous variables at entry year according to sex and the development of hypercholesterolemia

	Males				*P*	Females				*P*
	Not developed		Developed			Not developed		Developed		
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Total serum cholesterol (mg/dL)	180.2	25.3	216.0	15.8	<.001	182.2	26.0	207.6	22.3	<.001
Age	36.9	9.9	41.9	9.0	<.001	37.4	9.1	41.2	8.6	<.001
BMI (kg/m^2)	22.6	2.8	23.6	2.8	<.001	21.8	3.0	22.3	3.3	.124

Table 4
Frequencies of genotype and allele grouped according to sex and the development of hypercholesterolemia

Sex	Hypercholesterolemia	C825T (*GNB3*)	C1429T (*GNB3*)	A-350G (*GNB3*)	A1330V (*LRP5*)	Q89R (*LRP5*)
Males	Not developed	172 (TT, 21.7%)	18 (TT, 2.3%)	0 (AA, 0.0%)	64 (VV, 8.2%)	2 (RR, 0.3%)
		433 (CT, 54.7%)	249 (CT, 31.5%)	20 (AG, 2.5%)	315 (AV, 40.1%)	92 (QR, 11.6%)
		187 (CC, 23.6%)	524 (CC, 66.2%)	770 (GG, 97.2%)	406 (AA, 51.7%)	698 (QQ, 88.1%)
	Developed	41 (TT, 28.5%)	8 (TT, 5.6%)	0 (AA, 0.0%)	11 (VV, 7.7%)	0 (RR, 0.0%)
		61 (CT, 42.3%)	45 (CT, 31.2%)	3 (AG, 2.1%)	72 (AV, 50.3%)	23 (QR, 16.1%)
		42 (CC, 29.2%)	91 (CC, 63.2%)	140 (GG, 97.9%)	60 (AA, 42.0%)	120 (QQ, 83.9%)
	P	0.024	0.087	1.000	0.069	0.276
	Not developed	777 (T, 49.1%)	285 (T, 18.0%)	20 (A, 1.3%)	443 (V, 28.2%)	96 (R, 6.1%)
		807 (C, 50.9%)	1297 (C, 82.0%)	1560 (G, 98.7%)	1127 (A, 71.8%)	1488 (Q, 93.9%)
	Developed	143 (T, 49.7%)	61 (T, 21.2%)	3 (A, 1.0%)	94 (V, 32.9%)	23 (R, 8.0%)
		145 (C, 50.3%)	227 (C, 78.8%)	283 (G, 99.0%)	192 (A, 67.1%)	263 (Q, 92.0%)
	P	0.898	0.216	1.000	0.119	0.235
Females	Not developed	114 (TT, 20.2%)	14 (TT, 2.5%)	0 (AA, 0.0%)	45 (VV, 8.0%)	3 (RR, 0.5%)
		304 (CT, 54.0%)	181 (CT, 32.2%)	13 (AG, 2.3%)	222 (AV, 39.4%)	59 (QR, 10.5%)
		145 (CC, 25.8%)	367 (CC, 65.3%)	550 (GG, 97.7%)	296 (AA, 52.6%)	501 (QQ, 89.0%)
	Developed	20 (TT, 20.2%)	3 (TT, 3.0%)	0 (AA, 0.0%)	12 (VV, 12.1%)	0 (RR, 0.0%)
		57 (CT, 57.6%)	32 (CT, 32.3%)	4 (AG, 4.0%)	38 (AV, 38.4%)	16 (QR, 16.2%)
		22 (CC, 22.2%)	64 (CC, 64.6%)	95 (GG, 96.0%)	49 (AA, 49.5%)	83 (QQ, 83.8%)
	P	0.735	0.951	0.302	0.398	0.204
	Not developed	532 (T, 47.2%)	209 (T, 18.6%)	13 (A, 1.2%)	312 (V, 27.7%)	65 (R, 5.8%)
		594 (C, 52.8%)	915 (C, 81.4%)	1113 (G, 98.8%)	814 (A, 72.3%)	1061 (Q, 94.2%)
	Developed	97 (T, 49.0%)	38 (T, 19.2%)	4 (A, 2.0%)	62 (V, 31.3%)	16 (R, 8.1%)
		101 (C, 51.0%)	160 (C, 80.8%)	194 (G, 98.0%)	136 (A, 68.7%)	182 (Q, 91.9%)
	P	0.700	0.843	0.304	0.305	0.201

habits, habitual exercise, age, and BMI. The period of observation continued only as long as the subjects received an annual health check-up. The dummy variables for genotypes and other categorical variables were constructed in the analyses. The analyses were performed with SPSS 12.0J (SPSS Japan, Tokyo, Japan) with *P* values of less than .05 considered as statistically significant.

3. Results

3.1. Subject population characteristics

Table 1 shows the number of subjects and cumulative person-years recorded in the analyses. The total person-years of observation were 3398 for males and 2213 for females, whereas the mean observed years per person were 3.63 and 3.34 for males and females, respectively. The characteristics of the subjects at their entry year are shown in Tables 2 and 3, grouped according to the development of hypercholesterolemia. At entry to the study, total cholesterol level and age in both sexes and BMI in males were higher in subjects who developed hypercholesterolemia than in subjects who remained normal. Table 4 summarizes the frequencies of genotypes and alleles grouped according to sex and development of hypercholesterolemia. The numbers of subjects in whom genotype could not be determined because of the loss of sample were 1 (Q89R), 2 (C1429T), 3 (A-350G), and 7 (A1330V). In males, the frequency of the TT and CC genotype of *GNB3*/C825T was higher in subjects who developed hypercholesterolemia than in subjects who remained normal. In contrast, there was no other significant difference in frequencies for other variants.

Table 5
The results of pooled logistic regression on the development of hypercholesterolemia

Gene	Genotype	Males				Females			
		Odds ratio	95% CI		*P*	Odds ratio	95% CI		*P*
			Lower limit	Upper limit			Lower limit	Upper limit	
C825T (*GNB3*)	TT/CC	1.01	0.64	1.59	.963	1.03	0.55	1.93	.925
	CT/CC	0.67	0.44	1.01	.053	1.21	0.73	2.01	.463
C1429T (*GNB3*)	TT/CC	2.27	1.04	4.94	.040	1.23	0.37	4.09	.733
	CT/CC	1.11	0.77	1.61	.569	1.02	0.66	1.58	.932
A-350G (*GNB3*)	AG/GG	0.59	0.18	1.92	.380	1.63	0.57	4.69	.365
A1330V (*LRP5*)	VV/AA	1.13	0.58	2.20	.713	1.47	0.76	2.82	.253
	AV/AA	1.49	1.04	2.12	.029	1.07	0.69	1.66	.747
Q89R (*LRP5*)	QR + RR/QQ	1.23	0.77	1.97	.377	1.67	0.95	2.91	.074

Odds ratios, the ratio of the former to the latter, were estimated after adjusting for smoking, alcohol consumption, and habitual exercise.

3.2. Development of hypercholesterolemia

The number of males and females in the experimental cohort who developed hypercholesterolemia (Table 1) was 144 (15.4%) and 99 (15.0%), respectively, resulting in an overall incidence rate of hypercholesterolemia of 42.4 per 1000 person-years in males and 44.7 per 1000 person-years in females.

The results of pooled logistic regression analysis are summarized in Table 5. Some categories (nonsmoker and ex-smoker; smoking <20 cigarettes per day and ≥20 cigarettes per day; alcohol drinkers <100, 100-199, 200-299 g/wk; and drinkers ≥300 g/wk; moderate and heavy exercise) were combined because of the small numbers of corresponding subjects in females. After adjustment for the effects of other potential covariates, the risk of hypercholesterolemia in male subjects with the TT genotype in *GNB3*/C1429T was 2.27 times higher than in males with the CC genotype (95% confidence interval [CI], 1.04-4.94). The risk of hypercholesterolemia in male subjects with the AV genotype in *LRP5*/A1330V was 1.49 times higher than that in males with the AA genotype (95% CI, 1.04-2.12). In contrast, no significant risk of hypercholesterolemia was observed for the other genotypes in both sexes.

4. Discussion

In the present study, prospective and multivariate analysis using pooled logistic regression revealed that the TT genotype in *GNB3*/C1429T and the AV genotype in *LRP5*/A1330V were independent risk factors for the development of hypercholesterolemia in males. To our knowledge, this is the first demonstration for the effect of C1429T in *GNB3* and A1330V in *LRP5* on hypercholesterolemia. On the other hand, we could not detect a significant risk of the VV genotype in *LRP5*/A1330V, which may be due to the small number of subjects with the VV genotype. We selected these 2 genes because a previous cross-sectional study has shown they are related to the development of various lifestyle-related diseases such as hypertension, diabetes, and hypercholesterolemia. We investigated only one other single nucleotide polymorphism, the 1438A/G polymorphism in the 5-hydroxytryptamine receptor 2A gene, in the same cohort, and found that this polymorphism was not related to the development of hypercholesterolemia [39].

In the present study, the prevalence of subjects who developed hypercholesterolemia was 15% in both sexes, a rate which appeared to be rather high compared to that expected in the Japanese population. As dietary habits were not measured in the present study, we were unable to determine the reason for this high prevalence. However, several epidemiological studies have reported that cholesterol levels have been increasing for several decades in Japan [40-42]. The Ministry of Health, Labor, and Welfare has therefore conducted several decennial cross-sectional studies in more than 10 000 general inhabitants older than 30 years in Japan. They reported that the mean serum cholesterol level had increased from 186 mg/dL in 1980 to 200 mg/dL in 2000 in males and from 191 to 208 mg/dL in females. Consequently, the prevalence of hypercholesterolemia (≥240 mg/dL) had increased from 6.1% in 1980 to 12.0% in 2000 in males and from 8.9% to 17.4% in females [40,41]. Another epidemiological study carried out at the same time in thousands of general inhabitants older than 40 years in Hisayama, Japan, showed a higher prevalence of hypercholesterolemia (≥220 mg/dL), with a 9-fold increase in males (3% in 1961 to 28% in 1988) and a 6-fold increase in females (7% in 1961 to 42% in 1988). During the follow-up period, lifestyle including dietary habits in Hisayama had changed or become westernized, characterized by an increased intake of animal fats and proteins and a reduced intake of carbohydrate or rice without any change in total energy intake [42]. We surmise similar changes in dietary habits were the main reason for the unexpectedly high prevalence of subjects who developed hypercholesterolemia.

Important features of this study included the fact that it was a prospective cohort study with a long follow-up period of 5 years. In contrast, earlier studies that have investigated the association between target polymorphisms and hypercholesterolemia have been cross-sectional in nature [13-18,30]. The second feature of our study was the method of statistical analysis. We used pooled logistic regression analyses in which each examination interval of 1 year was treated as a mini follow-up study. The advantage of this method was that it allowed evaluation of repeated measurements of lifestyle that may have changed over time in multivariate models. This method of analysis has been adopted more frequently in recent years [43,44] and has been validated by D'Agostino et al [45]. Other studies on polymorphism in this gene have incorporated only univariate analyses [14-18]. We therefore consider that the design of our study would have resulted in improved epidemiological accuracy. Another notable feature of our study was that data were collected from more than 1500 Japanese workers who represented the general Japanese population. From an epidemiological point of view, it is important to establish the genotypic distribution and the association of polymorphisms with target diseases in the general population. Taken together, we consider that this study is highly informative from an epidemiological perspective.

For *GNB3*, no other report investigated the effect of *GNB3*/C1429T on hypercholesterolemia. Rosskopf et al [19,46] characterized the entire *GNB3* gene and defined new polymorphisms including C1429T and A-350G, some of which almost demonstrated linkage disequilibrium with C825T. Siffert [8] pointed out that the complex of these polymorphisms appeared to cause alternative splicing of the 825T allele.

For *LRP5*, Okubo et al [30] investigated this gene in 5 patients with type III hyperlipoproteinemia and 172 healthy

volunteers, and identified Q89R and V1330A variants. However, the result suggested that these variants were not associated with type III hyperlipoproteinemia. Because the target disorder was uncommon, the sample size was small, which consequently lowered the statistical power. Therefore, the negative results they reported may have been due to insufficient statistical power. The entire LRP5 gene has been identified by Hey et al [21], and its variants have been identified in many other studies [23-27,30,31,47-49]. Some of them showed linkage disequilibrium with the A1330V variant [24,27]. Our study does not exclude the possibility that other variants around the C825T and C1429T polymorphisms in *GB3* and the A1330V polymorphism in *LRP5* may have an influence on the development of hypercholesterolemia in the Japanese population. Therefore, future investigations should be conducted to determine whether haplotypes of *GB3* and *LRP5* are associated with hypercholesterolemia, in addition to functional analysis of variants in these genes. To clarify the etiology of the effects of these variants on hypercholesterolemia, studies should be performed to evaluate the influence of these variants on obesity, diabetes mellitus, or other metabolic diseases. These studies would allow us to draw more definitive conclusions because such diseases may affect hypercholesterolemia.

In conclusion, this study indicates clearly that the *GNB3*/C1429T and *LRP5*/A1330V polymorphisms are independent risk factors for hypercholesterolemia in the general Japanese population and suggests that measurement of these variants may be beneficial when attempting to prevent hypercholesterolemia in the general Japanese male population.

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