The Polymorphism of the β_3-Adrenergic Receptor Gene Is Associated With Reduced Low-Density Lipoprotein Particle Size

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People with a predominance of small, dense low-density lipoprotein (LDL) particles appear to be at increased risk for coronary disease, independent of LDL cholesterol levels. The Trp64Arg variant of the β_3-adrenergic receptor gene is reported to be associated with abdominal obesity and resistance to insulin, and as a consequence, this variant may be a genetic factor in the development of atherosclerosis. Therefore, we investigated whether the β_3-adrenergic receptor polymorphism contributes to the distribution of LDL particle size in 136 Japanese subjects, aged 33 to 59 years, who visited for a routine annual checkup. None of these subjects were taking any medication. The diameter of LDL particles was determined at their peak size using nondenaturing 2% to 16% polyacrylamide gradient gels using fresh plasma samples. The genotype frequencies were: Trp/Trp, 71.3%; Try/Arg, 22.1%; and Arg/Arg, 6.6%, with allele frequencies of 0.82 for Trp64 and 0.18 for Arg64. The subjects with the Arg/Arg genotype had significantly higher levels of fasting plasma insulin and triglycerides and an insulin resistance index of homeostasis model assessment (HOMA-R), and significantly smaller LDL particle size than did the subjects with the Trp/Trp genotype. After adjusting for fasting insulin, body mass index (BMI), and HOMA-R index, there was no longer an observed difference in LDL particle size. The number of the Arg64 allele in individuals was significantly related with fasting insulin, BMI, triglycerides, glycosylated hemoglobin (HbA_{1c}), and fasting glucose, and it was inversely related with LDL particle size. After adjusting for triglyceride, fasting insulin levels, and HOMA-R index, LDL particle size was no longer inversely correlated with the Arg allele. These findings suggest that the Trp64Arg variant in the β_3-adrenergic receptor gene may be associated with reducing LDL particle size, probably due to insulin resistance.

ALTHOUGH AN INCREASED plasma concentration of low-density lipoprotein (LDL) cholesterol is widely recognized as a risk factor for coronary artery disease,[1] there is considerable variation among individuals in the size, density, and chemical composition of LDL particles.[2,3] Individuals with a predominance of small, dense LDL particles appear to be at increased risk for coronary artery disease, independent of the absolute concentration of LDL cholesterol, sex, and age, as noted in several studies.[4,5] Small LDL particles have been associated with the presence of diabetes mellitus,[6,7] adiposity,[8] and insulin resistance.[9] With regard to circulating lipid concentrations, the smaller size of the LDL particle is associated with the higher plasma triglyceride levels and lower high-density lipoprotein (HDL) cholesterol levels.[10,11] LDL subclasses are divided into 2 LDL phenotypes according to size and are genetically influenced.[12,13]

Insulin resistance is characterized by resistance to insulin in skeletal muscle and has often been attributed to concomitant hypertriglyceridemia, lower HDL cholesterol,[9,14] and abdominal obesity.[15,16] Several putative candidates, such as the β_3-adrenergic receptor,[17] insulin-receptor substrate-1,[18] peroxisome proliferator-activated receptor-γ,[19] and fatty and binding protein-2,[20] have been proposed as indicators of a genetic predisposition to insulin resistance. The β_3-adrenergic receptor is expressed in adipose tissue and is considered responsible for increases in lipolysis. The polymorphism in the β_3-adrenergic receptor gene that results in the replacement of tryptophan by arginine at position 64 (Trp64Arg) is closely associated with abdominal obesity and insulin resistance.[21] Therefore, it is easily presumed that the Trp64Arg variant of the β_3-adrenergic receptor is related to changing LDL particle size. To test the hypothesis that the polymorphism of the β_3-adrenergic receptor gene could explain the link to small LDL particle size, we studied apparently healthy subjects without medication to detect any relationship between the prevalence of Trp64Arg of the β_3-adrenergic receptor and the distribution of LDL particle size.

MATERIALS AND METHODS

Study Subjects

Participants in this study were apparently healthy and visited the Nihonseimei Health Consultant in Nagoya for an annual routine checkup. A total of 136 Japanese subjects, 106 men, aged 27 to 66 years, and 30 women, aged 33 to 59 years, were recruited. None of these subjects was taking medications. The study protocol was approved by the Ethics Committee of Nagoya University, and all subjects gave their informed consent to participate in the study.

Determination of LDL Particle Size

The LDL particle size was determined at the peak size by electrophoresis using nondenaturing 2% to 16% polyacrylamide gradient gels in fresh plasma samples with a modified method,[11] as originally described by Krauss and Burke.[22] Electrophoresis was performed in TBE buffer (pH 8.3) that contained 90 mmol/L Tris (hydroxymethyl) aminomethane, 80 mmol/L boric acid, and 3 mmol/L disodium EDTA at 200 V for 24 hours at 12°C. Each gel was calibrated for the accuracy of size determinations by using the migration distance of standards of known diameters. These standards consisted of carboxylated latex beads (Magsphere, Pasadena, CA), thyroglobulin dimmer, thyroglobulin, and ferritin (Amersham Pharmacia Biotech, Uppsala, Sweden) with molecular diameters of 370 Å, 236 Å, 170 Å, and 122 Å, respectively.

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Table 1. Physical and Biochemical Characteristics of the Study Subjects by Gender

	Men	Women	*P*
No.	106	30	
Age (yr)	48.8 ± 8.3	47.1 ± 7.2	.32
BMI (kg/m^2)	23.7 ± 3.1	21.2 ± 2.2	<.0001
Systolic BP (mm Hg)	126 ± 15	117 ± 11	.0017
Diastolic BP (mm Hg)	78 ± 12	70 ± 11	.0032
Total cholesterol (mmol/L)	5.34 ± 0.97	5.53 ± 0.86	.33
HDL cholesterol (mmol/L)	1.33 ± 0.35	1.57 ± 0.42	.0018
Triglycerides (mmol/L)	1.72 ± 1.16	1.03 ± 0.59	.0021
LDL cholesterol (mmol/L)	3.22 ± 0.97	3.49 ± 0.86	.17
LDL size(Å)	262.1 ± 7.3	264.6 ± 8.5	.11
Fasting insulin (pmol/L)	47.0 ± 39.6	32.0 ± 14.2	.044
Fasting glucose (mmol/L)	5.51 ± 1.30	4.76 ± 0.54	.0026
HbA_{1c} (%)	5.81 ± 1.09	5.33 ± 0.43	.020
HOMA-R	0.95 ± 0.44	1.73 ± 2.01	.037

NOTE. Values are the mean ± SD.

Abbreviations: BMI, body mass index; HOMA-R, homeostasis model assessment for insulin resistance; BP, blood pressure.

Detection of the Trp64Arg Variant of the β_3-Adrenergic Receptor Gene

Leukocytes were isolated from blood samples containing EDTA disodium using Ficoll-Hypaque solution (Amersham Pharmacia Biotech). DNA was extracted from the leukocytes with the use of a QIAamp Blood Kit (Qiagen, Valencia, CA). The Trp64Arg β_3-adrenergic receptor polymorphism was determined by the polymerase chain reaction (PCR) method followed by the restriction enzyme BstOI specific for the sequence CC(A/T)GG.[21] As a consequence, digestion of the PCR product with BstOI produced fragments of 99 bp in the Trp64 allele and 161 bp in the Arg64 allele.

Baseline Data Collection

Samples of venous blood were collected from the antecubital vein in the morning after an overnight fasting beginning at 8 PM on the preceding day. The plasma level of HDL cholesterol was determined with enzymatic reagents after precipitating the apolipoprotein (apo) B-containing lipoproteins with heparin, Ca^{2+}, and Ni^{2+}. The concentration of LDL cholesterol was determined using the formula described by Friedewald et al.[23] Plasma glucose levels were measured by the glucose oxidase method, and insulin levels were determined by radioimmunoassay. The glycosylated hemoglobin (HbA_{1c}) levels in whole blood were determined by high-performance liquid chromatography. The normal levels of HbA_{1c} ranged from 4.0% to 6.0%. Blood pressure was measured using an automatic cuff with the subject seated; it was measured twice in the morning and then averaged. The body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). For estimation of insulin sensitivity, the insulin resistance index of homeostasis model assessment (HOMA-R) was calculated according to Matthews et al.[24]

Statistical Analysis

Data are presented as means ± SD. Data were collected and stored in an Apple computer (Apple Japan, Tokyo, Japan) using the Statview 5.0 software (SAS Institute, Cary, NC). To assess mean differences between 2 groups, we used an unpaired Student's *t* test or a 1-way analysis of variance (ANOVA) followed by the Student-Newman-Keul multiple range test. An analysis of covariance (ANCOVA) was used to compare the mean differences between 2 groups after adjusting a certain parameter. The Pearson (*r*) correlation coefficients were computed to identify the parameters that were significantly associated with variations in the LDL peak particle size. Forward stepwise multiple regression analysis was performed to examine significant contributions of the parameters to the prediction of the LDL size variation. A level of $P < .05$ was accepted as statistically significant.

RESULTS

The physical and biochemical characteristics of the 136 subjects by gender are shown in Table 1. The men showed significantly higher levels of plasma triglycerides, fasting glucose, fasting insulin, HbA_{1c}, BMI, and blood pressure and significantly lower HDL cholesterol levels. These data indicated that recruited male subjects for this study were more prevalently insulin resistant and diabetic than were recruited female subjects. The overall prevalence of fasting glucose levels higher than 7.0 mmol/L was 8.1% (10.4% and 0% in men and women, respectively). The frequency of the β_3-adrenergic receptor Arg allele was 0.176 (0.175 and 0.183 in men and women, respectively) (Table 2). The genotype distributions of the β_3-adrenergic receptor gene were 71.3%, 22.1%, and 6.6% for the Trp64 homozygote, Trp64Arg heterozygote, and Arg64 homozygote, respectively (Table 2). The allele distribution met Hardy-Weinberg equilibrium.

When the subjects were divided into 3 groups according to the Trp64Arg variant of the β_3-adrenergic receptor gene, triglyceride and fasting insulin levels were significantly higher in the Arg64 homozygote than in the Trp64 homozygote (Table 3). The results are consistent with a recent report in a Japanese sample.[25] On the other hand, LDL particle size was smaller than in the Trp64 homozygote. The homeostasis model assessment for insulin resistance (HOMA-R) index was also the highest in the Arg64 homozygote. Although these parameters were prominent in the Arg64 homozygote, we divided the subjects into 2 groups according to the presence of the Arg allele to assess the effect of the Arg64 variant of the β_3-adrenergic receptor gene because of the small number of the Arg64 homozygote (n = 9). LDL particle size was smaller in the subjects with the Arg64 allele than in those without the Arg64 allele (260.1 ± 8.5 *v* 263.6 ± 7.0 Å, $P = .015$). An analysis by ANCOVA adjusted for differences in triglyceride and HDL cholesterol levels between groups showed a consistently significant difference in LDL particle size. However, the significant difference in LDL particle size between the genotypes disappeared after adjustment for differences in fasting insulin, BMI, HbA_{1c}, or HOMA-R index (Table 4).

Because the effect of the Arg allele on LDL particle size was greater as the number of Arg alleles increased, we calculated

Table 2. Distribution of the β_3-Adrenergic Receptor Genotype and Allele Frequencies

	No. (men/women)	%
Genotype frequency		
Trp/Trp	97 (76/21)	71.3
Trp/Arg	30 (23/7)	22.1
Arg/Arg	9 (7/2)	6.6
Allele frequency		
Trp64	0.824	
Arg64	0.176	

Table 3. Clinical and Biochemical Characteristics Assigned to the β_3-Adrenergic Receptor Polymorphism

	Trp/Trp	Trp/Arg	Arg/Arg
No.	97	30	9
Gender (M/F)	76/21	23/7	7/2
Age (yr)	48.7 ± 8.1	46.5 ± 8.0	53.1 ± 4.1
BMI (kg/m^2)	22.7 ± 2.6	24.0 ± 4.3	24.5 ± 2.7
Systolic BP (mm Hg)	123.7 ± 12.2	123.8 ± 21.2	123.3 ± 10.3
Diastolic BP (mm Hg)	76.6 ± 10.5	74.9 ± 15.6	78.1 ± 9.4
Total cholesterol (mmol/L)	5.34 ± 0.95	5.38 ± 0.96	5.90 ± 0.70
HDL cholesterol (mmol/L)	1.41 ± 0.36	1.37 ± 0.41	1.15 ± 0.11
Triglycerides (mmol/L)	1.46 ± 0.94	1.63 ± 1.34	2.45 ± 1.43*†
LDL cholesterol (mmol/L)	3.25 ± 0.95	3.27 ± 0.98	3.63 ± 0.89
LDL size (Å)	263.5 ± 7.0	261.4 ± 8.9	256.9 ± 7.7*
Fasting insulin (pmol/L)	32.3 ± 17.2	44.5 ± 46.7	56.1 ± 55.1*
Fasting glucose (mmol/L)	5.21 ± 1.10	5.59 ± 1.28	5.88 ± 1.90
HbA_{1C} (%)	5.59 ± 0.86	5.91 ± 1.18	6.19 ± 1.53
HOMA-R	1.26 ± 0.75	2.06 ± 2.52	3.09 ± 4.61*

NOTE. Values are the mean ± SD.

Abbreviations: BMI, body mass index; BP, blood pressure; HOMA-R, homeostasis model assessment for insulin resistance.

*$P < .05$ *v* Trp/Trp and †$P < .05$ *v* Trp/Arg by S-N-K.

the Pearson correlation coefficients between LDL size and the number of Arg64 alleles, resulting in the significant effect of the Arg64 allele on decreasing LDL particle size (Table 5). After adjusting for triglyceride and fasting insulin levels, and HOMA-R index, LDL particle size was no longer significantly, inversely correlated with the Arg64 allele.

The forward stepwise multiple regression analysis, including apoE ϵ4 genotype[26] and the I405V polymorphism of cholesteryl ester transfer protein,[27] selected the following parameters as independent factors capable of influencing the LDL particle size: logarithms of the triglyceride levels, apoE ϵ4, and the HbA_{1c} levels (data not shown). Therefore, the Arg64 allele of the β_3-adrenergic receptor gene does not appear to be directly correlated with LDL particle size, but it may influence several features of the insulin resistance syndrome, such as triglyceride and fasting insulin levels, which in turn, affect LDL particle size.

DISCUSSION

The β_3-adrenergic receptor is associated with activating lipolysis and energy expenditure. The β_3-adrenergic receptor is chiefly expressed in visceral fat in humans[28] and is considered responsible for increases in lipolysis in response to catecholamines and the delivery of free fatty acids into the portal vein, suggesting the association of obesity with genetically altered function of the β_3-adrenergic receptor.[29] Careful consideration of the association analysis should be made.[30] A missense mutation at position 64 of the β_3-adrenergic receptor with a replacement of tryptophan to arginine (Trp64 Arg) in the first intracellular loop of the receptor protein has been implicated in the obesity-related phenotype as an obesity candidate gene.[31] However, the association of the Arg64 allele with body weight may be highly dependent on gender, age, and other environmental and genetic factors.[32,33] In addition, the Trp64Arg variant of the β_3-adrenergic receptor gene is also associated with insulin resistance and the early onset of non–insulin-dependent diabetes mellitus (NIDDM).[21] The frequency of the Arg64 allele is higher in the Japanese,[34] as well as Pima Indians compared with Caucasians.[21]

On the other hand, small LDL particles are considered to be part of the atherogenic lipid profile.[1] A possible explanation for

Table 4. Difference in LDL Particle Size Between the Groups Classified by the β_3-Adrenergic Receptor Genotype

	Trp/Trp (n = 95)	Trp/Arg+Arg/Arg (n = 39)	*P* Value
LDL particle size (Å)	263.6 ± 7.0	260.1 ± 8.5	.0145
After adjusting for			
Age			.0108
Gender			.0126
Triglycerides (Ln)			.0242
BMI			.0721
HbA_{1C}			.0621
Fasting insulin			.0781
HDL cholestetrol			.0305
HOMA-R			.1072

NOTE. *P* value was calculated by ANCOVA.

Abbreviations: BMI, body mass index; Ln, natural logarithm; HOMA-R, homeostasis model assessment for insulin resistance.

Table 5. Pearson Correlation Coefficients Between LDL Particle Size and β_3-Adrenergic Receptor Arg Alleles

	Correlation Coefficient (*r*) (n = 136)	*F* Value	*P* Value
LDL particle size and the number of Arg alleles	−.226	7.23	.0081
After adjusting for triglycerides (Ln)	−.164	3.70	.058
BMI	−.175	4.22	.043
HbA_{1C}	−.180	4.46	.037
IRI	−.168	3.87	.052
HDL	−.182	4.53	.036
HOMA-R	−.152	3.13	.078
Triglycerides (Ln), BMI, insulin, and HbA_{1C}	−.120	1.89	.18

Abbreviations: Ln, natural logarithm; HOMA-R, homeostasis model assessment for insulin resistance.

the atherogenecity of the LDL is that smaller, more dense LDL has a greater affinity for the LDL receptor[35] and binds to the arterial wall.[36] Another explanation is that the dense LDL subfraction is more susceptible to oxidative modification, which may further enhance its atherogenecity.[37,38] In fact, malondialdehyde-modified LDL, one of the oxidized forms of LDL, was highly correlated with LDL particle size.[39] It is generally believed that small LDL particles may be associated with insulin resistance,[9,40] despite the lack of a significant relationship reported by several studies.[41] The results of the present study support the notion of a close relationship between the two.

Distribution of LDL particle size was influenced by both environmental and genetic factors. Among genetic factors, we demonstrated significant relationships with the apoE ϵ4 allele[26] and the I405V polymorphism of the cholesteryl ester transfer protein gene.[27] With regard to environmental factors, there is evidence that small LDL particles were increased in diameter after a body weight loss of 10 kg.[42] The present study showed a significant association between LDL particle size and the Trp64Arg variant in the β_3-adrenergic receptor gene. Given that both small LDL particle size and the Trp64Arg gene are related to obesity, hypertriglyceridemia, and insulin resistance, we identified which factor is a predominant determinant among the parameters to influence the LDL particle size. The multiple regression analysis selected apoE ϵ4 genotype and HbA_{1c} as well as triglyceride levels, as independent factors. In fact, according to an analysis with ANCOVA adjusted for fasting insulin, BMI, HbA_{1c}, or HOMA-R index, there was no significant effect of the β_3-adrenergic receptor genotype on the LDL particle size. We noted that adjustment with lipid levels could not cancel the significant difference in LDL particle size. Furthermore, significant correlations between LDL particle size and the number of the Arg allele disappeared after adjusting for HOMA-R index, triglyceride or fasting insulin levels or were much weaker after adjusting for BMI. An analysis of our data indicates that the association between the β_3-adrenergic receptor and LDL particle size is attributed to the close relationship of the β_3-adrenergic receptor genotype to obesity or insulin resistance.[21]

The Trp64Arg variant of the β_3-adrenergic receptor gene has been shown to have a reduced amount of cyclic adenosine monophosphate (cAMP) compared with the wild-type receptor in response to catecholamines in adipocytes.[43] Therefore, the decreased signaling results in impaired activity of the β_3-adrenergic receptor and lipolysis, and it may promote body weight gain through reducing thermogenesis and basal energy expenditure.[44] Given that the β_3-adrenergic receptors are found predominantly in deep visceral adipose cells in adults, it has been postulated that the Trp64Arg variant may result in weight gain predominantly due to visceral obesity. The influence of the β_3-adrenergic receptor variant on obesity was much different between men and women.[45] The gender-specific difference can be explained by the greater lipolytic β_3-adrenergic receptor sensitivity and enhanced lipolysis induced by the stimulants in men.[46] Therefore, obesity may induce more metabolic disturbances in men. Our findings in the present study demonstrated that the significant effect of the Trp64Arg variant in the β_3-adrenergic receptor gene was statistically observed only in men. We also obtained the same levels of the correlation coefficients in women, but there was no statistical significance, because the recruited number of women was relatively small and the prevalence of insulin resistance was less than in women.

Apart from the effect on body weight, we found a close relationship of the HOMA-R index to the Trp64Arg variant in the β_3-adrenergic receptor gene. The HOMA-R index is calculated by fasting insulin and glucose levels, but not by body weight; however, insulin resistance is very closely associated with increasing body weight. The close relationship of insulin resistance with the β_3-adrenergic receptor may be attributed to an increase in body weight resulting from obesity, because weight loss improves insulin sensitivity,[47] and obesity induces insulin resistance.[48] Based on the analysis of stepwise multiple regression regarding LDL particle size, the effect of the Arg allele on the distribution of LDL particle size is implicated to be a resultant of the higher plasma triglyceride levels, which is often accompanied by obesity and the insulin resistance syndrome. This speculation is consistent with evidence that an increase in the plasma triglycerides is associated with a shift of LDL peaks to a higher density.[49]

In conclusion, we demonstrated that the Trp64Arg variant in the β_3-adrenergic receptor gene is associated with reducing LDL particle size. The relationship is attributed to the close association of obesity and insulin resistance with LDL particle size.

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