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ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1689 – 1693
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

The Trp64Arg polymorphism of the β_3-adrenergic receptor gene is associated with increased small dense low-density lipoprotein in a rural Japanese population: the Mima study

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Received 23 February 2007; accepted 10 July 2007

Abstract

The presence of small dense low-density lipoprotein (sdLDL) is closely associated with an increased risk of developing coronary artery disease. The Trp64Arg polymorphism of the β_3-adrenergic receptor (β_3-AR) gene is a genetic marker for obesity-related traits. However, any possible association between this polymorphism and sdLDL profiles is unclear. The objective of this study is to investigate the effects of the polymorphism of the β_3-AR gene on LDL particle size and sdLDL in a rural Japanese population. Among 277 subjects, body mass index, blood pressure, fasting serum insulin levels, and insulin resistance index (fasting glucose × fasting insulin/405) were determined. The polymorphism of the β_3-AR gene was assessed by polymerase chain reaction–restriction fragment length polymorphism using buccal samples. Low-density lipoprotein particle size and sdLDL were measured with the electrophoretic separation of lipoproteins on the LipoPrint System (Quantimetrix, Redondo Beach, CA). The frequency of the β_3-AR allele was 0.19. In *Arg* carriers (*Trp*/*Arg* or *Arg*/*Arg*), the mean value of LDL particle size was smaller than that of non-*Arg* carriers (*Trp*/*Trp*) ($P < .05$). The area percentage of sdLDL was higher in *Arg* carriers ($P < .05$) than in non-*Arg* carriers. A multiple regression analysis showed that the area percentage of sdLDL was correlated with the polymorphism of the β_3-AR gene ($P < .05$), independently of age, sex, body mass index, smoking, and insulin resistance index. The present findings suggest that the β_3-AR gene polymorphism plays a role in the genetic predisposition to increased sdLDL, independently of insulin resistance.

1. Introduction

In the β_3-adrenergic receptor (β_3-AR) gene, the replacement of tryptophan by arginine (Trp64Arg) was first demonstrated in 1995 in the Pima Indian population. Those with this polymorphism have an early onset of non–insulin-dependent diabetes mellitus and a tendency to have a low metabolic rate [1]. In a study on the association of the Trp64Arg polymorphism with body mass index (BMI) in the Japanese population, BMI was found to be significantly higher in *Arg* carriers (*Trp*/*Arg* or *Arg*/*Arg*) than in non-*Arg* carriers (*Trp*/*Trp*) [2]. This polymorphism is also associated with abdominal obesity and resistance to insulin [3], an increased capacity to gain weight [4], and difficulty in weight loss [5,6].

Individuals with a predominance of small dense low-density lipoprotein (sdLDL) particles appear to be at increased risk for coronary artery disease, independent of the absolute concentration of LDL cholesterol, sex, and age [7,8]. The size of sdLDL particles has been associated with the presence of diabetes mellitus [9,10] and diabetic nephropathy [11-13].

Heterogeneity in LDL was first identified in the late 1940s with an analytical ultracentrifuge [14] and later by density gradient ultracentrifugation and gradient gel

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doi:10.1016/j.metabol.2007.07.012

electrophoresis [15,16]. The combined application of these techniques established that LDL exists in the plasma of all individuals as a small number of discrete populations or subclasses and led to a consensus on the classification of LDL subclasses based on density.

Controversy exists regarding the association between the Trp64Arg polymorphism of the β_3-AR gene and triglyceride levels. In Japanese patients with non–insulin-dependent diabetes mellitus, this polymorphism is associated with higher serum triglyceride levels and decreased high-density lipoprotein (HDL) cholesterol levels [17]. On the other hand, this polymorphism had no apparent effect on triglyceride levels in Japanese patients with schizophrenia [18].

The association between the polymorphism of the β_3-AR gene and the sdLDL profile is unclear. Thus, we investigated the effect of the polymorphism on LDL particle size and area of sdLDL.

2. Subjects and methods

2.1. Study subjects

All participants were recruited through an annual health checkup in Mima city, Tokushima prefecture, Japan. A total of 287 Japanese subjects (127 men, aged 24-88 years; 160 women, aged 27-83 years) participated in this study. All subjects were recruited from among rural community–dwelling volunteers. Eligible subjects were subjectively healthy without any known history or clinical features of metabolic, cardiovascular, kidney, and liver disease. Subjects taking drugs known to influence sdLDL were excluded. However, subjects receiving a small amount of statin such as pravastatin were included because a population-based study previously found no effects of statin use on sdLDL [19]. Medical information was carefully checked through a questionnaire and medical records by trained professionals. The study protocol was approved by the ethics committee of the National Hospital Organization Kyoto Medical Center. All the subjects signed an informed consent form after being fully informed about all aspects of the study before enrolling. After an overnight fast, body weight and height were measured using a body fat analyzer (Omron, Kyoto City, Japan). Body mass index was calculated as weight divided by squared height (kilograms per square meter). Blood pressure was measured 3 times at 10-minute intervals using a mercury sphygmomanometer. Venous blood samples were then drawn to measure blood glucose and serum insulin levels. Blood glucose was measured by the hexokinase method (Shino-Test, Tokyo, Japan), and the serum insulin concentration was assayed by chemiluminescent immunoassay (Bayer Medical, Tokyo, Japan). Serum total cholesterol levels (Wako Pure Chemical Industries, Osaka City, Japan) and HDL cholesterol, LDL cholesterol, and triglyceride levels (Daiichi Pure Chemicals, Tokyo, Japan) were determined by enzymatic methods. The insulin resistance index was calculated according to Matthews et al [20] (fasting glucose × fasting insulin/405). Medical histories were confirmed by a public heath questionnaire or interview.

2.2. Determination of LDL subfractions and particle size

Both the area percentage of LDL and the mean size of LDL particles were measured with the LipoPrint System (Quantimetrix, Redondo Beach, CA) according to the procedure of Son et al [21]. Briefly, 25 μL of serum sample and 200 μL of LipoPrint loading gel were applied to each 3% polyacrylamide gel tube and then mixed several times. Afterward, these

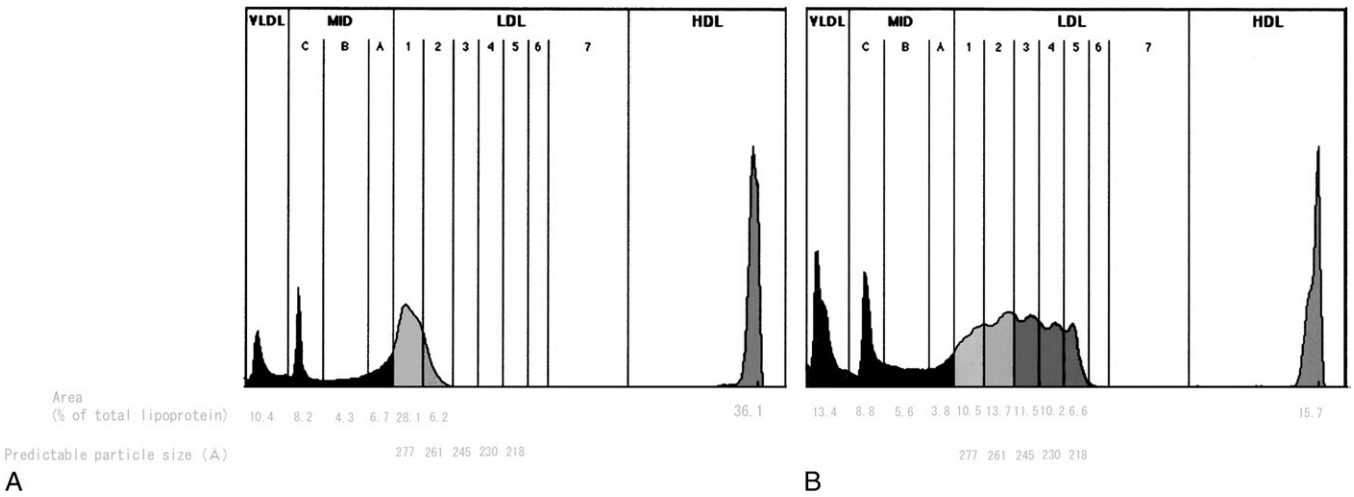

Fig. 1. A typical pattern representing sdLDL and not representing sdLDL imaged by the LipoPrint System. Lipoprotein profiles consist of 1 very low-density lipoprotein, 3 midband (MID), a maximum of 7 LDLs, and 1 HDL in the LipoPrint System. The subfractions have been named *LDL-1*, consisting of the largest particles, through *LDL-7*, consisting of the smallest particles. Individuals with mainly the larger, buoyant LDL-1 and LDL-2 subfractions have been designated as *type A* (A), whereas those with predominantly smaller subfractions (LDL-3 through LDL-7) have been designated as *type B* (B) in the LipoPrint System.

samples were photopolymerized at room temperature for 30 minutes and then electrophoresed for 65 minutes (3 mA per gel tube). After the electrophoresis was completed, scanning was done with a ScanMaker i900 (Microtek, Carson, CA); and the lipoprotein subfractions were analyzed by an iMac personal computer (Apple Computer, Cupertino, CA). Representative profiles of lipoprotein subfractions are shown in Fig. 1. Each of the LDL subfractions was calculated with an Rf between the very low-density lipoprotein fraction, whose Rf was 0.0, and the HDL fraction, whose Rf was 1.0. Low-density lipoprotein is distributed from Rf 0.32 to Rf 0.64 as 7 bands, whose Rfs are 0.32, 0.38, 0.45, 0.51, 0.56, 0.60, and 0.64; they are designated as *LDL-1* to *LDL-7*. The predictable LDL particle size from LDL-1 to LDL-7 was 27.7, 26.1, 24.5, 23.0, 21.8, 20.7, and 18.7 nm, respectively. Low-density lipoprotein 1 and LDL-2 are defined as large LDL, and LDL-3 to LDL-7 are defined as sdLDL. The determination of sdLDL was defined as a particle size of less than 25.5 nm from the LDL-3 to LDL-7 subfractions and calculated relative to the total sum of sdLDL.

2.3. Genotype measurements

Each DNA sample was obtained from the oral mucosa using a BuccalAmp DNA Extraction Kit (AR Brown, Tokyo, Japan). The Trp64Arg polymorphism of the β_3-AR gene was determined by a polymerase chain reaction (PCR)–restriction fragment length polymorphism analysis with the restriction enzyme *Mva*I according to our previously reported method [22]. The primers for PCR were 5′-CCAATACCGC-CAACACCAGT-3′ (upstream) and 5′-AGGAGTCCCAT-CACCAGGTC-3′ (downstream), which flank the whole of exon 1 of the β_3-AR gene. Genomic DNA (100 ng) in a total volume of 20 μL was used for PCR. Polymerase chain reaction was performed with initial denaturation at 94°C for 5 minutes; 30 cycles at 94°C for 30 seconds, 67°C for 30 seconds, and 72°C for 30 seconds; and a final extension at 72°C for 10 minutes. We then incubated 5 μL of the PCR product for 1 hour with 10 U of *Mva*I at 37°C in a final volume of 10 μL without further purification. The samples were run on a 3.0% agarose gel, stained with ethidium bromide, and analyzed under ultraviolet light. In the presence of the polymorphism, the restriction site of *Mva*I is lost; therefore, the allele of this polymorphism corresponds to the 158–base pair undigested band.

2.4. Statistical analysis

All of the statistical analyses were performed with the Statistical Package of Social Science (SPSS for Windows,

Table 1
Physical and biochemical characteristics of the study subjects by β_3-AR genotype

	Trp/Trp	*Trp/Arg*	*Arg/Arg*	*Trp/Arg + Arg/Arg*	*P*
n	180	89	8	97	
Sex (male/female)[a]	75/105	43/46	5/3	48/49	.212
Age (y)	64.3 ± 13.1	66.0 ± 13.0	69.0 ± 7.1	66.3 ± 12.6	.230
Smoking (none/past/current)[a]	141/13/26	61/15/13	7/0/1	68/15/14	.464
Use of statin (none/treatment)[a]	165/15	73/16	7/1	80/17	.030
BMI (kg/m^2)	24.2 ± 3.2	23.7 ± 2.9	22.8 ± 1.9	23.6 ± 2.8	.150
Fasting plasma glucose (mmol/L)	5.6 ± 1.7	5.4 ± 1.5	6.6 ± 4.4	5.5 ± 1.9	.644
Systolic blood pressure (mm Hg)	139.3 ± 20.9	137.2 ± 18.0	136.8 ± 19.8	137.2 ± 18.1	.392
Diastolic blood pressure (mm Hg)	77.9 ± 11.8	75.4 ± 10.7	76.8 ± 13.9	75.5 ± 11.0	.105
HbA_{1c} (%)	5.66 ± 1.20	5.54 ± 1.04	5.81 ± 1.01	5.56 ± 1.04	.489
HOMA-IR	2.01 ± 1.89	1.98 ± 3.80	4.02 ± 7.71	2.15 ± 4.23	.711
Total cholesterol (mmol/L)	4.79 ± 0.88	4.94 ± 0.88	4.73 ± 1.16	4.93 ± 0.91	.231
HDL cholesterol (mmol/L)	1.40 ± 0.03	1.39 ± 0.38	1.30 ± 0.45	1.39 ± 0.38	.728
Triglycerides (mmol/L)	1.10 ± 0.49	1.24 ± 0.74	1.10 ± 0.46	1.23 ± 0.72	.079
LDL subfractions (% of total lipoprotein)					
Large LDL					
LDL-1	19.01 ± 4.58	18.19 ± 4.95	18.24 ± 5.52	18.19 ± 4.97	.173
LDL-2	19.35 ± 4.59	19.86 ± 4.47	19.88 ± 4.88	19.86 ± 4.48	.373
Area of large LDL	38.35 ± 5.81	38.05 ± 6.63	38.11 ± 4.77	38.05 ± 6.48	.694
sdLDL					
LDL-3	5.46 ± 3.47	6.53 ± 3.73	6.34 ± 4.35	6.51 ± 3.76	.021
LDL-4	1.12 ± 2.07	1.91 ± 2.92 *	1.28 ± 2.37	1.86 ± 2.87	.015
LDL-5	0.24 ± 1.04	0.32 ± 0.98	0.14 ± 0.39	0.31 ± 0.95	.619
LDL-6	0.03 ± 0.20	0.01 ± 0.08	0.00 ± 0.00	0.01 ± 0.01	.352
LDL-7	0.01 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	.464
Area of sdLDL	6.86 ± 5.79	8.77 ± 6.97	7.75 ± 6.89	8.68 ± 6.93	.021
Mean of LDL particle size (nm)	26.51 ± 0.45	26.37 ± 0.50	26.46 ± 0.50	26.38 ± 0.50	.030

Data are n or means ± SD. *P* values were based on a comparison between non-*Arg* carriers (*Trp/Trp*) and *Arg* carriers (*Trp/Arg* + *Arg/Arg*). HbA_{1c} indicates hemoglobin A_{1c}.

[a] $\chi 2$ analysis was performed.

* $P = .040$ (vs *Trp/Trp*).

version 11.0, SPSS, Chicago, IL). Data are expressed as means ± SD. Student unpaired *t* test was used to analyze the difference in continuous variables between the non-*Arg* carriers (*Trp*/*Trp*) and the *Arg* carriers (*Trp*/*Arg* or *Arg*/*Arg*). A multiple regression analysis controlled for age, sex, BMI, and a history of cigarette smoking was performed to evaluate the effects of the β_3-AR gene polymorphism on LDL subfractions. $P < .05$ was accepted as statistically significant.

3. Results

The frequency of the Trp64Arg allele was 0.19 with respect to the β_3-AR polymorphism. One hundred eighty (65.0%) subjects were Trp64 homozygotes (*Trp*/*Trp*), 89 (32.1%) were Trp64Arg heterozygotes (*Trp*/*Arg*), and 8 (2.9%) were Arg64 homozygotes. This distribution was in Hardy-Weinberg equilibrium.

The physical and biochemical characteristics of all the subjects are listed in Table 1. There were no differences between non-*Arg* carriers (*Trp*/*Trp*) and *Arg* carriers (*Trp*/*Arg* or *Arg*/*Arg*) in sex distribution, age, BMI, fasting plasma glucose levels, systolic blood pressure, diastolic blood pressure, hemoglobin A_{1c}, total cholesterol levels, HDL cholesterol levels, and homeostasis model assessment of insulin resistance (HOMA-IR). Triglyceride levels were higher, but not significantly so, in the carriers ($P = .079$).

The mean size of LDL particles was significantly smaller in *Arg* carriers than in non-*Arg* carriers ($P < .05$, Table 1). In the LDL subfraction, the area of sdLDL was larger in the carriers (Table 1). Although the prevalence of the use of statin in *Arg* carriers (16/89 [18.0%] in *Trp*/*Arg*, 1/8 [12.5%] in *Arg*/*Arg* genotype) was higher than that in non-*Arg* carriers (15/180 [8.3%] in *Trp*/*Trp*) ($P = .030$, Table 1), the area of sdLDL was larger in *Arg* carriers than in non-*Arg*; and the sdLDL particle size was not different by carrying *Arg* among subjects with statin use.

When adjustments were made for age, sex, BMI, smoking history, and HOMA IR in a multiple regression analysis, a significant positive association was found between the Trp64Arg polymorphism of the β_3-AR gene and the area of sdLDL ($P < .05$, Table 2).

Table 2
The area of sdLDL and correlated variables in a multiple regression analysis

Explanatory variable	Regression coefficient (95% CI)	*P*
Intercept	5.023 (−2.837~12.883)	.209
Age	−0.003 (−0.063~0.058)	.925
Sex	1.388 (−0.444~3.220)	.137
BMI	0.056 (−0.193~0.305)	.660
Smoking	−0.154 (−2.317~2.010)	.889
HOMA IR	0.122 (−0.136~0.379)	.353
Arg allele	1.741 (0.164~3.319)	.031

Smoking was scored as follows: never/past = 0, current = 1. CI indicates coefficient interval.

4. Discussion

First, we investigated the relationship between the Trp64Arg polymorphism of the β_3-AR gene and the area percentage of sdLDL. The allelic frequency of Trp64Arg was 0.19. This result is consistent with previous studies on Japanese subjects [6,22-26]. This polymorphism may play an important role in Japanese people as well as Pima Indians.

Second, the mean size of LDL particles was smaller in *Arg* carriers than in non-*Arg* carriers (Table 1). This result is consistent with a previous study [27], although the method used in the determination of LDL particle size (at the peak size by electrophoresis using nondenaturing 2% to 16% polyacrylamide gradient gels in plasma samples [28], as originally described by Krauss and Burke [15]) was different. For the LipoPrint System, this is the first description that the area of sdLDL was larger in *Arg* carriers than in non-*Arg* carriers, as shown in Table 1. In this study, whereas the prevalence of the use of statin was higher in *Arg* carriers than in non-*Arg* carriers, the area of sdLDL was larger in *Arg* carriers than in non-*Arg* carriers. In taking together the previous result on weak effects of statin on sdLDL in a general population [19], we believe that the statin use did little to affect our study results. Moreover, the Trp64Arg polymorphism of the β_3-AR gene is significantly associated with the area of sdLDL, independently of insulin resistance. Okumura et al [27] reported that the significant correlation between LDL particle size and the number of *Arg* alleles disappeared after adjusting for the HOMA-IR, triglyceride level, or fasting insulin level, or was weaker after adjusting for BMI. These results indicated that the association between the β_3-AR gene and LDL particle size is attributable to a close relationship of the polymorphism of the β_3-AR gene to obesity or insulin resistance. This discrepancy between our findings and the data of Okumura et al [27] may be explained by the difference in study subjects and the environment where they live. Packard and Shepherd [29] explained the mechanism for the generation of sdLDL, but the underlying details were unclear. The Trp64Arg polymorphism of the β_3-AR gene is reported to be associated with weaker lipolytic activity [30]. Therefore, as a possible mechanism, we speculate that the Trp64Arg polymorphism acts as a dominant negative mutation in a heterozygous manner and that this contributes to further triglyceride accumulation in the liver with a decrease in lipolytic activity, resulting in the production of sdLDL.

In conclusion, the present findings suggest that the amount of sdLDL may increase in *Arg* carriers, independently of insulin resistance. Further studies will be needed to clarify the relationship between the Trp64Arg polymorphism of the β_3-AR gene and sdLDL.

Acknowledgments

We thank Mr Masaru Asahara (Mitsubishi-Kagaku Bio-Clinical laboratory) for technical advice on the LipoPrint

LDL System and helpful discussions, and Ms Hiroko Tabe (Cantabery) for statistical analysis. We also thank Ms Megumi Harada, Ms Junko Izumi (Mima City Koyadaira General Office), Mr Fumiaki Eguchi, Ms Kazue Amaki, Ms Akemi Kawaguchi, Ms Kiyoko Sako, and Mr Makoto Hote (Mima City National Health Insurance Koyadaira Clinic) for technical assistance. This study was supported in part by a grant-in-aid from the Foundation for the Development of the Community, Japan.

References

[1] Walston J, Silver K, Bogardus C, Knowler WC, Celi FS, Austin S, et al. Time of onset of non–insulin-dependent diabetes mellitus and genetic variation in the beta 3-adrenergic-receptor gene. N Engl J Med 1995;333:343-7.

[2] Kadowaki H, Yasuda K, Iwamoto K, Otabe S, Shimokawa K, Silver K, et al. A mutation in the beta 3-adrenergic receptor gene is associated with obesity and hyperinsulinemia in Japanese subjects. Biochem Biophys Res Commun 1995;215:555-60.

[3] Widen E, Lehto M, Kanninen T, Walston J, Shuldiner AR, Groop LC. Association of a polymorphism in the β_3-adrenergic receptor gene with features of the insulin resistance syndrome in Finns. N Engl J Med 1995;333:348-51.

[4] Clement K, Vaisse C, Manning BSJ, Basdevant A, Guy-Grand B, Ruiz J, et al. Genetic variation in the β_3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. N Engl J Med 1995;333:352-4.

[5] Yoshida T, Sakane N, Umekawa T, Sakai Y, Takahashi T, Kondo M. Mutation of β3-adrenergic receptor gene and response to treatment of obesity (letter). Lancet 1995;346:1433-4.

[6] Sakane N, Yoshida T, Umekawa T, Kogure A, Takakura Y, Kondo M. Effects of Trp64Arg mutation in the beta 3-adrenergic receptor gene on weight loss, body fat distribution, glycemic control, and insulin resistance in obese type 2 diabetic patients. Diabetes Care 1997;20:1887-90.

[7] Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 1988;260:1917-21.

[8] Crouse JR, Parks JS, Schey HM, Kahl FR. Studies of low density lipoprotein molecular weight in human beings with coronary artery disease. J Lipid Res 1985;26:566-74.

[9] Haffner SM, Mykkanen L, Stern MP, Paidi M, Howard BV. Greater effect of diabetes on LDL size in women than in men. Diabetes Care 1994;10:1164-71.

[10] Singh AT, Rainwater DL, Haffner SM, VandeBerg JL, Shelledy WR, Moore Jr PH, et al. Effect of diabetes on lipoprotein size. Arterioscler Thromb Vasc Biol 1995;15:1805-11.

[11] Yoshino G, Hirano T, Kazumi T. Atherogenic lipoproteins and diabetes mellitus. J Diabetes Complications 2002;16:29-34 [Review].

[12] Matsui J, Tamasawa N, Tanabe J, Kasai N, Murakami H, Yamato K, et al. LDL particle size and lipid composition are risk factors for microalbuminuria in normotensive and normocholesterolemic patients with type 2 diabetes. Diabetes Res Clin Pract 2004;66: 229-36.

[13] Jenkins AJ, Rowley KG, Lyons TJ, Best JD, Hill MA, Klein RL. Lipoproteins and diabetic microvascular complications. Curr Pharm Des 2004;10:3395-418.

[14] Gofman JW, Frank T, Lindgren H. Ultracentrifugal studies of lipoproteins of human serum. J Biol Chem 1949;179:973-9.

[15] Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J Lipid Res 1982;23: 97-104.

[16] Griffin BA, Caslake MJ, Yip B, Tait GW, Packard CJ, Shepherd J. Rapid isolation of low density lipoprotein (LDL) subfractions from plasma by density gradient ultracentrifugation. Atherosclerosis 1990;83:59-67.

[17] Sakane N, Yoshida T, Yoshioka K, Nakamura Y, Umekawa T, Kogure A, et al. Beta 3-adrenoreceptor gene polymorphism: a newly identified risk factor for proliferative retinopathy in NIDDM patients. Diabetes 1997;46:1633-6.

[18] Nagano T, Matsuda Y, Tanioka T, Yoshioka T, Hiroi T, Yoshikawa K, et al. No association of the Trp64Arg mutation of the beta3-adrenergic receptor gene with obesity, type 2 diabetes mellitus, hyperlipidemia, and hypertension in Japanese patients with schizophrenia. J Med Invest 2005;52:57-64.

[19] Blake GJ, Albert MA, Rifrai N, Ridker PM. Effect of pravastatin on LDL particle concentration as determined by NMR spectroscopy: a substudy of a randomized placebo controlled trial. Eur Heart J 2003;24:1843-7.

[20] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.

[21] Son JE, Lee W, Min WK, Chun S, Lee YW, Park H. Distribution of low-density lipoprotein particle size in healthy Korean adult males. Clin Chem Lab Med 2004;42:235-7.

[22] Sakane N, Yoshida T, Umekawa T, Kondo M, Sakai Y, Takahashi T. Beta 3-adrenergic-receptor polymorphism: a genetic marker for visceral fat obesity and the insulin resistance syndrome. Diabetologia 1997;40:200-4.

[23] Kadowaki H, Yasuda K, Iwamoto K, Otabe S, Shimokawa K, Silver K, et al. A mutation in the beta 3-adrenergic receptor gene is associated with obesity and hyperinsulinemia in Japanese subjects. Biochem Biophys Res Commun 1995;215:555-60.

[24] Fujisawa T, Ikegami H, Yamato E, Takekawa K, Nakagawa Y, Hamada Y, et al. Association of Trp64Arg mutation of the beta3-adrenergic-receptor with NIDDM and body weight gain. Diabetologia 1996;39:349-52.

[25] Yoshioka K, Yoshida T, Sakane N, Umekawa T, Takahashi T, Sakai Y, et al. Association of Trp64Arg mutation of the beta 3-adrenergic receptor gene with NIDDM, current and maximal body mass index. Diabetologia 1996;39:1410-1.

[26] Ueda K, Tanizawa Y, Oota Y, Inoue H, Kizuki N, Inoue H, et al. Prevalence of the Trp64Arg missense mutation of the beta3-adrenergic receptor gene in Japanese subjects. Metabolism 1997;46:199-202.

[27] Okumura K, Matsui H, Ogawa Y, Takahashi R, Matsubara K, Imai H, et al. The polymorphism of the beta3-adrenergic receptor gene is associated with reduced low-density lipoprotein particle size. Metabolism 2003;52:356-61.

[28] Okumura K, Matsui H, Kawakami K, Numaguchi Y, Hayakawa M, Morishima I, et al. Low density lipoprotein particle size is associated with glycosylated hemoglobin levels regardless of plasma lipid levels. Intern Med 1998;37:273-9.

[29] Packard CJ, Shepherd J. Lipoprotein heterogeneity and apolipoprotein B metabolism. Arterioscler Thromb Vasc Biol 1997;17:3542-56.

[30] Umekawa T, Yoshida T, Sakane N, Kogure A, Kondo M, Honjyo H. Trp64Arg mutation of beta3-adrenoceptor gene deteriorates lipolysis induced by beta3-adrenoceptor agonist in human omental adipocytes. Diabetes 1999;48:117-20.