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Association between I/D Polymorphism of Human LRPAP1 Gene and Body Mass Index in Korean General Population

Byung Yong Kang[1], Hak Gyoon Bae[2], Hae Kyung Jhin[3], Kyung Soon Lee[4] and Kang Oh Lee[5]

[1]*Research Institute for Life Science, Sahmyook University, Seoul 139-742, Korea*
[2]*Graduate School of Health Science and Social Welfare, Sahmyook University, Seoul 139-742, Korea*
[3]*Dept. of Child and Adolescent Psychiatry, Seoul National Hospital, Seoul 143-711, Korea*
[4]*Dept. of Nursing, Sahmyook University, Seoul 139-742, Korea*
[5]*Dept. of Life Science, Sahmyook University, Seoul 139-742, Korea*

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Abstract. The aim of this study was to estimate the influence of 37 bp insertion/deletion (I/D) polymorphism of the low density lipoprotein receptor-related protein-associated protein 1 (LRPAP1) gene on anthropometrical or biochemical parameters in korean general population. To determine the frequency of the genotype, we analyzed 244 samples of Korean origin. The frequency of the I allele was 0.55 in men and 0.56 in women, which were significantly higher than the frequency (0.26) that was reported in Czech population of Caucasian origin. In addition, the I allele of this polymorphism was significantly associated with higher value of body mass index (BMI) in our subjects by ANOVA test ($P<0.05$), and this association was maintained after controlling for age and gender by ANCOVA test ($P<0.05$). Thus, our results suggest that the I/D polymorphism of the LRPAP1 gene may be useful as a genetic marker for obesity in Korean general population.

Keywords: Body Mass Index, Genotype, LRPAP1.

INTRODUCTION

The lipoprotein receptor-associated protein (RAP) is a 39 kD intracellular protein discovered as a subunit of lipoprotein receptor-related protein (LRP) isolated by affinity purification (Ashcom *et al.*, 1990; Jensen *et al.*, 1989), and serves as a molecular chaperone within the endoplasmic reticulum (EM) to assist the folding of certain LDL receptor family members (Willnow *et al.*, 1996).

The human low-density lipoprotein receptor-related protein-associated protein (LRPAP1) gene encoding for this protein is located in chromosome 4p16.3, and composed of 8 exons seperated by 7 introns (Korenberg *et al.*, 1994; Van Leuven *et al.*, 1995). To date, numerous genetic variations in LRPAP1 gene have been documented (Van Leuven *et al.*, 1998), but there was a few report on clinical role of these genetic variations.

Beneš *et al.* (2000) firstly reported that the 37 bp insertion/deletion (I/D) polymorphism of this LRPAP1 gene was associated with plasma apolipoprotein AI (apoAI) and high dernsity lipoprotein (HDL) cholesterol concentrations in 515 male patients with angiographically proven coronary artery disease (CAD) of Caucasian Czech origin, suggesting the probable role of this gene in plasma lipid and lipoprotein metabolism. However, no other studies have confirmed those findings.

The goals of our present study were: (1) to determine the gene frequencies of I/D polymorphism in LRPAP1 gene for men and women samples taken from ethnically homogeneous Korean general population, (2) to investigate the impact of this polymorphism on plasma lipid levels, and (3) to evaluate that this polymorphism confer susceptibility to obesity.

MATERIALS AND METHODS

Study Subjects

A total of 244 unrelated subjects were randomly chosen from the Dept. of Clinical Pathology, Seoul Hygiene Hospital, Seoul, Korea.

The body mass index (BMI) value was calculated by

Correspondence to: Kang Oh Lee, Dept. of Life Science, Sahmyook University, San 26-21, Kongreung-2Dong, Nowon-Gu, Seoul 139-742, Korea
E-mail: leeko@syu.ac.kr

the body weight (kg) divided by the square of the height (m^2). Obese group was defined as BMI value above 25 kg/m^2. Plasma triglyceride (TG), total cholesterol (TC) and high density lipoprotein (HDL) cholesterol concentrations were measured using enzymatic methods and a HITACHI 7150 automatic analyzer. Low density lipoprotein (LDL) cholesterol concentration was calculated by Friedewald's formula (Friedewald *et al.*, 1972).

DNA Analysis

Genomic DNA was isolated using Wizard® Genomic DNA purification kit (Promega Co. Ltd., Madison, WI, USA) from leukocyte. Polymerase chain reaction(PCR) techniques were used for I/D polymorphism of LRPAP1 gene (Beneš *et al.*, 2000). Briefly, total 50 ul of the reaction mixture contained 200~400 ng of genomic DNA, 10 *p*mol of each primer, 200 μM of each dNTP, 0.25 U of *Taq* DNA polymerase (Promega Co. Ltd., Madison, WI, USA) and buffers recommended by the manufacturer. The primer sequences for I/D polymorphism studied were; sense, 5'-GGTGTTTCTGGACACAAAGGA-3', and anti-sense, 5'-AGTGTGCGTGGAGCCTATG-3' (Beneš *et al.*, 2000). PCR amplification was performed with automated thermal cycler: one cycle at 94°C for 5 min, 30 cycles at 94°C for 1 min, at 60°C for 1 min and 72°C for 2 min with a final extension at 72°C for 10 min. Final PCR amplicons were visualized by 2% agarose gel with ethidium bromide staining. The gels were directly photographed on an UV transilluminator. The PCR amplicon of the I/D polymorphism in the LRPAP1 gene yielded a long fragment (I allele of 222 bp) or a short fragment (D allele of 185 bp) (Fig. 1).

Data Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 11.0 for Windows. Allele frequencies were calculated from genotype frequencies, and the distribution of observed genotypes was tested for Hardy-Weinberg equilibrium using the χ^2-fitness test.

The unbiased heterozygosity index (H) and polymorphism information content (PIC) value were calculated according to the formula by Bostein *et al.* (1980). H is a probability measure of the likelihood that a randomly selected subject is heterozygous for any two alleles at a given gene locus. The PIC value is an indicator of the probability that a marker locus is informative. It is estimated from the frequency of heterozygotes that are determined by the number of marker alleles and their respective frequencies.

Categorical data were evaluated using the χ^2- independence test, and continuous data by the Student's *t* - test, One-way analysis of variance (ANOVA) test or analysis of covariance (ANCOVA) test. Statistical significance was established at the P=0.05 level.

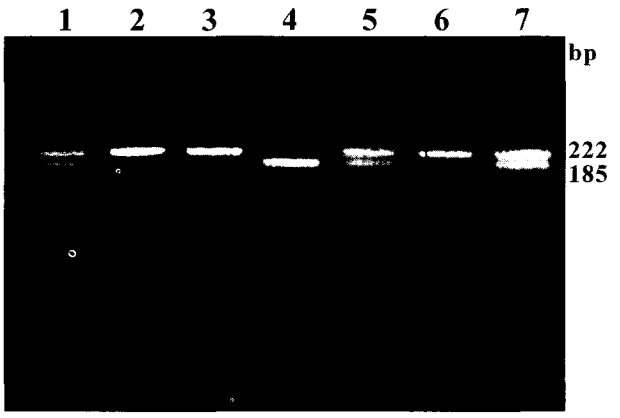

Fig. 1. I/D polymorphism of human LRPAP1 gene. Lanes 1, 5 and 7, ID heterozygotes; lanes 2, 3 and 6, II homozygotes; lane 4, DD homozygote.

RESULTS

Basic Demographics

Table 1 summarized the basic demographics of our subjects. BMI value, plasma TG, and HDL-cholesterol levels were similar between men and women, respectively. Age, plasma TC and LDL-cholesterol levels were significantly higher in women compared with men, respectively (P<0.05).

Genotype Distribution

Table 2 summarized the gene frequencies of the I/D polymorphism in the LRPAP1 gene from Korean subjects categorized by gender. The genotype distribution of the II, ID and DD were 33%, 47% and 20% in men, and 28, 53 and 19% in women, respectively, giving a I allele frequency of 0.55 for men and 0.56 for women. In

Table 1. Basic demographics of study subjects

Variables	Mean ± SD[1] (Number)		Student's *t* - test P-value
	Men	Women	
Age (year)	54.7 ± 10.5(115)	62.4 ± 11.1(129)	<0.001***
BMI (kg/m^2)[2]	23.8 ± 1.9(110)	23.5 ± 2.7(117)	0.369
TG (mg/dl)[3]	125.7 ± 83.1(83)	129.1 ± 67.5(96)	0.766
TC (mg/dl)[4]	144.8 ± 39.6(83)	156.3 ± 34.2(96)	0.038*
LDL-chol (mg/dl)[5]	91.3 ± 39.2(83)	103.2 ± 32.7(96)	0.029*
HDL-chol (mg/dl)[6]	28.1 ± 10.4(83)	27.4 ± 9.2(96)	0.604

Abbreviations: [1]Standard deviation, [2]body mass index, [3]triglyceride, [4]total cholesterol, [5]low density lipoprotein cholesterol and [6]high density lipoprotein cholesterol. *P<0.05 and ***P<0.001.

Table 2. Genotype and allele frequencies of I/D polymorphism in the LRPAP1 gene between men and women

	Genotype No.(%)			Allele No.(%)			
	II	ID	DD	I	D	H^1	PIC^2
Men	32(33)	61(47)	22(20)	127(55)	105(45)	0.4976	0.3727
Women	42(28)	61(53)	26(19)	145(56)	113(44)	0.4942	0.3711
Sum	74(30)	122(50)	48(20)	270(55)	218(45)	0.4943	0.3721
X^2		0.8843		0.0546			
Degree of freedom		2		1			
Probability[3]		0.6427		0.8152			

Abbreviations: [1]Heterozygosity and [2]Polymorphism Information Content. [3]There were no significant differences in genotype and allele frequencies between men and women groups, respectively (P>0.05).

all samples, the distribution of I/D polymorphism did not deviated from Hardy-Weinberg equilibrium. The PIC estimation of the I/D polymorphism represented the values of 0.3727 for men, and 0.3711 for women, respectively. According to the PIC value, the I/D polymorphism in the LRPAP1 gene showed relatively high genetic diversity in the both group, suggesting the usefulness of this polymorphism in performing case-control study or pedigree-based linkage analysis. When the genotype and allele frequencies were analyzed between men and women, neither the genotype nor allele frequencies of this polymorphism differed between two groups, respectively.

Effect of I/D Polymorphism on Clinical Phenotypes

Table 3 showed the comparison of clinical phenotypes among the I/D polymorphism of LRPAP1 gene in our subjects. We found a significant association between this polymorphism and BMI value (one-way ANOVA trst, P<0.05). Mean BMI values and SD were 24.6±2.1, 23.2±2.5 and 23.4±2.1 for individuals with II (n=67), ID (n=115) and DD (n=45) genotypes, respectively. To exclude the effects of other factors, which might confound the relationship between I/D polymorphism and BMI value, ANCOVA test that controlled for gender and age showed that this polymorphism was also significantly associated with BMI value (P< 0.05).

DISCUSSION

Unlike Mendelian traits, complex traits can be defined as phenotypes in which there is no a simple one-to-one relationship between genotype and phenotype (Lander and Schork, 1994). Although the identification of gene mutations linked to Mendelian traits is still a challenging task, it is relatively achievable goal and depends mostly on the availability of informative pedigrees (Deschepper *et al.*, 2002). However, genetic studies of complex traits such as obesity remain challenging because of the multiplicity of genes underlying complex traits, the modesty of the effect of each gene and the heterogeneity that occurs within human populations (Stoll *et al.*, 2000). Some estimates suggest that 40% to 70% of the variation in obesityrelated phenotypes in humans is heritable and probably the result of the interaction of multiple genes. Consequently, the effect of each single gene will be rather limited, making the search for obesity genes in humans especially challenging (Acton *et al.*, 1999). Several common polymorphisms in candidate genes studied have been associated with BMI values in humans (Bouchard and Perusse, 1996; Comuzzie and Allison, 1998; Friedman *et al.*, 1991).

In the present study, our finding showed that the I/D polymorphism of LRPAP1 gene was significantly associated with BMI value in Korean general population. Thus, this polymorphism may be useful as a genetic

Table 3. Clinical characteristics of subjects according to genotypes of the I/D polymorphism in the LRPAP1 gene

Variables	Genotypes			ANOVA[7] P-value	ANCOVA[8] P-value
	II (No.)[6]	ID (No.)	DD (No.)		
BMI(kg/m^2)[1]	24.6 ± 2.1(67)	23.2 ± 2.5(115)	23.4 ± 2.1(45)	0.001**	0.007**
TG(mg/dl)[2]	129.0 ± 75.9(58)	128.7 ± 81.7(85)	122.4 ± 55.6(36)	0.901	0.929
TC(mg/dl)[3]	148.7 ± 33.3(58)	150.2 ± 39.8(85)	156.5 ± 37.2(36)	0.593	0.633
LDL-chol(mg/dl)[4]	95.8 ± 33.7(58)	96.9 ± 37.6(85)	102.4 ± 37.4(36)	0.671	0.760
HDL-chol(mg/dl)[5]	27.1 ± 8.8(58)	27.9 ± 10.5(85)	28.2 ± 9.6(36)	0.819	0.591

[1]body mass index, [2]triglyceride, [3]total cholesterol, [4]low density lipoprotein-cholesterol, [5]high density lipoprotein-cholesterol, [6]number, [7]analysis of variance and [8]analysis of covariance. Values are mean ± SD (Standard Deviation). **P<0.01.

Table 4. Genotype and allele frequencies of I/D polymorphism in the LRPAP1 gene between lean and obese groups

	Genotype Number(%)			Allele Number(%)	
	II	ID	DD	I	D
Lean (BMI<25 kg/m2)	41(24)	93(55)	35(21)	175(52)	163(48)
Obese (BMI>25 kg/m2)	26(45)	22(38)	10(17)	74(64)	42(36)
×2		8.9426		4.5630	
Degree of freedom		2		1	
Probability		0.0114*		0.0327*	
OR (95%CI)1		II vs. ID+DD: 2.54 (1.36~4.74)			
AdOR (95%CI)2		II vs. ID+DD: 2.57 (1.36~4.84)			

[1]Odds ratio (95% confidence interval) and [2]adjusted odds ratio (95% confidence interval). *P<0.05.

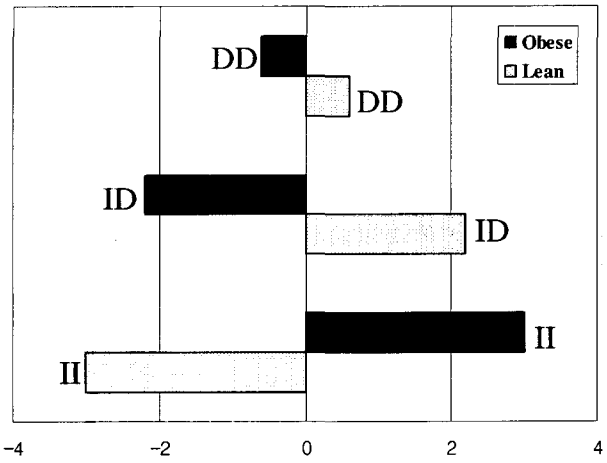

Fig. 2. Adjusted residuals of I/D polymorphism in the LRPAP1 gene between lean and obese groups.

marker to predict BMI value in Korean general population. When our subjects were divided to lean (BMI< 25 kg/m^2) and obese groups (BMI>25 kg/m^2) by BMI value, there were also significant differences in genotype and allele frequencies of I/D polymorphism between two groups, respectively (P<0.05) (Table 4). The odd ratio and 95% CI for obesity of the II genotype was 2.54 (1.36~4.74), and after adjusted for age and gender, 2.57 (1.36~4.84). From our observation, the II genotype may carry a risk for obesity by about 2.5 times in Korean general population. Fig. 2 displayed the adjusted residuals for an association between the obesity and the I/D polymorphism in the LRPAP1 gene. In this case, the II genotype indicated the highest adjusted residual value (3.0) compared with other genotypes. It appears, according to these data, that the effect of the I allele on obesity is dominant.

It is difficult to explain the mechanism for an association between the I/D polymorphism in the LRPAP1 gene and obesity in our subjects. Beneš *et al.* (2000) did not analyze the relationship between I/D polymorphism and obesity in Czech population, but described a significant association between the I/D polymorphism and plasma apoAI or HDL-cholesterol levels in CAD group of Czech origin. Unfortunately, we could not measure the plasma apoAI level. However, similar to the report by Beneš *et al.* (2000), there was a trend toward higher plasma HDL-cholesterol levels according to the number of D alleles in our subjects. The individuals with the DD genotype had the highest mean plasma HDL-cholesterol level, and ID heterozygotes had an intermediate value between those of both homozygotes, although this was not statistically significant. By Pearson correlation analyses, BMI value showed a negative correlation with plasma HDL-cholesterol level in our subjects ($r = -0.172$, $P<0.05$) (Table 5). It is therefore probable that the I/D polymorphism in the LRPAP1 gene might influence on the BMI value in Korean general population through the difference in plasma HDL metabolism among the genotypes. Another possibility is due to the location of the I/D polymorphism in intron 5 of the LRPAP1 gene (Van Leuven *et al.*, 1998). Because the I/D polymorphism in the LRPAP1 gene does not modify any amino acid sequence, the significance association between this polymorphism and the BMI value in our subjects may be explained by linkage disequilibrium with other functional polymorphisms of this gene or nearby causative gene.

However, we cannot reject the possibility that some of the differences observed in our study could be due to type I errors by multiple comparisons. Moreover, it remains to be determined whether these associations can be replicated in other populations with different ethnic and/or environmental background. Nevertheless, this study is the first report for the probable role of LRPAP1 gene in the inter-individual difference of BMI value. Thus, our data suggest that further studies should focus on weight regulation, in addition to plasma HDL metabolism in order to clarify the precise function of LRPAP1 gene.

The relative allele frequencies of I/D polymorphism in the LRPAP1 gene were remarkably different between

Table 5. Pearson correlations among clinical parameters

	Age	BMI	TG	TC	LDL-chol	HDL-chol
Age (year)		6r=0.048 7P=0.470	r=-0.018 P=0.812	r=0.062 P=0.410	r=0.109 P=0.145	r=-0.169 P=0.024*
BMI1 (kg/m2)			r=0.146 P=0.056	r=-0.043 P=0.573	r=-0.069 P=0.366	r=-0.172 P=0.024*
TG2 (mg/dl)				r=0.022 P=0.771	r=-0.302 P<0.001***	r=-0.216 P=0.004**
TC3 (mg/dl)					r=0.913 P<0.001***	r=0.397 P<0.001***
LDL-chol4 (mg/dl)						r=0.236 P=0.001**
HDL-chol5 (mg/dl)						

[1]Body mass index, [2]triglyceride, [3]total cholesterol, [4]low density lipoprotein cholesterol, [5]high density lipoprotein cholesterol, [6]Pearson correlation and [7]probability. *P<0.05, **P<0.01 and ***P<0.001.

Table 6. The distribution of I/D polymorphism in the LRPAP1 gene between Czech and Korean ethnic groups

Population	Genotype Number (%)			Allele Number(%)	
	II	ID	DD	I	D
Czech[1]	40(8)	191(37)	284(55)	271(26)	759(74)
Korean[2]	74(30)	122(50)	48(20)	270(55)	218(45)
X^2		110.4278		120.2862	
Degree of freedom		2		1	
Probability		1.0493×10^{-24}***		5.4762×10^{-28}***	

[1]Beneš *et al.*, (2000) and [2]our study. ***P<0.001.

Koreans and Caucasians, possibly due to random genetic drift and/or natural selection in this gene (P< 0.05) (Table 6). The I allele frequency (0.55) in Korean population was significantly higher than that of previously reported in Caucasian Czech population (0.26), suggesting that this increase in BMI value effects a large percentage of the Korean population.

In summary, the I/D polymorphism in the LRPAP1 gene appears to show an association with BMI value in Korean general population. Also, the I allele frequency of this polymorphism in Korean population was different from that in Caucasian population, reflecting a difference in ethnic background. Further comparative studies of this polymorphism in other racial or ethnic groups should therefore prove to be of value.

REFERENCES

Acton, S., Osgood, D., Donoghue, M., Corella, D., Pocovi, M., Cenarro, A., Mozas, P., Keity, J., Squazzo, S., Woolf, E.A. and Ordovas, J.M. (1999): Association of polymorphisms at the SR-BI gene locus with plasma lipid levels and body mass index in a white population, *Arterioscler. Thromb. Vasc. Biol.*, **19**, 1734-1743.

Ashcom, J.D., Tiller, S.E., Dickerson, K., Cravens, J.L., Argraves, W.S. and Strickland, D.K. (1990): The human alpha 2-macroglobulin receptor: Identification of a 420-kD cell surface glycoprotein specific for the activated conformation of alpha 2-macroglobulin, *J. Cell Biol.*, **110**, 1041-1048.

Beneš, P., Mužik, J., Benedik, J., Elbl, L., Znojil, V. and Vácha, J. (2000): Relation between the insertion/deletion polymorphism in the gene coding for receptor associated protein (RAP) and plasma apolipoprotein AI (apoAI) and high-density lipoprotein cholesterol (HDL) levels, *Clin. Genet.*, **57**, 309-310.

Bostein, D., White, R.L., Skolnick, M. and Davis, R.W. (1980): Construction of a genetic linkage map using restriction fragment length polymorphisms, *Am. J. Hum. Genet.*, **32**, 314-331.

Bouchard, C. and Perusse, L. (1996): Current status of the human obesity gene map, *Obes. Res.*, **4**, 81-90.

Comuzzie, A.G. and Allison, D.B. (1998): The search for human obesity genes, *Science*, **80**, 1374-1377

Deschepper, C.F., Boutin-Ganache, I, Zahabi, A. and Jiang, Z. (2002): In search of cardiovascular candidate genes: Interactions between phenotypes and genotypes, *Hypertension*, **39**, 332-336.

Friedewald, W.T., Levy, R.L. and Friedrickson, D.S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge, *Clin. Chem.*, **18**, 499-502.

Frideman, J.M., Leibel, R.L., Bahary, N., Siegel, D.A. and Truett, G. (1991): Genetic analysis of complex disorders: molecular mapping of obesity genes in mice and humans, *Ann. N. Y. Acad. Sci.*, **630**, 100-115.

Jensen, P.H., Moestrup, S.K. and Gliemann, J. (1989): Purification of the human placental alpha 2-macroglobulin receptor, *FEBS Lett.*, **255**, 275-280.

Korenberg, J.R., Argraves, K.M., Chen, X.N., Tran, H., Strickland, D.K. and Argraves, W.S. (1994): Chromosomal localization of human genes for the LDL receptor family member glycoprotein 330 (LRP2) and its associated protein RAP (LRPAP1), *Genomics*, **22**, 88-93.

Lander, E.S. and Schork, N.J. (1994): Genetic dissection of complex traits, *Science*, **265**, 2037-2048.

Stoll, M., Kwitek-Black, A.E., Cowley, A.W. Jr., Harris, E.L., Harrap, S.B., Krieger, J.E., Printz, M.P., Provoost, A.P., Sassard, J. and Jacob, H.J. (2000): New target regions for human hypertension via comparative genomics, *Genome*

Res., **10**, 473-482.

Van Leuven, F., Thiry, E., Stas, L. and Nelissen, B. (1998): Analysis of the human LRPAP1 gene coding for the lipoprotein receptor-associated protein: Identification of 22 polymorphisms and one mutation, *Genomics*, **52**, 145-151.

Van Leuven, F., Hilliker, C., Serneels, L., Umans, L., Overbergh, L., De Strooper, B., Fryns, J.P. and Van den Berghe, H. (1995): Cloning, characterization, and chromosomal localization to 4p16 of the human gene (LRPAP1) coding for the alpha 2-macroglobulin receptor-associated protein and structural comparison with the murine gene coding for the 44-kDa heparin-binding protein, *Genomics*, **25**, 492-500.

Willnow, T.E., Rohlman, A., Horton, J., Otani, H., Braun, J.R., Hammer, R.E. and Herz, J. (1996): RAP, a specialized chaperone, prevents ligand-induced ER retention and degradation of LDL receptor-related endocytic receptors, *EMBO J.*, **15**, 2632-2639.