Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1345 – 1349
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Association of Pro12Ala polymorphism in the peroxisome proliferator–activated receptor γ2 gene with small dense low-density lipoprotein in the general population

Taku Hamada[a], Kazuhiko Kotani[a,b], Kokoro Tsuzaki[a], Yoshiko Sano[a], Takashi Murata[c], Masahiko Tabata[d], Shigeaki Sato[e], Naoki Sakane[a,e,*]

[a]*Department of Preventive Medicine and Diabetes Education, Clinical Research Institute for Endocrinology and Metabolic Disease, National Hospital Organization Kyoto Medical Center, Kyoto 612-8555, Japan*
[b]*Division of Health Administration and Promotion, Faculty of Medicine, Tottori University, Tottori 683-8503, Japan*
[c]*Center for Diabetes, National Hospital Organization Kyoto Medical Center, Kyoto 612-8555, Japan*
[d]*Himeji National Health Insurance Ieshima Clinic, Himeji 672-0102, Japan*
[e]*Division of Molecular Epidemiology, Graduate School of Medicine, Kobe University, Kobe 650-0017, Japan*

Received 13 February 2007; accepted 4 May 2007

Abstract

The Pro12Ala polymorphism of the peroxisome proliferator–activated receptor γ2 (PPARγ2) gene has been reported to predict a lower risk for developing type 2 diabetes mellitus. However, its effect on the lipid profile has been disputable. Among low-density lipoproteins, small dense low-density lipoprotein (sdLDL) particles have been linked to a greater risk for coronary artery disease. The purpose of this study was to investigate the genetic effect of the Pro12Ala polymorphism in the PPARγ2 gene on the presence of sdLDL in the general Japanese population. In 379 subjects (aged 54 ± 13 years), body mass index, percentage of body fat, blood pressure, and biochemical profiles were measured. Pro12Ala polymorphism was genotyped by polymerase chain reaction–restriction fragment length polymorphism. The area of sdLDL subfractions (sdLDL4-7) was analyzed by high-resolution polyacrylamide gel electrophoresis. The frequency of the Ala12 allele in PPARγ2 was 0.04. There was no difference in total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol levels between genotypes. However, subjects with the X/Ala genotype (Pro/Ala + Ala/Ala) had significantly higher serum triglyceride levels ($P = .001$) and a larger area of sdLDL4-7 ($P = .002$) than those with the Pro/Pro genotype. Multiple regression analysis revealed that the Ala12 allele was a significant variable contributing to the variance in the increased area of sdLDL4-7 ($P = .040$). In conclusion, the Pro12Ala polymorphism in the PPARγ2 gene was positively associated with an enlarged area of sdLDL4-7. This polymorphism may play a role in the genetic predisposition to increases in sdLDL4-7.
© 2007 Elsevier Inc. All rights reserved.

1. Introduction

Peroxisome proliferator–activated receptor γ (PPARγ) plays an important role in adipocyte differentiation, glucose, lipid metabolism, and fat-specific gene expression [1]. A common Pro12Ala polymorphism in the PPARγ2 gene has been well documented to predict lower risk for developing type 2 diabetes mellitus. In fact, a recent classic meta-analysis based on data from nondiabetic individuals has revealed that in selected groups, such as whites and obese subjects, the Ala variant was associated with higher insulin sensitivity [2]. However, studies on the association between the Pro12Ala polymorphism and lipid profiles have been controversial. Several studies have shown the effect of Pro12Ala polymorphism on lower lipid and lipoprotein levels [3-6], but others have yielded inconsistent results [7-9].

Previous studies have demonstrated the clinical importance of small dense low-density lipoprotein (sdLDL) particles that are highly predictive of increased risk for coronary artery disease [10-12] and a risk factor for the

* Corresponding author. Department of Preventive Medicine and Diabetes Education, Clinical Research Institute for Endocrinology and Metabolic Disease, National Hospital Organization Kyoto Medical Center, Kyoto 612-8555, Japan. Tel.: +81 75 641 9161; fax: +81 75 645 2781.
E-mail address: nsakane@kyotolan.hosp.go.jp (N. Sakane).

0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.05.017

future development of type 2 diabetes mellitus [13]. sdLDL particles possess a lower binding affinity for cellular low-density lipoprotein (LDL) receptors and are more easily oxidized in vitro [14,15]. These findings suggest that sdLDL particles are more atherogenic than larger LDL particles because their lower binding affinity for LDL receptor reflects a longer plasma residence time for them to be oxidized and taken up by macrophages in extravascular spaces. In line with these findings in vitro, case-control studies have indicated that sdLDL particles are predominant in a high proportion of coronary artery disease patients, even those with normal LDL levels [10,16]. Subjects with sdLDL particles have a 2- to 3-fold greater risk of developing coronary artery disease [10,11]. To the best of our knowledge, no previous study has investigated whether the Pro12Ala polymorphism contributes to the presence of sdLDL. In the present study, the association of the Pro12Ala polymorphism in the PPARγ2 gene with serum lipoprotein profiles accompanied by the predominance of sdLDL was investigated in the general Japanese population.

2. Methods

2.1. Subjects

A total of 379 subjects, aged 54 ± 13 years (mean ± SD), were recruited from community-dwelling volunteers in an annual health checkup of residents of Ieshima town on the Inland Sea of Japan. The study protocol was approved by the ethics committee of Kobe University. All subjects were instructed about the study and gave their informed consent to participate. To evaluate lifestyle habits, each subject completed a self-administered questionnaire regarding the medical history, alcohol drinking, smoking, exercise habits, medications, and other lifestyle-related factors [17]. Alcohol drinking was assessed from the frequency of drinking on a weekly basis. With respect to smoking, individuals were classified according to whether the respondent was a non-smoker, a past smoker, or a current smoker. Exercise habits were determined from the frequency of physical exercise of more than 3 metabolic equivalents on a weekly basis. Other lifestyle factors including the consumption of balanced meals, breakfast, snacks between meals, and beverages containing caffeine were also checked. Eligible subjects had to be subjectively healthy without any known history and clinical features of metabolic, cardiovascular, kidney, or liver disease. After an overnight fast, body weight and height were measured using a body fat analyzer (Omron, Kyoto, Japan). Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. 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 other serum profiles. Blood glucose was measured by the hexokinase method (Shino-Test, Tokyo, Japan). The serum total cholesterol (Wako Pure Chemical Industries, Tokyo, Japan), high-density lipoprotein (HDL) cholesterol, LDL cholesterol, and triglyceride levels were determined by enzymatic methods (Daiichi Pure Chemicals, Tokyo, Japan).

2.2. Determination of serum lipoprotein profiles

The lipoprotein profiles were measured with a LipoPrint system (Quantimetrix, Redondo Beach, CA) [18]. The profiles consist of 1 fraction of very low-density lipoprotein (VLDL), 3 of midbands, 7 of LDL, and 1 of HDL in the LipoPrint system. Briefly, 25 μL of serum sample and 200 μL of liquid loading gel containing a lipophilic dye to stain cholesterol in each lipoprotein were applied to a 3% polyacrylamide gel tube and then mixed several times by vigorously inverting. Afterward, these samples were photopolymerized at room temperature for 30 minutes and then electrophoresed for 65 minutes (3 mA per gel tube). After the electrophoresis, scanning was done with a ScanMaker i900 (Microtek, Osaka, Japan); and the analysis of lipoprotein subfractions, VLDL, midbands, LDL, and HDL was simultaneously performed by using a Quantimetrix proprietary software program. The various stained bands of lipoprotein fractions were identified by their mobility (relative mobility) using VLDL as the starting reference point (VLDL = 0) and HDL as the leading reference point (HDL = 1). *sdLDL* was defined as the LDL4 through LDL7 subfractions and was calculated relative to the total area proportion of the sdLDL subfraction.

2.3. Genotyping of the Pro12Ala polymorphism in the PPARγ2 gene

Genomic DNA was extracted from peripheral blood leukocytes. Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) amplification of the segment with the Pro12Ala polymorphism in the PPARγ2 gene was carried out with a total volume of 25 μL containing 100 ng of genomic DNA, 5 pmol of each primer, and Taq DNA polymerase. The PCR-RFLP conditions were as follows: initial denaturation at 94°C for 3 minutes followed by 40 cycles of denaturation for 30 seconds, annealing at 53°C for 30 seconds, and extension at 72°C, and a final extension at 72°C for 9 minutes. The polymorphism was genotyped using the following primers: 5′-GCCAATTCAAGCCCAGTC-3′ (forward) and 5′-GATATGTTTGCCTTTCCG-3′ (reverse). The samples were run on a 3.0% agarose gel, stained with ethidium bromide, and analyzed under ultraviolet light. The PCR product size is 273 base pairs. The RFLP was detected after digestion overnight with 2 units of *Bst*U1 [19].

2.4. Statistical analysis

All of the statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS for Windows, version 11.0; SPSS, Chicago, IL). Data are expressed as means ± SD. The Student unpaired *t* test was used to analyze the difference in continuous variables between the genotypes. Allele frequencies were estimated

by the gene-counting method. Sex and smoking between the genotypes were compared with the χ^2 test. Hardy-Weinberg equilibrium was also checked by χ^2 test. A multiple regression analysis was used to evaluate the genetic impact of the Pro12Ala polymorphism in the PPARγ2 gene on the area of sdLDL4-7. $P < .05$ was accepted as statistically significant.

3. Results

In Table 1, the distribution of the Pro/Pro, Pro/Ala, and Ala/Ala genotypes was 353, 25, and 1, respectively. The frequency of the Ala12 allele was 0.04. All genotypes were in Hardy-Weinberg equilibrium. Because the number of subjects with the Ala/Ala genotype was only one, the Ala/Ala genotype was combined with subjects with the Pro/Ala genotype (X/Ala genotype). There was no significant difference in age, BMI, percentage of body fat, blood pressure, total cholesterol, LDL cholesterol, and HDL cholesterol between the 2 different genotypes. In this study, only 5% of diabetes was classified by the American Diabetes Association criteria [20]; and there was no difference in fasting blood glucose, hemoglobin A_{1c}, and the prevalence of diabetes between genotypes. However, subjects with the X/Ala genotypes had a significantly higher serum triglyceride level than those with the Pro/Pro genotype ($P < .001$). There was no significant difference in HDL, VLDL, and the area of large LDL (LDL1 and LDL2) between the 2 different genotypes. In the X/Ala genotypes, the area of sdLDL subfractions (sdLDL4-7) was significantly larger than that in the Pro/Pro genotype ($P < .01$). The average LDL particle size tended to be smaller than that in the X/Ala genotype, but there was no statistical significance. In addition, there was no difference between genotypes in lifestyle-related factors including exercise habits, alcohol intake, smoking, balanced meals, daily breakfast, snacks between meals, beverages containing caffeine, and medications for hyperlipidemia (data not shown).

In Table 2, a multiple regression analysis revealed that BMI and the Ala12 allele were significantly associated with an enlarged area of sdLDL4-7 ($P < .05$) when adjustments were made for age, sex, BMI, exercise habits, alcohol intake, smoking, and serum lipid concentration.

Table 1
Physical, clinical, and lipoprotein profiles of subjects according to genotype of the PPARγ2 Pro12Ala polymorphism

Genotype	Pro/Pro	X/Ala	*P*
n	353	26	
Sex (male/female)	75/278	3/23	.237
Smoking (never, past/current)	289/64	22/4	.725
Age (y)	54 ± 13	53 ± 16	.177
BMI (kg/m^2)	24.1 ± 4.6	24.4 ± 5.5	.265
Body fat (%)	29.4 ± 6.1	30.7 ± 5.7	.865
Systolic blood pressure (mm Hg)	130 ± 17	129 ± 16	.542
Diastolic blood pressure (mm Hg)	75 ± 9	73 ± 9	.722
Hemoglobin A_{1c} (%)	5.2 ± 0.5	5.3 ± 0.5	.781
Fasting blood glucose (mmol/L)	5.44 ± 0.88	5.56 ± 0.88	.437
Total cholesterol (mmol/L)	5.66 ± 0.97	5.61 ± 0.96	.699
HDL cholesterol (mmol/L)	1.60 ± 0.37	1.64 ± 0.46	.673
LDL cholesterol (mmol/L)	2.86 ± 0.75	2.77 ± 0.70	.711
Triglyceride (mmol/L)	3.20 ± 2.27	3.78 ± 3.55	.001
Area of large LDL (%)			
LDL1	12.84 ± 3.75	11.73 ± 4.08	.677
LDL2	10.40 ± 4.29	10.39 ± 3.78	.067
LDL 1-2	23.24 ± 4.67	22.12 ± 4.57	.821
Area of sdLDL (%)			
sdLDL3	2.18 ± 2.27	2.10 ± 2.38	.903
sdLDL4	0.39 ± 0.90	0.60 ± 1.43	.048
sdLDL5	0.06 ± 0.29	0.22 ± 0.77	.001
sdLDL6	0.00 ± 0.04	0.07 ± 0.025	.001
sdLDL7	0	0	–
sdLDL 4-7	0.45 ± 1.16	0.88 ± 2.38	.002
VLDL (%)	16.50 ± 4.79	17.07 ± 5.60	.051
LDL particle size (nm)	26.77 ± 0.43	26.65 ± 0.56	.266

Values are the means ± SD. *P* values compare subjects carrying the wild genotype (Pro/Pro) with those carrying the X/Ala genotype, which is a combination of the heterozygous genotype (Pro/Ala) and homozygous genotype (Ala/Ala). Sex and smoking were compared between the genotypes with the χ^2 test.

Table 2
Multiple regression analysis with sdLDL as the response variable

Explanatory variable	Regression coefficient (95% CI)	*P*
Age	−0.006 (−0.015 to 0.003)	.218
Sex	0.489 (0.023 to 0.955)	.150
BMI	0.038 (0.009 to 0.067)	.011
Exercise habits	0.016 (−0.011 to 0.042)	.381
Alcohol drinking	0.070 (−0.285 to 0.425)	.700
Smoking	0.109 (−0.423 to 0.460)	.545
HDL cholesterol	−0.013 (−0.021 to 0.005)	.002
LDL cholesterol	0.014 (0.009 to 0.018)	.001
Ala12 allele	0.489 (0.023 to 0.955)	.040

Habitual exercise was scored as follows: almost every day = 0, twice a week = 1, once a week = 2, once a month = 3, and no exercise = 4. Alcohol drinking was scored as follows: never/sometimes = 0 and almost everyday = 1. Smoking was scored as follows: never/past = 0 and current = 1. CI indicates coefficient interval.

4. Discussion

The present study provided, for the first time, new information regarding the effect of the Pro12Ala polymorphism on sdLDL profiles among community-dwelling Japanese. The most important findings were that Pro12Ala polymorphism in its homozygous and heterozygous forms may itself increase the area of sdLDL4-7 independent of potential confounding factors such as age, sex, BMI, habitual exercise, alcohol intake, and lipid concentration. The present finding that subjects with the X/Ala genotype had a lipid atherogenic profile is consistent with some previous results [8,9] but not all [3-6]. It has been proposed that the PPARγ2 gene is one of the mediators of gene-environmental interactions. For example, the Ala12 allele has been supposed to be slightly protective against type 2 diabetes

mellitus [2], whereas there are findings that the Ala12 allele may predispose obese subjects to the development of type 2 diabetes mellitus without intervention through diet and exercise [21]. It could be that beneficial changes in diet, increased physical activity, and weight loss modify the genetic impact of the Ala12 allele, possibly because of a lower incidence of type 2 diabetes mellitus and improved insulin sensitivity [21]. Therefore, differences in confounding factors such as lifestyle or in the experimental design between the present and previous studies may also partly explain the divergent results.

Although the underlying mechanisms by which PPARγ2 and variation in its gene might actually influence sdLDL4-7 remain to be elucidated, the Pro12Ala polymorphism may be linked to previous findings indicating the role of PPARγ in the lipid accumulation in atherogenesis through transcriptional induction of the scavenger receptor CD36 [22,23]. CD36 is expressed in adipose tissue and is directly induced during the differentiation of preadipocytes evoked by PPARγ. It is activated by fatty acids and may act as a transmembrane transporter of long-chain fatty acids in adipose tissue [24]. CD36 functions in a feedback loop as a receptor for oxidized LDL in which lipid components of oxidized LDL activate PPARγ and thereby induce CD36 expression [8,22,23]. In this respect, the Pro12Ala polymorphism may be associated with a reduced level of PPARγ2 activity [3]; and it is conceivable that this has an effect on the area of sdLDL4-7 in the X/Ala genotype. Furthermore, the PPARγ2 agonist pioglitazone improves LDL particle characteristics in a manner similar to diet/exercise without changing triglyceride, total cholesterol, or LDL cholesterol levels in obese, insulin-resistant, nondiabetic adults [25]. Because this effect may have implications for atherosclerotic risk [25,26], further examinations using PPARγ2 agonists in humans are needed to elucidate this point for the effect of the Pro12Ala polymorphism on the area of sdLDL4-7.

Body mass index was a significant and independent variable contributing to the variance in the increased area of sdLDL4-7. This suggests that the higher BMI as a marker of obesity may result in the increase in sdLDL4-7 independent of genetic factors. The exact mechanism behind this association is unclear. Obesity has been a major risk factor for insulin resistance, and sdLDL production may become an integral feature of insulin resistance [27,28]. Consequently, the finding raises the possibility that the area of sdLDL4-7 may increase through insulin resistance.

Based on a meta-analysis of nondiabetic individuals [2], the frequency of the PPARγ2 Ala12 allele in the present study was similar to that reported previously in Asians including Japanese and Koreans and in African American populations, but was lower than that reported in white populations including Spanish, Finnish, Danish, and Germans and in Pima Indians [2]. The prevalence of the Ala12 allele varies from 4% in Asian populations to 28% in white populations [2]. Thus, the difference in frequency of the Ala12 allele among study populations may reflect the importance of different ethnic, environmental, or other factors.

In conclusion, the Pro12Ala polymorphism in the PPARγ2 gene was positively associated with an increased area of sdLDL4-7 in the general Japanese population. The present findings suggest that the Pro12Ala polymorphism may play a role in the genetic predisposition to increases in sdLDL4-7. Because only one homozygous genotype was identified among 379 subjects, the result must be interpreted with caution.

Acknowledgment

This study was supported in part by a grant-in-aid from the Foundation for the Development of the Community, Japan.

References

[1] Auwerx J. PPARγ, the ultimate thrifty gene. Diabetologia 1999;42:1033-49.
[2] Tonjes A, Scholz M, Loeffler M, et al. Association of Pro12Ala polymorphism in peroxisome proliferator–activated receptor–γ with pre-diabetic phenotypes: meta-analysis of 57 studies on nondiabetic individuals. Diabetes Care 2006;29:2489-97.
[3] Deeb SS, Fajas L, Nemoto M, et al. A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 1998;20:284-7.
[4] Gonzalez Sanchez JL, Serrano Rios M, Fernandez Perez C, et al. Effect of the Pro12Ala polymorphism of the peroxisome proliferator–activated receptor–γ2 gene on adiposity, insulin sensitivity and lipid profile in the Spanish population. Eur J Endocrinol 2002;147:495-501.
[5] Iwata E, Matsuda H, Fukuda T, et al. Mutations of the peroxisome proliferator–activated receptor γ (PPARγ) gene in a Japanese population: the Pro12Ala mutation in PPAR γ2 is associated with lower concentrations of serum total and non-HDL cholesterol. Diabetologia 2001;44:1354-5.
[6] Iwata E, Yamamoto I, Motomura T, et al. The association of Pro12Ala polymorphism in PPARγ2 with lower carotid artery IMT in Japanese. Diabetes Res Clin Pract 2003;62:55-9.
[7] Pinterova D, Cerna M, Kolostova K, et al. The frequency of alleles of the Pro12Ala polymorphism in PPARγ2 is different between healthy controls and patients with type 2 diabetes. Folia Biol 2004;50:153-6.
[8] Meirhaeghe A, Fajas L, Helbecque N, et al. Impact of the peroxisome proliferator activated receptor γ2 Pro12Ala polymorphism on adiposity, lipids and non–insulin-dependent diabetes mellitus. Int J Obes Relat Metab Disord 2000;24:195-9.
[9] Zietz B, Barth N, Spiegel D, et al. Pro12Ala polymorphism in the peroxisome proliferator–activated receptor–γ2 (PPARγ2) is associated with higher levels of total cholesterol and LDL-cholesterol in male Caucasian type 2 diabetes patients. Exp Clin Endocrinol Diabetes 2002;110:60-6.
[10] Campos H, Genest Jr JJ, Blijlevens E, et al. Low density lipoprotein particle size and coronary artery disease. Arterioscler Thromb 1992;12:187-95.
[11] Gardner CD, Fortmann SP, Krauss RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA 1996;276:875-81.
[12] Lamarche B, Tchernof A, Moorjani S, et al. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Circulation 1997;95:69-75.

[13] Austin MA, Mykkanen L, Kuusisto J, et al. Prospective study of small LDLs as a risk factor for non–insulin dependent diabetes mellitus in elderly men and women. Circulation 1995;92:1770-8.
[14] Arad Y, Rumsey SC, Galeano NF, et al. LDL has optimal size for maximal binding to the LDL receptor. Circulation 1992;86:I-551.
[15] Tribble DL, Holl LG, Wood PD, et al. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. Atherosclerosis 1992;93:189-99.
[16] Coresh J, Kwiterovich Jr PO, Smith HH, et al. Association of plasma triglyceride concentration and LDL particle diameter, density, and chemical composition with premature coronary artery disease in men and women. J Lipid Res 1993;34:1687-97.
[17] Tsukinoki R, Morimoto K, Nakayama K, Association between lifestyle factors and plasma adiponectin levels in Japanese men. Lipids Health Dis 2005;2:4:27
[18] Hoefner DM, Hodel SD, O'Brien JF, et al. Development of a rapid, quantitative method for LDL subfractionation with use of the Quantimetrix Lipoprint LDL System. Clin Chem 2001;47:266-74.
[19] Radha V, Vimaleswaran KS, Babu HN, et al. Role of genetic polymorphism peroxisome proliferator–activated receptor–γ2 Pro12Ala on ethnic susceptibility to diabetes in South-Asian and Caucasian subjects: evidence for heterogeneity. Diabetes Care 2006; 29:1046-51.
[20] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes care 2004;27:S5-S10.
[21] Lindi VI, Uusitupa MI, Lindstrom J, et al. Association of the Pro12Ala polymorphism in the PPAR-γ2 gene with 3-year incidence of type 2 diabetes and body weight change in the Finnish Diabetes Prevention Study. Diabetes 2002;51:2581-6.
[22] Tontonoz P, Nagy L, Alvarez JG, et al. PPARγ promotes monocyte/ macrophage differentiation and uptake of oxidized LDL. Cell 1998;17: 241-52.
[23] Nagy L, Tontonoz P, Alvarez JG, et al. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ. Cell 1998;93:229-40.
[24] Abumrad NA, el-Maghrabi MR, Amri EZ, et al. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. J Biol Chem 1993;268:17665-8.
[25] Shadid S, LaForge R, Otvos JD, et al. Treatment of obesity with diet/ exercise versus pioglitazone has distinct effects on lipoprotein particle size. Atherosclerosis 2006;188:370-6.
[26] Nagashima K, Lopez C, Donovan D, et al. Effects of the PPARγ agonist pioglitazone on lipoprotein metabolism in patients with type 2 diabetes mellitus. J Clin Invest 2005;115:1323-32.
[27] Reaven GM, Chen YD, Jeppesen J, et al. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. J Clin Invest 1993;92:141-6.
[28] Lee W, Min WK, Chun S, et al. Low-density lipoprotein subclass and its correlating factor in diabetics. Clin Biochem 2003;36:657-61.