

## HindIII Polymorphism of Lipoprotein Lipase Gene and its Contribution to Coronary Artery Disease in Egyptians

<sup>1</sup>Azza K. Amer, <sup>1</sup>Manal S.Z. Moustapha, <sup>1</sup>Mehry S. EL-Sobky, <sup>2</sup>Safa Refaat, <sup>3</sup>Hany Negm,  
<sup>4</sup>Manal H. Abu-Elela, <sup>5</sup>Shahira R. Nowier

Departments of <sup>1</sup>Clinical & Chemical Pathology, <sup>2</sup>Internal Medicine, <sup>3</sup>Cardiology, <sup>4</sup>Public Health and <sup>5</sup>Genetics; Research Institute of Ophthalmology

**Abstract:** Coronary artery disease (CAD) is one of the main leading causes of death worldwide. The aim of this study is the detection of the HindIII polymorphism in patients with coronary artery disease (CAD) to determine the importance of this mutation as a risk factor. The study included forty CAD patients and twenty healthy subjects. They were genotyped for HindIII LPL using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) technique, and the findings were analyzed for association with CAD and with plasma levels of lipid parameters; total cholesterol (T-chol), low density lipoprotein- cholesterol (LDL- C), high density lipoprotein – cholesterol (HDL-C) and triglycerides (TG). Patients with HindIII +ve allele were at higher risk of developing CAD than those with -ve allele (odd's ratio: 2.8 & 95% CI 0.89-8.92). Significant predictors of CAD were HDL-C followed by serum level of TG then LDL-C. In conclusion HindIII polymorphism in LPL gene was associated with CAD severity, due to its significant implication on lipid metabolism. Further studies are required, in particular looking at the influence on clinical outcome.

**Key words:** HindIII polymorphism – Lipoprotein lipase –Coronary artery disease (CAD)

### INTRODUCTION

Coronary artery disease (CAD) is the main cause of death in the industrial nations. In the vast majority of cases, atherosclerosis, a process influenced by both environmental and genetic factors, underlies the development of CAD. Disorders of lipoprotein metabolism are considered important risk factors in the pathogenesis of atherosclerosis (Wang *et al.*, 1994). Hypertriglyceridemia is a well-established independent risk factor for CAD and the influence of several genetic variants in genes related to triglyceride (TG) metabolism has been described, including lipoprotein lipase (LPL), APOA5 and APOE genes (Ariza *et al.*, 2010).

The LPL, an enzyme discovered in 1943 (Hahn 1943), plays a central role in lipid metabolism by hydrolyzing triglyceride-rich particles in muscle, adipose tissue and macrophages, thereby generating free fatty acids and glycerol for energy utilization and storage. LPL also plays a non-catalytic bridging role as a ligand in lipoprotein-cell surface interactions and receptor-mediated uptake of lipoproteins with its ability to bind simultaneously to both lipoproteins and cell surface receptors (Goldberg 1996).

Lipoprotein lipase (LPL) is an enzyme that hydrolyzes the core triglycerides of chylomicrons and very low density lipoproteins, thereby playing a role in their metabolism. It also hydrolyzes triglycerides in the core of low density lipoprotein, thereby remodeling this lipoprotein class as well. Zilversmit first proposed that LPL may be important in atherosclerosis by hypothesizing that hydrolysis of the triglyceride-rich lipoproteins by LPL at the endothelial lining of arteries could lead to the formation of atherogenic remnants, which might be taken up by arterial wall cells and lead to cholesterol deposition.

LPL protein was detected in association with macrophage-derived foam cells, endothelial cells, adventitial adipocytes, and medial smooth muscle cells, and, to a lesser extent, in intimal smooth muscle cells and media underlying well-developed plaque. These results indicate that macrophage-derived foam cells are the primary source of LPL in atherosclerotic plaques and are consistent with a role for LPL in the pathogenesis of atherosclerosis. (O'Brien *et al.*, 1992).

The LPL gene is located on chromosome 8p22, spanning approximately 35 kilobases. It contains 10 exons and encodes a 448-amino acid mature protein (Fisher *et al.*, 1997).

**Corresponding Author:** Azza K. Amer, Departments of 1Clinical & Chemical Pathology, Research Institute of Ophthalmology

Several restriction fragment length polymorphisms in the *LPL* gene have been documented and associated with various lipid traits. Despite growing evidence that the *LPL* gene is involved in the predisposition to dyslipoproteinemia, its impact on CAD is less clearly established. Furthermore, several questions remain open about the underlying biological mechanisms, in particular about a possible mediation of the effects of HindIII mutation (Zhang *et al.*, 2003). HindIII polymorphism of intron 8 of this gene results from replacement of a thymine (T) with a guanine (G) base at position +495 in intron 8 and abolishes a HindIII restriction enzyme recognition site (Gotoda *et al.*, 1992). It has been hypothesized that the more common H+ve allele (presence of cutting site) is associated with a lower LPL activity compared with the rare H-ve allele (absence of the restriction site) (Larson *et al.*, 1999).

DNA polymorphism is a useful marker to analyze disorders with genetic backgrounds, even though the genetic cause of the disease has not been elucidated. A number of DNA polymorphisms have been used to examine their possible linkage with a hereditary predisposition to common polygenic disorders such as dyslipidemia (Lusis 1988) and diabetes mellitus (Bell *et al.*, 1991).

Previously, the DNA polymorphism at the *LPL* gene locus has been detected only with the southern blot procedure, because detailed DNA sequences at and around the polymorphic sites have been unavailable. This conventional procedure requires considerable time, labor and skill as well radio labeled *LPL* gene probes. Therefore, the technique is inappropriate for a large scale study in an ordinary laboratory, however, polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) technique has developed a simple procedure for the detection of DNA polymorphisms in the human *LPL* gene (Gotoda *et al.*, 1992).

#### ***Aim of the Work:***

In the current study, subjects were genotyped using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique to detect *LPL* HindIII polymorphism in patients with atherosclerotic CAD to determine the importance of this mutation as a risk factor for the disease.

#### ***Subjects and Methods:***

This study was conducted on sixty Egyptian subjects. They were subdivided into the following groups:

Group 1: Included 19 non diabetic patients presenting with atherosclerotic coronary artery disease. They were 10 males & 9 females, their ages ranging from 47 up to 70 years.

Group 2: Included 21 diabetic patients presenting with atherosclerotic coronary artery disease. They were 13 males & 8 females their ages ranging from 48 up to 72 years.

Group 3: Included 20 healthy subjects with normal lipid profile with comparable ethnic background, they were 12 males & 8 females their ages ranging 47 up to 70 years.

Our patient population, in this study was enrolled from the Cardiac Outpatient Clinic of the Cardiac Unit in the Research Institute of Ophthalmology and Kasr El-Eini Hospital, Cairo University. Patients and normal controls were subjected to the following:

I- *Patient's medical history* was obtained using a questionnaire with standardized choices of answers. The presence or absence of hypertension, diabetes, angina and previous myocardial infarction, current medication particularly the usage of lipid lowering drugs and beta-adrenergic blocking agents were recorded. Smoking habits were recorded as well.

II- *Patients were subjected to routine clinical examination in addition to:*

***Blood Pressure Measurements:*** blood pressure was measured using mercury sphygmomanometer, while the patient was lying in the semi-sitting position, using korokoff phase 5 for diastolic reading.

***Electrocardiogram:*** Electrocardiogram was performed using a 3-channel ECG (Sciller, Cardiovit AT-1). Interpretation of the ECG data was done by a consultant cardiologist.

***Echocardiogram:*** Transthoracic Echocardiogram was performed in all patients using Kontron Sigma 44 HVCD machine with a 3.0 MHz phased array transducer for both 2 dimensional and Doppler study. Ejection fraction was used as parameter to assess the left ventricular (LV) systolic function, while trans-mitral inflow pattern was used to assess diastolic function.

Patients with evidence of atherosclerotic coronary artery disease were referred to cardiac catheter angiography to perform a diagnostic coronary angiography. Patients with other causes of heart disease such

as valvular heart disease, congenital heart disease, vasculitis coagulopathy and anti-cardiolipin syndrome were excluded from the study.

### **III-Assessment of CAD Severity:**

The severity of CAD was determined after the coronary angiogram was performed and studied by two cardiologists who were unaware that the patients were to be included in the study.

Each angiogram was classified either as revealing no coronary lesion with >50% luminal stenosis or as having one, two or three epicardial coronary arteries with > 50% luminal obstructions. The use of the Green Lane coronary artery scoring system helped provide numerical values for lesion severity and took into account the amount of myocardium supplied by the affected vessel, the maximum score was 15.

### **IV- Laboratory Assessment:**

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of LPL intron 8 HindIII polymorphism; DNA was isolated from frozen EDTA whole blood using commercial kit (QIAamp DNA blood Mini kit, QIAGEN Inc. USA).

The primer set Forward primer, 5'GATGTCTACCTGGATAATCAAAG -3' Reverse primer: 5'-CTTCAGCTAGACATTGCTAGTGT-3'

Amplification of genomic DNA: 0.2-0.5µg of genomic DNA was amplified in 25 µl reaction mixture containing 10 mmol/l Tris-HCL (pH 8.3); 50 mmol/l KCl; 200µmol/l each of dATP, dCTP, dGTP and dTTP; 4mmol/l MgCl<sub>2</sub>; 0.5µmol/l of each primer and 0.5µ Taq DNA polymerase (Finnzyme). Blank controls containing no genomic DNA were run with each set of amplifications. The amplification cycle entailed 5 minutes of denaturation at 96°C followed by 30 cycles of 1 minute at 98°C, 1 minute at 55°C and 1 minute at 72°C. This was followed by 7 minutes of extension at 72°C (Mattu et al., 1994).

**Digestion and Electrophoresis:** 0.5 µl of digestion mixture containing the manufacturer's restriction buffer and 10 U of the HindIII restriction enzyme were added to amplification product and incubated at 37°C overnight. Subsequently, the samples were electrophoresed using 2% agarose in TBE buffer (89mmol/l Tris borate, 2 mmol/l EDTA, pH;8.3) at 100V for 60 minutes. DNA was visualized by staining the gel with ethidium bromide (0.5 µg/ml) and transillumination with UV light. Presence of HindIII site yielded fragments 140 and 210 bp.

**Measurement of Serum Lipids:** For the evaluation of serum levels of lipids, blood samples were obtained from subjects between 8 am and 11 am after 12-14 hours fasting. Serum levels of total cholesterol (T-chol) high density lipoprotein-cholesterol (HDL-C) and triglycerides (TG) were measured by standard enzymatic methods. The serum levels of low density lipoprotein-cholesterol (LDL-C) were calculated with Friedwald formula.

**Statistical Analysis:** Data were computerized and analyzed using SPSS (Statistical Package for Windows XP version 10). Chi-square was calculated to compare categorical variables and level of significance was taken at (p<0.05). ANOVA test was used to determine the significance in the difference between means. Odds ratio was also calculated to assess possible risks.

### **Results:**

Characteristics of the subjects were summarized in table (1). There was no significant difference in age between CAD patients and normal controls (P> 0.84).

The serum levels of TG, T-Chol and LDL-C were significantly increased (p <0.0001) while HDL-C level was significantly decreased in CAD patients when compared to controls (p<0.01). The genotype distribution of LPL gene was summarised in table (2). The frequency of LPL HindIII (+/+) genotype was higher in CAD patients than controls (45% Vs 25%). There was no difference between the percentage (%) of the LPL HindIII (+/-) genotype among CAD patients and controls (32.5% Vs 30%). The frequency of LPL HindIII (-/-) genotype was lower in CAD patients than controls (22.5% Vs 45 %). For HindIII (+/+, +/- and -/-) LPL genotypes, the odd's ratio between CAD patients and normal controls was 2.82 (95% CI:0.89- 8.92) but the difference was not statistically significant (Table 3). The relation between HindIII +ve allele and serum levels of the lipid parameters (T-chol, LDL-C, HDL-C and TG) was analyzed using ANOVA test. It showed a significant difference in serum levels of HDL-C and TG between the three LPL HindIII genotypes (+/+, +/-, & -/-), P<0-001 (Table 4).

**Table 1:** Characteristics of CAD patients and normal controls.

Parameter	CADN=40		ControlsN=20	P-Value
	Non-diabetic	Diabetic		
Age (years) mean ±S.D	58.4±7.7	58.5±7.8	59.6±6.7	0.845
Total -Chol (mg/dl) mean +S.D	251.3±21.2	243.8±42.5	172.4±13.4	0.0001
HDL-C (mg/dl) mean ±S.D	59.2±22.1	44.2±13.4	53.5±7	0.010
TG (mg/dl) Mean±S.D	162.8+ 5.3	163.1+ 24.2	96.4+ 11.1	0.0001
LDL-C (mg/dl) mean ±S.D	158.6 + 18.5	199.4 + 28.3	115.1+19.1	0.0001

-Non- Significant:>0.05.

-Significant: <0.01.

**Table 2:** Frequency Distribution of LPL HindIII polymorphism of CAD patients and normal controls.

LPL HindIII Genotype	CAD (n=40) n(%)	Control (n=20) n(%)
-/-	9 (22.5%)	9 (45%)
+/-	13 (32.5%)	6 (30%)
+/+	18 (45%)	5 (25%)

CAD: Coronary artery disease.

LPL: Lipoprotein lipase.

**Table 3:** Odds ratio of HindIII of LPL Genotypes between CAD patients and controls.

	Value	95%CI	
		Lower	Upper
Odd's ratio for case and controls	2.82	0.89	8.92
HindIII LPL polymorphism (+/+, + / -)	1.41	0.92	2.17
HindIII LPL polymorphism ( -/-)	0.50	0.24	1.06

**Table 4:** Mean +/- SD and analysis of variance of serum levels of some parameters of lipids of LPL genotypes in CAD patients.

	(-/-)n=9	(+/-)n=13	(+/+)N=18	F	P
T- Chol	248.1±7.2	240.4±45.1	252 ± 33.4	0.43	0.6
HDL-C	73.1±14.6	53.2±17.8	39.1±11.1	17.1	<0.01
LDL-C	152.3±20.7	159.2±19.6	170.9±27.5	2.089	0.139
TG	145.2±11.3	175±21.9	201.01±27.6	17.98	<0.001

-Non- Significant: >0.05.

-Significant: <0.01.

### Discussion:

In the current study we explored the association between HindIII polymorphism of LPL gene with the lipid profile and CAD severity as well as its impact as a risk factor on CAD patients. We determined CAD severity from the number of significantly stenosed (50%) major coronary arteries and a commonly used scoring system.

Reports about the relationship between LPL polymorphism and severity of CAD have been controversial (Mattu *et al* 1994). Thorn *et al.*, (1990) found a significant association between HindIII (+ve) allele and increase in severity of CAD, while on the other hand Peacock *et al.*, (2003) reported that HindIII (+ve) allele was associated with decreased severity (Wang *et al* 1996). To our knowledge, to date, no data are available from CAD Egyptians. The results of this study showed that the frequency of LPL HindIII (+ve) allele was higher in CAD patients than in controls (77.5% VS 55%), so the +ve allele of HindIII RFLP of LPL gene could be linked to the occurrence of CAD (Odd's ratio 2.8 & 95% CI 0.89 - 8.92). The aforementioned data lends a support to previous findings which suggest a novel function of HindIII positive allele as a useful genetic marker associating the CAD (Gigek *et al.*, 2007 and Pasalic *et al*, 2006).

It is noteworthy to mention that Chen *et al.*, (2007) reported that HindIII polymorphism site of the LPL gene is functional and identified seven regulatory elements in intron 8 including the consensus sequence of TATA that is affected by the HindIII polymorphism.

Pursuing the same subject, Shimo–Nakanishi *et al.*, (2001) found that LPL HindIII (+/+) genotype was strongly associated with severe cerebrovascular disease, another form of atherosclerotic diseases.

The lipoprotein lipase (LPL) gene encodes a rate-limiting enzyme protein that has a key role in the hydrolysis of triglycerides. Hypertriglyceridemia, one widely prevalent syndrome of LPL deficiency and dysfunction, may be a risk factor in the development of dyslipidemia, type II diabetes (T2D), essential hypertension (EH), CAD and Alzheimer's disease (AD). Findings from earlier studies indicate that LPL may have a role in the pathology of these diseases and therefore is a common or shared biological basis for these common complex diseases (Xie *et al.*, 2010). Consistent with this suggestion are our findings denoting a significant increase in plasma levels of atherogenic lipid parameters such as TG, total cholesterol and LDL-C as well as significant decrease in plasma levels of HDL-C in CAD patients especially those suffering from diabetes mellitus when compared to healthy controls. On the other hand, Araujo *et al.*, (2010) reported that HindIII in intron 8 of LPL gene in adult man has been associated with lowered TG levels and cardiovascular risk in adult man.

On the other hand, Lee *et al.*, (2000) found that HindIII +ve allele is associated with insulin resistance in non diabetic CAD, the polymorphism may affect the regulation of LPL by insulin. Insulin is an important regulator of the LPL enzyme and this hormone may regulate LPL at the mRNA (Ong *et al.*, 1988) and / or post transcriptional level (Semenkovich *et al.*, 1989). For example, the +ve allele could be associated with a functional LPL enzyme that is less sensitive to insulin and may result in a reduced response of LPL to insulin in obese (Vohl *et al.*, 1995, Lopez-Miranda *et al.*, 2004). These findings could explain the increased frequency of HindIII +ve allele in non diabetic CAD patients found in this study.

Pasalich *et al.*,(2001) reported that there is an association marker between genetic variation at the locus of lipoprotein lipase HindIII +ve allele and high levels of TG. This might prove useful in the detection of individual susceptibility to the development of hypertriglyceridemia as well as a marker of the analysis of this genetic defect in patients family.

There was an effect of the HindIII polymorphism on the variation of plasma concentrations of HDL-C. The H+ve allele of the HindIII site was significantly associated with lower concentration of HDL cholesterol. Our results on the HindIII association with HDL-C and TG concentrations are in accordance with those of Ahn *et al.*, (1993) who found a statistically significant effect of the H+ve allele on both HDL-C and TG.

In contrast, Mattu *et al.*, (1994) found that the H+ve allele was associated with higher plasma cholesterol, we could not demonstrate any significant effect of the H+ve allele on the total plasma cholesterol or non HDL-cholesterol.

In conclusion, the present study suggest that the presence of H+ve allele of LPL gene plays an important role in vascular atherogenesis that in turn promotes the occurrence of CAD especially in diabetic Egyptians.

## REFERENCES

- Ahn, Y.I., M.I. Kamboh, R.F. Hamman, S.A. Cole, R.E. Ferrell, 1993. Two DNA polymorphism in the lipoprotein lipase gene and their association with factors related to cardiovascular disease. *J Lipid Res.*, 34: 421-428.
- Araújo, L.M.Q., M.S. Cendoroglo, C.O. Gígek, E.S. Chen and A.C. Smith Mde, 2010. Association of lipase lipoprotein polymorphisms with high-density lipoprotein and triglycerides in elderly men. *Genet. Mol. Res.*, 9(1): 89-96.
- Ariza, M.J., S. Miguel-Angel, Anchez-Angel, F.J. Baron, A.M. Horonos, E.C. Bonacho, J. Rioja, P. Valdivielso, J.A. Gelpi and P. Gonzalea-Santos, 2010. Additive effects of LPL, APOA5 and APOE variant combinations on triglyceride levels and hypertriglyceridemia: results of the ICARIA genetic sub-study. *BMC Medical Genetics*, 11: 1166-1186.
- Bell, G.I., K.S. Xiang, M.V. Newman, S.H. Wu, L.G. Wright, S.S. Fajans, R.S. Spielman and N.J. Cox, 1991. Gene for non- insulin-dependent diabetes mellitus (maturity-onset diabetes of the young subtype) is linked to DNA polymorphism on human chromosome 20q. *Proc Natl Acad Sci USA*, 88(4):1484-1488.
- Chen, Q., H. Razzaghi, F.Y. Demirci, M.I. Kamboh, 2008. Functional significance of lipoprotein lipase HindIII polymorphism associated with the risk of coronary artery disease. *Atherosclerosis*, 200(1): 102-8.
- Fisher, R.M., S.E. Humphries, P.J. Talmud, 1997. Common variation in the lipoprotein lipase gene: effects on plasma lipids and risk of atherosclerosis. *Atherosclerosis*; 135(2): 145-159.
- Gígek Cde, O., E.S. Chen, M.S. Cendoroglo, L.R. Ramos, L.M. Araujo, S.L. Payão and A. Smith Mde 2007. Association of lipase lipoprotein polymorphisms with myocardial infarction and lipid levels. *Clin Chem Lab Med.*, 45(5): 599-604.



- Goldberg, I.J., 1996. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J Lipid Res.*, 37(4): 693-707.
- Gotoda, T., N. Yamada, T. Murase, H. Shimano, M. Shimada, K. Harada, M. Kawamura, K. Kozaki and Y. Yazaki, 1992. Detection of three separate DNA polymorphisms in the human lipoprotein lipase gene by gene amplification and restriction endonuclease digestion. *J lipid Res.*, 33: 1067-1072.
- Hahn, P.F., 1943. Abolishment of alimentary lipemia following injection of heparin. *Science*, 98: 19-20.
- Larson, I., M.M. Hoffmann, J.M. Ordovas, E.J. Schaefer, W. März, J. Kreuzer, 1999. The Lipoprotein Lipase HindIII Polymorphism: Association with Total Cholesterol and LDL-Cholesterol, but not with HDL and Triglycerides in 342 females. *Clinical Chemistry*, 45: 963-68.
- Lee, W.J., W.H. Sheu, C.Y. Jeng, M.S. Young Y.T. Chen, 2000. Associations between lipoprotein lipase gene polymorphisms and insulin resistance in coronary heart disease. *Zhonghua Yi Xue Za Zhi(Taipei)*, 63(7): 563-72.
- López-Miranda, J., G. Cruz, P. Gómez, C. Marín, E. Paz, P. Pérez-Martínez, F.J. Fuentes, J.M. Ordovas and F. Pérez-Jiménez, 2004. The Influence of Lipoprotein Lipase Gene Variation on Postprandial Lipoprotein Metabolism. *J Clin Endo Met.*, 89(9): 4721-4728.
- Lusis ,A.J., 1988. Genetic factors affecting blood lipoproteins: the candidate gene approach. *J Lipid Res.*, 29(4): 397-429.
- Mattu, R.K., E.W.A. Needham, R. Morgan, A. Rees, A.K. Hackshaw, J. Stocks, P.C. Elwood, D.J. Galton, 1994. DNA variants at the LPL gene locus associate with angiographically defined severity of atherosclerosis and serum lipoprotein levels in a Welsh population . *Arterioscler Thromb.* 14(7): 1090-1097.
- O'Brien, K.D., D. Gordon, S. Deeb, M. Ferguson and A. Chait, 1992. Lipoprotein Lipase Is Synthesized by Macrophage derived Foam Cells in Human Coronary Atherosclerotic Plaques. *J. Clin. Invest.*, 89: 1544-1550.
- Ong, J.M., T.G. Kirchgessner, M.C. Schotz and P.A. Kern, 1988. Insulin increases the synthetic rate and messenger RNA level of lipoprotein lipase in isolated rat adipocytes. *J Biol Chem.*, 263: 12933-12938.
- Pasalić, D., G. Ferencak, B. Grsković, M. Sesto and A. Stavljenić-Rukavina, 2006. Association of two genetic variations of lipoprotein lipase, S447X and Hind III, with coronary artery disease and hypertriglyceridemia. *Coll Antropol.*, 30(3): 549-554.
- Pasalic, D., J. Sertic, B. Kunovic, Z. Milicevic, A. Pasic and R. Zrinski-Topic, 2001. Lipoprotein lipase gene polymorphism and lipid profile in patients with hypertriglyceridemia. *Croat Med J.*, 42: 517-522.
- Peacock, R.E., A. Hamsten, P. Nilsson-Ehle, S.E. Humphries, 1992. Associations between lipoprotein lipase gene polymorphisms and plasma correlations of lipids, lipoproteins and lipase activities in young myocardial infarction survivors and age-matched healthy individuals from Sweden. *Atherosclerosis*, 97(2-3): 171-185.
- Semenkovich, C.E., M. Wims, L. Noe, J. Etienne and L. Chan., 1989. Insulin regulation of lipoprotein lipase activity in 3T3-L1 adipocytes is mediated at posttranscriptional and posttranslational levels. *J Biol Chem.*, 264: 9030-9038.
- Shimo-Nakanishi, Y., T. Urabe, N. Hattori, Y. Watanabe, T. Nagao, M. Yokochi, M. Hamamoto and Y. Mizuno, 2001. Polymorphism of the Lipoprotein Lipase Gene and Risk of Atherothrombotic Cerebral Infarction in the Japanese. *Stroke*, 32: 1481-1486.
- Thorn, J.A., J.C. Chamberlain, J.C. Alcolado, K. Oka, L. Chan, J. Stocks and D.J. Galton, 1990. Lipoprotein and hepatic lipase gene variants in coronary atherosclerosis. *Atherosclerosis*, 85(1): 55-60.
- Vohl, M.C., B. Lamarche, S. Moorjani, D. Prud'homme, A. Nadeau, C. Bouchard, P.J. Lupien and J.P. Després, 1995. The lipoprotein lipase *HindIII* polymorphism modulates plasma triglyceride levels in visceral obesity. *Arterioscler Thromb Vasc Biol.*, 15: 714-720.
- Wangm, X.L., R.M. McCredie and D.E. Wilcken, 1996. Common DNA polymorphisms at the lipoprotein lipase gene: association with severity of coronary artery disease and diabetes. *Circulation*, 93(7): 1339-1345.
- Wang, X.L., C. Tam, R.M. McCredie and D.E.L. Wilcken, 1994. Determinants of severity of coronary artery disease in Australian men and women . *Circulation*, 89: 1974-1981.
- Xie, C., Z.C. Wang, X.F. Liu and M.S. Yang, 2010. The common biological basis for common complex diseases: evidence from lipoprotein lipase gene. *Eur J Hum Genet.*, 18(1): 3-7.
- Zhang, R., Y. Liu, L.C. Yang, H. Bai and B.W. Liu, 2003. Study on lipoprotein lipase gene Hind III polymorphism in Chinese type II b hyperlipoproteinemia [in Chinese]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi.*, 20(6): 539-541.
- Zilverstmit, D.B., 1973. A proposal linking atherogenesis to the interaction of endothelial lipoprotein lipase with triglyceride-rich lipoproteins. *Circ. Res.*, 33: 633-638.