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LPL gene Pvu II polymorphism and hypertriglyceridemia: a meta-analysis involving 1,640 subjects

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Background/Aims: Although lipoprotein lipase *(LPL)* gene Pvu II polymorphism has been associated with an increased risk of hypertriglyceridemia (HT), there is no clear consensus within the scientific community.

Methods: A meta-analysis of 1,640 subjects from six individual studies was conducted to better elucidate the potential relationship between the *LPL* gene Pvu II polymorphism and HT within the Chinese population. Pooled odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were evaluated by using fixed effect models.

Results: Our analysis indicated a significant association between *LPL* gene Pvu II polymorphism and HT within the Chinese population under allelic (OR, 1.550; 95% CI, 1.320 to 1.830; $p = 1.158 \times 10^{-7}$), recessive (OR, 0.540; 95% CI, 0.390 to 0.750; $p = 0.0002$), dominant (OR, 1.889; 95% CI, 1.501 to 2.377; $p = 5.960 \times 10^{-8}$), homozygous (OR, 2.167; 95% CI, 1.531 to 3.067; $p = 1.242 \times 10^{-5}$), heterozygous (OR, 1.810; 95% CI, 1.419 to 2.309; $p = 1.842 \times 10^{-6}$), and additive genetic models (OR, 1.553; 95% CI, 1.320 to 1.828; $p = 1.158 \times 10^{-7}$).

Conclusions: Because *LPL* gene Pvu II restriction fragment length polymorphism polymorphism was associated with an elevated risk of HT, the P+ allele carriers of the LPL gene might be predisposed to HT.

Keywords: Lipoprotein lipase; Pvu II; Polymorphism; Hypertriglyceridemia

INTRODUCTION

Hyperlipidemia (HL) is an umbrella term referring to several acquired or genetic disorders that result in high level of lipids circulating in the blood. The increased prevalence of Western diets and sedentary lifestyles contributed to increasing morbidities and mortalities associated with HL, but the complex interactions between hereditary and environments makes the disease difficult to treat.

One of the disorders categorized under HL is hypertriglyceridemia (HT), which, more specifically, describes elevated levels of triglycerides (TGs), the most abundant lipid in the human body [1]. HT is strongly associated with atherosclerosis, even in the absence of hypercholesterolemia, and diabetes mellitus. Contemporary research also identifies HT as one of the most potent risk factors for coronary artery disease [2]. These findings sparked the interest of many researchers into better understanding the hereditary and environmental factors that contribute to development of HT among patients.

Lipoprotein lipase (LPL) is an area of particular interest. LPL plays a key role in TG metabolism by catalyzing digestion of TGs in chylomicron emulsions and very low density lipoprotein (VLDL)'s into free fatty acids and glycerol to meet the energy expenditure and storage

needs for a variety of high-energy tissues (adipocytes, cardiomyocytes, skeletal muscle cells, macrophages, etc) [3]. The importance of LPL in HT is further accentuated when we look at patients. Patients with HT always exhibit abnormal apolipoprotein levels with the significantly elevated plasma TG and VLDL level.

It has been postulated that variation within the genetic material coding this protein may also contribute to HT. The *LPL* gene, located in 8p22, is comprised of 10 exons and nine introns. The Pvu II polymorphism (rs285) is caused by the C-T base transition in the 6th intron. Although this polymorphism does not affect the structure of the encoded protein, it could still affect general TG esterlysis by altering the gene expression and regulation through linkage disequilibrium [4].

Many studies seeking to elucidate the relationship between this gene polymorphism and HT have been conducted, but they fail to present a clear consensus on the subject. This may be due to the small samples sizes of the each study individually. For example, while Fan et al. [5] and Wang et al. [6] both found that the P+P+ genotype in the *LPL* gene was associated with HT in separate Chinese populations, but Huang et al. [7] found no genetic risk among HT subjects. Nevertheless, only 328, 464, and 310 sample size were included in the three individual studies respectively (Table 1). Our current meta-analysis seeks to combine the information gathered thus far and present a clear representation of the combined results.

METHODS

Publication search and inclusion criteria

Our primary search was executed through a search of the Wan Fang database, PubMed, the China National Knowledge Infrastructure, the VIP database, the China Biological Medicine Database, Embase, and the Web of Science using the terms "*LPL*," "Pvu II," "hypertriglyceridemia," and "polymorphism." Our initial search yielded papers published between 1999 and 2007 with the most recent update on May 28, 2016.

Studies selected for our meta-analysis had to meet inclusion criteria as follows. Studies selected must (1) evaluate the association of HT and *LPL* gene Pvu II polymorphism, (2) diagnose HT as defined by TG ≥ 2.5 mmol/L, and (3) have control groups that follow Hardy-Weinberg equilibrium (HWE). Results of the studies must also be published in official journals as either cohort or case-control studies.

Data extraction

The data was drawn out using a standardized protocol by three investigators. Two were responsible for identifying duplicate studies with the third serving as an arbitrator to settle any differences. Those studies that deviated from the inclusion criteria, repeatedly published, or did not provide sufficient data were removed. Similar data sets presented in different publications by the same author group were used once in our study. The listed data table should include such items as the first author's name, publication year, region, genotyping method, matching crite-

Table 1. Characteristics of the investigated studies of the association between the *LPL* gene Pvu II polymorphism and HT in the Chinese population

Study	Region	Genotype	HT			Control			Matching criteria	Sample size (HT/control)
			P+P+	P+P–	P–P–	P+P+	P+P–	P–P–		
Fan et al. (2001) [5]	Sichuan	PCR-RFLP	62	60	13	65	88	40	Ethnicity	135/193
Wang et al. (2006) [6]	Xinjiang	PCR-RFLP	20	35	23	49	193	144	Ethnicity	78/386
Zhu et al. (2002) [11]	Beijing	PCR-RFLP	31	26	3	43	73	20	Ethnicity	60/136
Huang et al. (2007) [7]	Shanghai	PCR-RFLP	70	63	23	60	72	22	Age, Ethnicity	156/154
Liu et al. (1999) [12]	Tianjin	PCR-RFLP	15	10	3	61	78	22	Ethnicity	28/161
Zhu et al. (1998) [13]	Jiangsu	PCR-RFLP	42	12	4	39	48	8	Age, sex, ethnicity	58/95

PCR-RFLP method and case-control study design were adopted in the above studies.
LPL, lipoprotein lipase; HT, hypertriglyceridemia; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

ria, the genotype number in the HT and control groups, and sample size of cases and controls.

Statistical analyses

Six genetic models were used in the present meta-analysis: allelic (P+ allele distribution frequency), recessive (P–P– vs. P+P+ and P+P–), dominant (P+P+ vs. P–P– and P+P–), homozygous (P+P+ vs. P–P–), heterozygous (P+P+ vs. P+P–), and additive (P+ vs. P–). The odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were used to compare the relationship of *LPL* gene Pvu II polymorphism and HT. The heterogeneity among the studies was calculated by using the chi-square-based Q-tests with the significance set at $p < 0.05$ level [8]. The fixed-effect model (the Mantel-Haenszel method) was used [9] because no heterogeneity among the included studies. The pooled OR was assessed by Z test with the significance set at $p < 0.05$ level.

HWE was evaluated by using the Fisher exact test with the significance set at $p < 0.05$ level. Potential publication bias was assessed using a funnel plot. Funnel plot symmetry was evaluated by using the Egger's linear regression test on a natural log scale of the OR with the significance set at $p < 0.05$ level [10]. Statistical analyses were performed by using Stata version 12.0 (StataCorp., College Station, TX, USA) and Review Manager 5.0.

RESULTS

Studies and populations

All of information was abstracted from 515 HT cases and 1,125 controls (Table 1) [5-7,11-13]. Twelve papers were obtained through the retrieval process, among which six papers were included in the present meta-analysis. Among the six excluded studies, two papers were reviews and two papers deviated from the HWE [14,15], and two papers have nothing with *LPL* gene Pvu II polymorphism or HT.

Pooled analyses

A significant association between *LPL* gene Pvu II polymorphism and HT was detected in the Chinese population under all genetic models: allelic (OR, 1.550; 95% CI, 1.320 to 1.830; $p = 1.158 \times 10^{-7}$), recessive (OR, 0.540; 95% CI, 0.390 to 0.750; $p = 0.0002$), dominant (OR, 1.889; 95% CI, 1.501 to 2.377; $p = 5.960 \times 10^{-8}$), homozygous (OR, 2.167; 95% CI, 1.531 to 3.067; $p = 1.242 \times 10^{-5}$), heterozygous (OR, 1.810; 95% CI, 1.419 to 2.309; $p = 1.842 \times 10^{-6}$), and additive genetic models (OR, 1.553; 95% CI, 1.320 to 1.828; $p = 1.158 \times 10^{-7}$) (Table 2, Figs. 1-6).

Bias diagnostics

There was no visible publication bias in the funnel plot

Table 2. Summary of meta-analysis of association between *LPL* gene Pvu II polymorphism and HT in the Chinese population

Genetic model	Pooled OR (95% CI)	Z value	p value[a]	Study number	HT size[b]	Control size[c]	P heterogeneity $(I^2 \%)$
Allelic genetic model[d]	1.550 (1.320–1.830)	5.30	1.158×10^{-7}	6	515	1,125	0.21 (31.0%)
Recessive genetic model[e]	0.540 (0.390–0.750)	3.72	0.0002	6	515	1,125	0.17 (36.0%)
Dominant genetic model[f]	1.889 (1.501–2.377)	5.42	5.960×10^{-8}	6	515	1,125	0.166 (36.2%)
Homozygous genetic model[g]	2.167 (1.531–3.067)	4.37	1.242×10^{-5}	6	515	1,125	0.296 (18.1%)
Heterozygous genetic model[h]	1.810 (1.419–2.309)	4.77	1.842×10^{-6}	6	515	1,125	0.149 (38.5%)
Additive genetic model[i]	1.553 (1.320–1.828)	5.30	1.158×10^{-7}	6	515	1,125	0.207 (30.5%)

LPL, lipoprotein lipase; HT, hypertriglyceridemia; OR, odds ratio; CI, confidence interval.

[a]$p \leq 0.05$.
[b]HT size: the total number of HT cases.
[c]Control size: the total number of control group.
[d]Allelic genetic model: P+ allele distribution frequency.
[e]Recessive genetic model: P–P– vs. P+P+ + P+P–.
[f]Dominant genetic model: P+P+ vs. P–P– + P+P–.
[g]Homozygous genetic mode: P+P+ vs. P–P–.
[h]Heterozygous genetic model: P+P+ vs. P+P–.
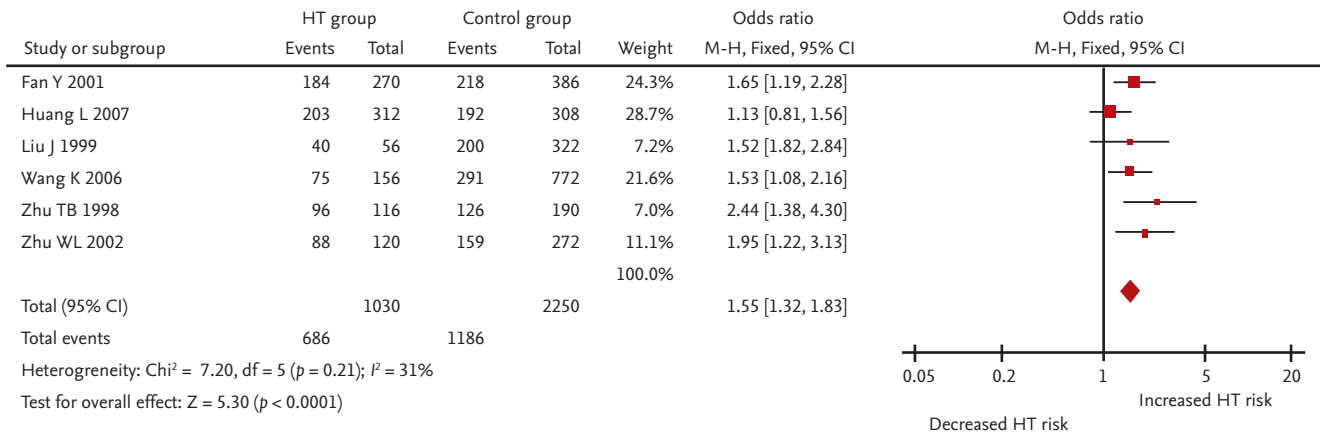[i]Additive genetic model: total P+ allele vs. total P– allele.

| Study or subgroup | HT group | | Control group | | Weight | Odds ratio | Odds ratio |
	Events	Total	Events	Total		M-H, Fixed, 95% CI	M-H, Fixed, 95% CI
Fan Y 2001	184	270	218	386	24.3%	1.65 [1.19, 2.28]	
Huang L 2007	203	312	192	308	28.7%	1.13 [0.81, 1.56]	
Liu J 1999	40	56	200	322	7.2%	1.52 [1.82, 2.84]	
Wang K 2006	75	156	291	772	21.6%	1.53 [1.08, 2.16]	
Zhu TB 1998	96	116	126	190	7.0%	2.44 [1.38, 4.30]	
Zhu WL 2002	88	120	159	272	11.1%	1.95 [1.22, 3.13]	
					100.0%		
Total (95% CI)		1030		2250		1.55 [1.32, 1.83]	
Total events	686		1186				

Heterogreneity: Chi² = 7.20, df = 5 (*p* = 0.21); I² = 31%
Test for overall effect: Z = 5.30 (*p* < 0.0001)

Figure 1. Forest plot of hypertriglyceridemia (HT) associated with lipoprotein lipase (*LPL*) gene Pvu II polymorphism under an allelic genetic model (distribution of P+ allelic frequency of *LPL* gene Pvu II polymorphism). CI, confidence interval.
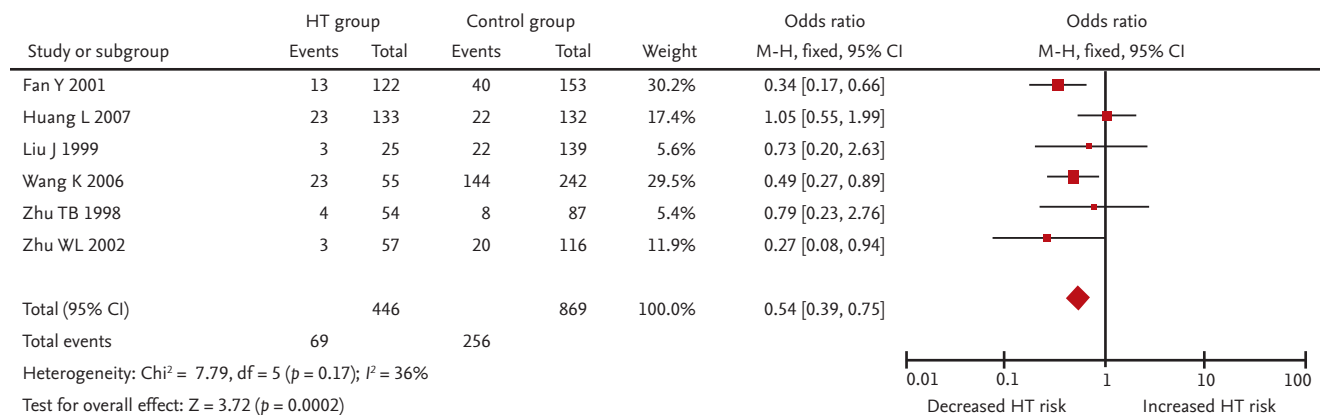
| Study or subgroup | HT group | | Control group | | Weight | Odds ratio | Odds ratio |
	Events	Total	Events	Total		M-H, fixed, 95% CI	M-H, fixed, 95% CI
Fan Y 2001	13	122	40	153	30.2%	0.34 [0.17, 0.66]	
Huang L 2007	23	133	22	132	17.4%	1.05 [0.55, 1.99]	
Liu J 1999	3	25	22	139	5.6%	0.73 [0.20, 2.63]	
Wang K 2006	23	55	144	242	29.5%	0.49 [0.27, 0.89]	
Zhu TB 1998	4	54	8	87	5.4%	0.79 [0.23, 2.76]	
Zhu WL 2002	3	57	20	116	11.9%	0.27 [0.08, 0.94]	
Total (95% CI)		446		869	100.0%	0.54 [0.39, 0.75]	
Total events	69		256				

Heterogeneity: Chi² = 7.79, df = 5 (*p* = 0.17); I² = 36%
Test for overall effect: Z = 3.72 (*p* = 0.0002)

Figure 2. Forest plot of hypertriglyceridemia (HT) associated with lipoprotein lipase (*LPL*) gene Pvu II polymorphism under a recessive genetic model (P–P– vs. P+P+ + P+P–). CI, confidence interval.

under the allelic genetic model (Fig. 7). Additionally, there was no significant difference in the Egger's test yet, which suggested that no publication bias existed in the present meta-analysis by using additive genetic model (*T* = 1.57, *p* = 0.192).

DISCUSSION

Our meta-analysis found a significant relationship between the *LPL* gene Pvu II polymorphism and HT under all genetic models, which suggested that the gene polymorphism might predispose P+ allele carriers to HT.

However, the precise molecular mechanism behind this relationship remains unclear. Because the Pvu II restriction enzyme digestion site is located in an intron, its alteration would not directly affect the protein structure and its baseline activity. However, it could influence the LPL activity or regulate the gene expression by the chain effects [16]. Hayden et al. [17] also suggested that the base variation in the intron might interact with environmental factors such as excessive drinking, pregnancy, and diabetes mellitus to reduce the *LPL* gene expression and lead to the elevated TG level.

In 2002, Ukkola et al. [18] gave excessive dietary intervention to 12 pairs of healthy identical twins for 100 days and they found that the *LPL* gene Pvu II polymorphism was positively correlated with body mass index and LPL activity. Nicklas et al. [19] found that the *LPL* gene Pvu
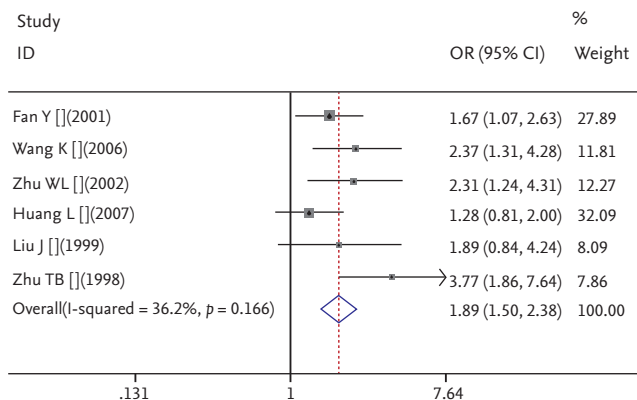
Figure 3. Forest plot of hypertriglyceridemia associated with lipoprotein lipase (*LPL*) gene Pvu II polymorphism under a dominant genetic model (P+P+ vs. P–P– + P+P–). OR, odds ratio; CI, confidence interval.
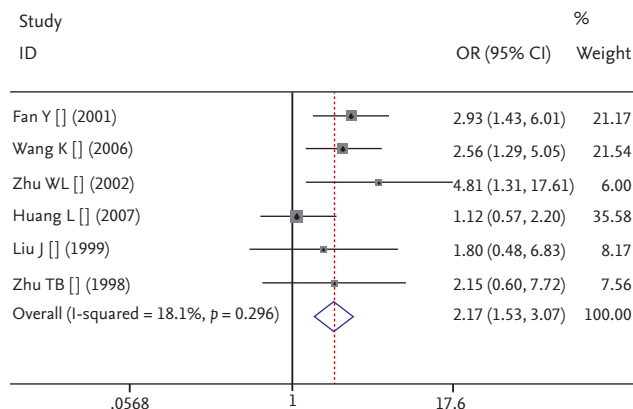
Figure 4. Forest plot of hypertriglyceridemia associated with lipoprotein lipase (*LPL*) gene Pvu II polymorphism under a homozygous genetic model (P+P+ vs. P–P–). OR, odds ratio; CI, confidence interval.
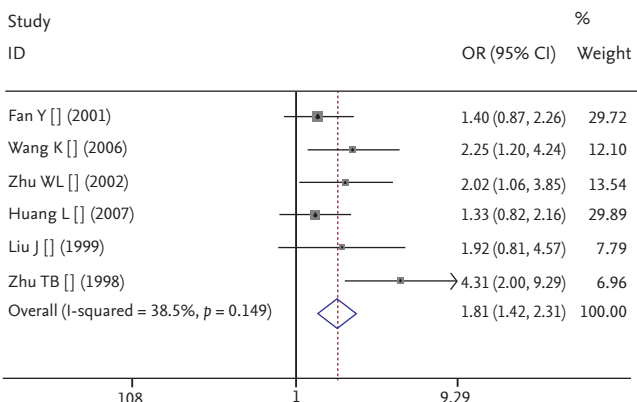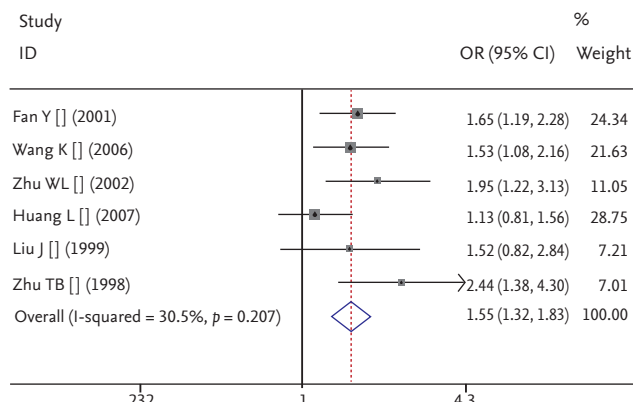
Figure 5. Forest plot of hypertriglyceridemia associated with lipoprotein lipase (*LPL*) gene Pvu II polymorphism under a heterozygous genetic model (P+P+ vs. P+P–). OR, odds ratio; CI, confidence interval.

Figure 6. Forest plot of hypertriglyceridemia associated with lipoprotein lipase (*LPL*) gene Pvu II polymorphism under an additive genetic model (total P+ allele vs. total P– allele). OR, odds ratio; CI, confidence interval.
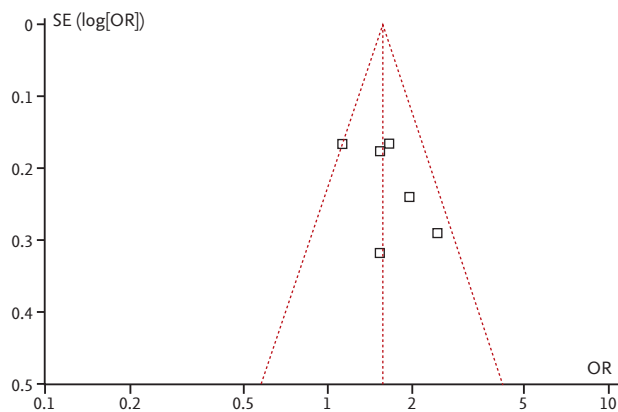
Figure 7. The funnel plot for studies of the association of hypertriglyceridemia associated with lipoprotein lipase (*LPL*) gene Pvu II polymorphism under an allelic genetic model (distribution of P+ allelic frequency of *LPL* gene Pvu II polymorphism). The horizontal and vertical axis correspond to the odds ratio (OR) and confidence limits. SE, standard error.

II polymorphism was not only associated with the LPL activity, but also with the serum lipid level.

Our meta-analysis improves on the presenting the current state of research on the topic. An admirable meta-analysis performed by Cui et al. [20] in 2010 also found the *LPL* gene Pvu II polymorphism to be significantly associated with HL. However, two studies with controls deviating from the HWE were included in their meta-analysis [20]. Hence, its results might not reach the objective standard of our presented work.

Even so, our current meta-analysis does not present a large-scale study necessary to fully understand the relationship between the *LPL* gene Pvu II polymorphism and HT. Our study also fails to take into account environmental factors that play a significant role in the

development HT. It also fails to take into account the micro-effects of many other genes involved in lipid metabolism, transport, and storage (i.e., *CRTC2* gene rs8450 polymorphism, *MTTP* 297 H polymorphisms) [21,22].

In conclusion, *LPL* gene Pvu II polymorphism was significantly associated with increased HT risk in the Chinese population. The persons with the P+ allele of *LPL* gene Pvu II polymorphism might be easy to suffer from HT. The significant association between them suggested that more studies clarifying more about this complex relationship would be valuable to developing our understanding of this subject.

KEY MESSAGE

1. Lipoprotein lipase (LPL) gene Pvu II restriction fragment length polymorphism was associated with an elevated risk of hypertriglyceridemia (HT).
2. The P+ allele carriers of the LPL gene might be predisposed to HT.

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