The lipoprotein lipase gene serine 447 stop variant influences hypertension-induced left ventricular hypertrophy and risk of coronary heart disease

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ABSTRACT

LVH [LV (left ventricular) hypertrophy] is an independent risk factor for CHD (coronary heart disease). During LVH, the preferred cardiac energy substrate switches from FAs (fatty acids) to glucose. LPL (lipoprotein lipase) is the key enzyme in triacylglycerol (triglyceride) hydrolysis and supplies FAs to the heart. To investigate whether substrate utilization influences cardiac growth and CHD risk, we examined the association between the functional LPL S447X (rs328) variant and hypertension-induced LV growth and CHD risk. LPL-X447 has been shown to be more hydrolytically efficient and would therefore release more free FAs than LPL-S447. In a cohort of 190 hypertensive subjects, LPL X447 was associated with a greater LV mass index [85.2 (1.7) in S/S compared with 91.1 (3.4) in S/X + X/X; P = 0.01], but no such association was seen in normotensive controls (n = 60). X447 allele frequency was higher in hypertensives with than those without LVH [0.14 (95% CI, 0.08–0.19) compared with 0.07 (95% CI, 0.05–0.10) respectively; odds ratio, 2.52 (95% CI, 1.17–5.40); P = 0.02]. The association of LPL S447X with CHD risk was then examined in a prospective study of healthy middle-aged U.K. men (n = 2716). In normotensive individuals, compared with S447 homozygotes, X447 carriers were protected from CHD risk [HR (hazard ratio), 0.48 (95% CI, 0.23–1.00); P = 0.05], whereas, in the hypertensives, X447 carriers had increased risk [HR, 1.54 (95% CI, 1.13–2.09) for S/S (P = 0.006) and 2.30 (95% CI, 1.53–3.45) for X447+ (P < 0.0001)] and had a significant interaction with hypertension in CHD risk determination (P = 0.007). In conclusion, hypertensive LPL X447 carriers have increased risk of LVH and CHD, suggesting that altered FA delivery constitutes a mechanism through which LVH and CHD are associated in hypertensive subjects.

Key words: coronary heart disease, fatty acid, genetics, hypertrophy, hypertension, lipoprotein lipase (LPL), LPL S447X variant.

Abbreviations: apoAI, apolipoprotein AI; apoB, apolipoprotein B; BMI, body mass index; BP, blood pressure; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; DBP, diastolic BP; FA, fatty acid; FAO, FA oxidation; HDL, high-density lipoprotein; HR, hazard ratio; IHD, ischaemic heart disease; LPL, lipoprotein lipase; LV, left ventricular; LVH, LV hypertrophy; LVMI, LV mass indexed to body surface area; MI, myocardial infarction; NPHSII, Second Northwick Park Heart Study; OR, odds ratio; SBP, systolic BP; SNP, single nucleotide polymorphism.

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INTRODUCTION

LVH (LV (left ventricular) hypertrophy) is an independent risk factor for CVD (cardiovascular disease) [1–3]. The association between LVH and CVD remains poorly understood, but hypertension is a common stimulus for LVH [4] and is itself a powerful risk factor for CVD [5] and heart failure [6].

In pathological situations, such as ischaemia and hypertrophy, the heart switches substrate utilization from predominantly FAO [FA (fatty acid) oxidation] [7] to glucose and lactate catabolism [8,9]. Substrate switching is thought to be an oxygen-sparing mechanism, as glucose utilization requires less oxygen/mol ATP generated than FAO. A number of lines of evidence suggest a direct role for FA metabolism in the pathogenesis and progression of LVH. First, dyslipidaemia and an unfavourable FA profile predict LVH over a 20-year period [10]. Furthermore, cardiac FA metabolism is an independent predictor of LV mass in hypertensive heart disease [11], whereas FAO inhibitors prevent LV dilatation in the pressure-overloaded rat heart [12] and show promising results in human studies on the treatment of CHD (coronary heart disease) and heart failure [13].

LPL (lipoprotein lipase) is the key enzyme in plasma triacylglycerol (triglyceride) hydrolysis, liberating FAs for energy generation in the heart. LPL is highly expressed in adult heart [14], and is down-regulated in the hypertrophied heart of SHRs (spontaneously hypertensive rats) [15,16] and in endothelin-treated neonatal rat cardiomyocytes [15]. Variation in the LPL gene is associated with altered plasma triacylglycerol levels, and variants associated with lower LPL activity [D9N (rs1801177) and N291S (rs268)] are associated with increased risk of CHD [17,18]. The Ser447 stop [S447X (rs328)] variant deletes two amino acids from the C-terminus of LPL, and LPL-X447 is more active [19]. The X447 allele is associated with increased CHD risk compared to S447 in vitro [19]. The X447 allele is associated with increased LPL activity, lower plasma triacylglycerols, higher HDL (high-density lipoprotein) and reduced risk of CHD [17,18,20,21].

We have observed an association between PPARα (peroxisome proliferator-activated receptor α), a key regulator of cardiac fatty acid catabolism [22], and exercise- and hypertension-induced LVH [23], suggesting that cardiac FA utilization directly influences the cardiac growth response. To investigate further the role of FA utilization in LV growth, we examined the association between the LPL S447X variant and hypertension-induced LVH [24], and the hypertension-associated risk of CHD in 2716 healthy middle-aged U.K. men participating in the prospective NPHSII (Second Northwick Park Heart Study) [25].

MATERIALS AND METHODS

Study subjects

Leeds LVH study

A total of 60 normotensive volunteers (30 male and 30 female) were recruited to determine the normal range of LV mass. Patients (n = 205; 201 Caucasians and four Asians) with essential hypertension, defined either as a history of essential hypertension (85 %) or newly diagnosed with two sitting readings of SBP [systolic BP (blood pressure)] ≥160 mmHg and/or DBP (diastolic BP) ≥100 mmHg, were recruited from cardiology outpatient clinics. Genotype data were available for 57 normotensive and 190 hypertensive subjects. All were Caucasian. Patients with a history of valvular heart disease, previous MI (myocardial infarction) and arrhythmias were excluded. Cardiac MRI (magnetic resonance imaging) was performed to accurately determine LV mass, as reported previously [24]. Written informed consent was obtained from all subjects and the local Ethics Committee approved the study.

NPHSII

NPHSII is a prospective study of unrelated healthy middle-aged U.K. men (mean age, 56.1 ± 3.5 years) recruited from nine U.K. general practices and followed prospectively for a median of 10.8 years (range, 7 days to 13.2 years) [25]. Exclusion criteria were a history of MI, cerebrovascular disease, life-threatening malignancy or regular medication with aspirin or anticoagulants. CHD was defined as sudden cardiac death, symptomatic or silent (ECG) MI or coronary revascularization, and time to first event was recorded. In the 2716 eligible Caucasian subjects for whom LPL S447X genotype data were available, there were 219 events, comprising 156 (71 %) acute MI, 42 (19 %) coronary surgery and 21 (10 %) silent MI. Plasma lipid and lipoprotein levels were determined at baseline. At entry, SBP and DBP were recorded twice with a random zero mercury sphygmomanometer after 5 min of sitting. LPL S447X (rs328) genotype was determined by PCR-RFLP (restriction-fragment-length polymorphism) analysis using HinfI digestion (New England Biolabs) [26]. Written informed consent was obtained from all subjects and approval was given by the Institutional Ethics Committees.

Statistical analysis

The maximum number of genotypes available for each parameter was analysed. Hardy–Weinberg equilibrium was examined using χ² test. In the Leeds LVH study, the X447 homozygotes were combined with X447 carriers (S/X + X/X) for all analyses. SPSS Version 12 was used for statistical analysis. The effect of S447X genotype
on measures of BP and LV mass were examined using univariate ANOVA and linear regression. Genotype and covariates, including age, BMI (body mass index), current smoking, gender and, in analysis of LV mass, the number of antihypertensive medications taken and SBP, were added. Factors and covariates were all included in the initial model and removed in a stepwise manner to generate the most parsimonious model. Binary logistic regression analysis was used to examine the effect of genotype on the presence of LVH.

In NPHSII, analysis was performed using 'Intercooled' STATA (version 8.2). Values are means (S.D.). Plasma concentrations of triacylglycerol, apoB (apolipoprotein B) and HDL were not normally distributed and were therefore log-transformed. Comparisons of baseline characteristics were made using ANOVA or $\chi^2$ tests. Adjustments were made by the inclusion of covariates in the models. The effects of hypertension and genotype on lipids were assessed by two-way ANOVA. Survival analysis was carried out using Cox proportional hazards model. Values are presented as HRs (hazard ratios) with 95 % CIs (confidence intervals). A $P$ value of 0.05 was taken as statistically significant. Estimated probabilities were obtained from logistic regression models and are plotted to illustrate the increase in risk with BP.

### RESULTS

#### LPL S447X variant and hypertension-induced cardiac growth

The characteristics of the Leeds LVH study are shown in Table 1. LPL S447X genotype data were available in 57 normotensive and 190 hypertensive subjects. Genotype distributions were in Hardy–Weinberg equilibrium in the entire study and in the normotensive and hypertensive subgroups separately. The X447 allele frequency was no different in the normotensive subjects compared with the hypertensives (Table 1).

The association between S447X genotype and LVMI (LV mass indexed to body surface area) was examined. In the hypertensive subjects, there was no heterogeneity of effect between men and women (results not shown) and, in the group as a whole, X447 carriers had significantly ($P=0.013$) higher LVMI than S447 homozygotes after adjustment for age, gender, SBP and the number of antihypertensive medications taken, all independent predictors of LVMI (Table 2). The $\beta$-coefficient for the X447 allele was +7.2 (3.2) g/m$^2$. The S447X genotype did not influence LVMI in normotensive subjects (69.5 +1.7 and 67.8 +3.0 g/m$^2$ in S/S and S/X + X/X carriers respectively; $P=0.67$).

In the hypertensive group, 74 subjects (39.0 %) had LVH, defined as greater than the mean of normotensive individuals +2 S.D. [77.8 (9.1) and 61.5 (7.5) g/m$^2$ in males and females respectively]. X447 carriers had significantly greater prevalence of LVH than S447 homozygotes (57.5 % compared with 35 % respectively; $P=0.013$) with an OR (odds ratio) for LVH of 2.52 (95 % CI, 1.17–5.40; $P=0.02$) compared with S447 homozygotes. This effect was observed both in males [OR, 2.95 (95 % CI, 1.09–7.96); $P=0.03$] and females [OR, 2.05 (95 % CI, 0.56–7.43)], although this did not reach statistical significance ($P=0.26$).

#### LPL S447X affects hypertension-induced LVH and CHD risk

The association between the LPL S447X genotype and hypertension-induced LVH indicated that LPL S447X may also modulate the risk of CHD in hypertensive subjects. This hypothesis was investigated in NPHSII. The baseline characteristics of participants are shown in Table 3. The X447 allele frequency was 0.103 (95 % CI, 0.094–0.111) and genotype distribution was in Hardy–Weinberg equilibrium in the whole study and in normotensive and hypertensive subgroups. X447 allele frequency did not differ between the Leeds LVH study and NPHSII ($P=0.42$) nor between normotensive [0.11, (95 % CI, 0.09–0.12)] and hypertensive individuals [0.10 (95 % CI, 0.09–0.11)] in NPHSII ($P=0.45$).
Table 3 Baseline characteristics of NPHSII by control or IHD case status

Values are means (S.D.) and frequency (95 % CI), except for triacylglycerol, apoB and fibrinogen where geometric means (interquartile ranges) are provided.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls</th>
<th>IHD cases</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2497</td>
<td>219</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.0 (3.4)</td>
<td>56.6 (3.6)</td>
<td>0.009</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>137.9 (19.1)</td>
<td>143.7 (19.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>84.3 (11.2)</td>
<td>87.8 (11.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>27.5</td>
<td>36.1</td>
<td>0.007</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4 (3.5)</td>
<td>27.1 (3.5)</td>
<td>0.004</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.71 (1.01)</td>
<td>6.04 (1.01)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerol (mmol/l)</td>
<td>1.77 (1.23–2.53)</td>
<td>2.10 (1.43–2.98)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoB (mg/l)</td>
<td>0.86 (0.72–1.01)</td>
<td>0.92 (0.78–1.10)</td>
<td>0.0008</td>
</tr>
<tr>
<td>ApoAI (mg/l)</td>
<td>1.64 (0.32)</td>
<td>1.57 (0.27)</td>
<td>0.008</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>2.70 (2.38–3.02)</td>
<td>2.83 (2.47–3.19)</td>
<td>0.0005</td>
</tr>
<tr>
<td>LPL S447X variant (n)</td>
<td>2014; 456; 27</td>
<td>175; 41; 3</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Association was examined between LPL S447X and plasma lipid and apolipoprotein levels (Table 4). The X447 carriers had 6.6 % lower plasma triglycerides ($P=0.008$), 5 % higher HDL-cholesterol ($P=0.01$) and 3 % higher apoAI (apolipoprotein AI; $P=0.005$) than S447 homozygotes.

The impact of the LPL S447X variant on prospective CHD risk was examined. Overall, the S447X genotype did not influence CHD risk, with S447X allele frequencies being the same in CHD cases and healthy control subjects (Table 3). This was reflected in survival analysis, where X447 carriers had an HR of 1.06 ([95 % CI, 0.76–1.48]; $P=0.72$).

Table 4 Plasma lipid, apolipoprotein and BP measurements in NPHSII according to the LPL S447X genotype

Values are means (S.D.), except for triacylglycerol, apoB and HDL-cholesterol where geometric means (interquartile ranges) are provided. *P value was adjusted for age, practice, BMI and smoking. Triacylglycerol and apolipoprotein measurements were adjusted additionally for cholesterol; BP was adjusted additionally for triglycerol.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>S/S</th>
<th>S/X + X/X</th>
<th>P value</th>
<th>Adjusted P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2189</td>
<td>527</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerol (mmol/l)</td>
<td>1.82 (1.26–2.61)</td>
<td>1.70 (1.20–2.36)</td>
<td>0.008</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.72 (1.01)</td>
<td>5.79 (1.01)</td>
<td>0.16</td>
<td>0.06</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>0.79 (0.65–0.97)</td>
<td>0.83 (0.69–1.02)</td>
<td>0.01</td>
<td>0.009</td>
</tr>
<tr>
<td>ApoAI (mg/l)</td>
<td>1.62 (0.32)</td>
<td>1.67 (0.34)</td>
<td>0.005</td>
<td>0.04</td>
</tr>
<tr>
<td>ApoB (mg/l)</td>
<td>0.86 (0.73–1.02)</td>
<td>0.86 (0.71–1.01)</td>
<td>0.73</td>
<td>0.73</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4 (3.5)</td>
<td>26.6 (3.5)</td>
<td>0.38</td>
<td>0.09</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>138.4 (19.2)</td>
<td>138.4 (19.0)</td>
<td>0.97</td>
<td>0.99</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>84.6 (11.4)</td>
<td>84.6 (10.8)</td>
<td>0.99</td>
<td>0.88</td>
</tr>
<tr>
<td>Presence of hypertension (%)</td>
<td>53.1</td>
<td>51.3</td>
<td>0.46</td>
<td>—</td>
</tr>
</tbody>
</table>

Figure 1 Relationship between cardiovascular risk and SBP in NPHSII in (a) 2716 subjects with genotype data and (b) subjects stratified by LPL S447X genotype

S447 homozygotes are represented by the solid line, and X447 allele carriers (S/X + X/X) are represented by the broken line. In (A), 95 % CI is shown as a dotted line.

The relationship between CHD risk and SBP as a continuous variable was examined. There was a linear relationship between increasing SBP and increasing CHD risk (Figure 1a). SBP was an independent risk...
factor for CHD in NPHSII ($P = 0.0003$), and the risk associated with a 1 S.D. (19.2 mmHg) increase in SBP was 1.35 ([95% CI 1.19–1.53]; $P < 0.0001$). The influence of the LPL S447X genotype on the relationship between CHD risk and SBP was then considered (Figure 1b). In normotensive X447 carriers, increasing SBP had a greater effect on CHD risk in carriers than in S447 homozygotes. CHD risk was examined in subjects stratified by hypertensive status (SBP $\geq 140$ mmHg, DBP $\geq 90$ mmHg or antihypertensive medication), which divided the study into approximately equally sized groups (normotensive, $n = 1282$; and hypertensive, $n = 1432$). Unadjusted survival analysis of the interaction between the S447X genotype and hypertension on risk of CHD over time is presented as a Kaplan–Meier survival plot (Figure 2). There was a significant interaction between S447X genotype and hypertension with regard to CHD risk (unadjusted $P = 0.007$). With normotensive S447 homozygotes as the reference group, after adjustment for traditional risk factors of age, practice, BMI, cholesterol, triacylglycerols and smoking status, normotensive X447 carriers had a reduced risk of CHD [HR, 0.46 (95% CI, 0.24–0.94); $P = 0.02$]. However, this effect was reversed in hypertensive subjects. Compared with normotensive subjects, hypertensive S447 homozygotes had an increased risk of CHD [HR, 1.22 (95% CI 0.89–1.68); $P = 0.21$], but this was not statistically significant, whereas hypertensive X447 carriers had a significant increase in CHD risk [HR, 1.83 (95% CI, 1.20–2.77); $P = 0.005$] (Figure 3), with a significant interaction between the S447X genotype and hypertension with regard to CHD risk ($P = 0.007$). In X447 carriers, the presence of hypertension was associated with an HR of 4.78 (95% CI, 2.22–10.28; $P = 0.00002$).

To study whether the impact of the LPL genotype on CHD risk was mediated through effects on plasma triacylglycerol concentrations, we examined the association of S447X with plasma lipid levels in normotensive and hypertensive subjects (Table 5). Overall, plasma triacylglycerol levels were significantly higher in hypertensive than in normotensive subjects (1.95 ± 1.04 and 1.64 ± 0.84 mmol/l respectively; $P < 0.0001$). A borderline significant interaction was observed between S447X genotype and hypertension with regard to plasma triacylglycerols, with X447 associated with lower triacylglycerols in hypertensive subjects (1.99 ± 1.08 mmol/l in S/S compared with 1.78 ± 0.88 mmol/l in S/X).

### Table 5 Plasma lipid and apolipoprotein levels in normotensive and hypertensive subjects from NPHSII

Values are means (S.D.), except for triacylglycerol, apoB and HDL-cholesterol where geometric means (interquartile ranges) are provided.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Normotensive subjects</th>
<th>Hypertensive subjects</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPL S447X variant</td>
<td>LPL S447X variant</td>
<td>Genotype</td>
</tr>
<tr>
<td></td>
<td>S/S</td>
<td>S/X + X/X</td>
<td>Hypertension</td>
</tr>
<tr>
<td>$n$</td>
<td>1026</td>
<td>256</td>
<td>1162</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/l)</td>
<td>1.65 (1.17–2.26)</td>
<td>1.63 (1.11–2.23)</td>
<td>1.99 (1.35–2.86)</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.61 (0.98)</td>
<td>5.70 (0.98)</td>
<td>5.81 (1.03)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>0.81 (0.66–0.99)</td>
<td>0.86 (0.71–1.07)</td>
<td>0.78 (0.63–0.94)</td>
</tr>
<tr>
<td>ApoAI (mg/l)</td>
<td>1.63 (0.33)</td>
<td>1.63 (0.34)</td>
<td>1.62 (0.31)</td>
</tr>
<tr>
<td>ApoB (mg/l)</td>
<td>0.86 (0.72–1.02)</td>
<td>0.85 (0.70–1.00)</td>
<td>0.87 (0.73–1.01)</td>
</tr>
</tbody>
</table>
in S/X + X/X; \( P = 0.002 \)), but not in normotensive subjects (1.65 ± 0.84 mmol/l in S/S compared with 1.63 ± 0.82 mmol/l in S/X + X/X; \( P = 0.73 \), \( P \) for interaction = 0.05; Table 5). When the CHD risk analysis was then adjusted for the effect of plasma triacylglycerols, the X447 allele was protective in normotensive subjects [HR, 0.48 (95% CI, 0.23–1.01); \( P = 0.05 \)]. However, in hypertensive subjects, X447 carriers had a marked increase in CHD risk [HR, 2.22 (95% CI, 1.48–3.33); \( P < 0.001 \)], whereas hypertensive S447 allele homozygotes had a borderline increased risk of CHD [HR, 1.38 (95% CI, 1.01–1.88); \( P = 0.04 \)] and the interaction remained significant (\( P = 0.004 \))

**DISCUSSION**

The results in the present study demonstrate that, in hypertensive subjects, the \( LPL \) X447 allele is associated with both greater cardiac growth and higher risk of CHD, whereas, in normotensive subjects, the X447 allele did not influence cardiac mass and was protective against the risk of CHD. LVH is an independent risk factor for cardiovascular morbidity and mortality [27], and is associated with increased prevalence of CHD [4], stroke [28], congestive heart failure [29] and sudden cardiac death [3]. Hypertension is a major risk factor for these disorders, and our present observations provide genetic evidence that cardiac energy metabolism may constitute a link between hypertension, LVH and cardiovascular disease.

There is increasing evidence that cardiac substrate utilization directly influences cardiac function and structure. We have demonstrated previously that genetic variation in PPARα, a master regulator of cardiac FAO, influenced hypertension-induced cardiac growth [23]. Mutations in \( \alpha \) genes or mitochondrial DNA cause hypertrophic cardiomyopathy and/or heart failure [30]. Myocardial FAO is an independent predictor of LV mass in hypertensive heart disease [11]. Conversely, in Type 2 diabetic subjects, whose hearts are almost totally reliant on FAO [31], LV mass is greater [32] and LVH is more prevalent [33] than in non-diabetic subjects. Partial FAO inhibitors, such as ranolazine, trimetazidine and perhexiline, have beneficial effects on angina and heart failure [34]. These data indicate that the appropriate regulation of substrate utilization is central to the maintenance of appropriate cardiac mass, and dysregulation is detrimental to cardiac structure and function. The association between the X447 allele and increased LVMI and CHD risk in hypertensives supports this hypothesis. Furthermore, these data indicate that the detrimental effect of the X447 allele in hypertensive subjects is not mediated through systemic effects on plasma lipids. Higher plasma triacylglycerols are associated with increased LV mass [10] and are an independent risk factor for CHD [35]. The X447 allele is consistently associated with lower plasma triacylglycerols [17,18], and our present results demonstrate that hypertensive X447 carriers have significantly lower triacylglycerols than hypertensive S447 homozygotes. The X447 allele has been inconsistently associated with moderately lower CHD risk in some, but not all, studies, with an OR of 0.8 in two meta analyses [17,18]. Our present observation that the X447 allele is associated with a 50% decreased risk in normotensive subjects, but 2.3-fold increased risk in hypertensive subjects, may explain some of the inconsistencies observed previously, as interaction with hypertension was not examined previously. Thus our present studies suggest a direct cardiac effect in which increased cardiac FA availability in the overloaded hypertensive heart of X447 carriers may be detrimental to cardiac function, thus further driving cardiac growth and increasing cardiovascular risk.

Templeton et al. [36] resequenced the \( LPL \) gene and identified 69 SNPs (single nucleotide polymorphisms) defining 88 haplotypes. They concluded that recombinational and mutational hotspots played significant roles in determining the haplotype variation at the \( LPL \) locus and, although the central genomic region had common SNPs, the 5′ region had population-specific variability, which makes tSNP (tagging SNP) analysis difficult. Three functional \( LPL \) SNPs have been well studied and association studies have identified a role for these \( LPL \) variants in the determination of IHD risk. D9N and N291S have been associated with increased IHD risk [17,18], with the N9 allele having a strong interaction with smoking status [37]. The functional basis for these associations has been identified: although the S291N variant leads to a loss of \( LPL \) dimer stability and, therefore, reduced hydrolytic function (since the \( LPL \) monomer is inactive) [38], the substitution of asparagine for aspartic acid (D9N) leads to an altered charge on the protein, which enhances binding to heparin sulfate proteoglycans and increased bridging function [39]. These two SNPs have rare allele frequencies ranging from 0.03–0.06 and have no linkage disequilibrium [40]. The S447S variant has increased enzyme activity and, as such, has been used to rescue the lethality in \( Lpl \) knockout mice, and does so more effectively than wild-type \( LPL \) [41]. The rare allele frequency of S447X is close to 0.20 and, although it shows strong negative linkage disequilibrium with S291N and D9N, the latter two are rare and are unlikely to play a major part in this analysis.

In NPHSII, X447 was associated with lower triacylglycerols in hypertensive, but not normotensive, subjects. Thus the effect of the \( LPL \) S447X genotype on plasma triacylglycerols could not explain the increased risk in hypertensive X447 allele carriers, as this subgroup has the highest CHD risk but significantly lower plasma triacylglycerols than hypertensive S447 homozygotes. This was confirmed when CHD risk adjusted for plasma triacylglycerols produced the same pattern of results. At recruitment, no subjects were taking ACE (angiotensin-converting enzyme) inhibitors, ARBs (angiotensin II
receptor blockers) or statins and, although a proportion of those who developed CHD during follow-up may have been prescribed such medication, this is unlikely to have confounded the genetic effect on risk observed in the present study, but would rather have the effect of diluting it.

The mechanisms underlying the increased risk of CHD in hypertensive X447 allele carriers remain unclear. Transgenic experiments indicate that global LPL overexpression is atheroprotective [42], whereas macrophage LPL expression is pro-atherogenic [43], smooth muscle cell overexpression causes vascular dysfunction [44], skeletal and cardiac muscle overexpression causes severe myopathy, weight loss and premature death [45], and cardiomyocyte membrane-bound LPL overexpression causes dilated cardiomyopathy [46]. However, such ectopic or overexpression experiments may be non-physiological with limited relevance to human disease.

The limitation of the study is the small number of normotensive individuals in the Leeds study and, as with the genotype effect in the hypertensive group, these results need to be confirmed in a larger study. However, as a hypothesis-generating study, it does raise some interesting questions about the role of LPL in the heart. These results support a role for the LPL X447-associated increase in cardiac growth and risk of LVH in response to hypertension. Since the truncation of LPL by two amino acids (LPL-X447) leads to increased hydrolytic efficiency [47], this would generate increased cardiac FA availability, which the results suggest is pro-hypertrophic. This suggests that substrate switching in LVH and ischaemia is indeed a beneficial adaptive response. Furthermore, hypertensive X447 carriers had an increased prospective risk of CHD, promoting the possibility that the heart may be more susceptible to ischaemia in situations of increased FA supply. These results provide genetic evidence for a role of substrate utilization in cardiac structure, function and risk. Human substrate utilization studies are needed to clarify the mechanisms underlying these effects.

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