

# **Lipoprotein Lipase (LPL) gene variants and coronary heart disease.**

*Protocol for a HuGE association review*

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## *Summary Statement*

The aim of this systematic review is to produce up-to-date meta-analyses of the association between specific polymorphisms in the lipoprotein lipase gene and coronary heart disease. We shall also quantify associations between the polymorphisms and measures of lipid metabolism.

## *Background*

Lipoprotein lipase (LPL) plays a central role in lipid metabolism through hydrolysing triglyceride-rich particles in muscle, adipose tissue and macrophages and generating free fatty acids and glycerol for energy utilisation and storage (1). In addition, it performs a bridging role as a ligand in lipoprotein-cell surface interactions and receptor-mediated uptake of lipoproteins (2). The LPL gene is located on chromosome 8p22 (OMIM 238600) and encodes a 448 amino acid mature protein (3). When present in homozygous form, amino acid changing mutations in LPL may lead to chylomicronemia syndrome (4), characterised by severe hypertriglyceridemia and extremely low levels of high density lipoprotein (HDL) and possibly ischemic heart disease (IHD). Heterozygosity for amino-acid changing mutations in *lpl* may lead to intermediate levels of HDL and triglycerides and an increased risk of coronary artery disease (CAD) or myocardial infarction (MI). Seven polymorphisms have been proposed as playing a role in the modulation of plasma HDL and triglyceride levels (T-93G, Asp9Asn/D9N, Gly188Glu/G188E, PvuII, Asn291Ser/N291S, HindIII, and Ser447Ter/S447X). The evidence for an association of these seven polymorphisms with CHD remains controversial, with recent studies suggesting that the Asp9Asn and Ser447Ter alleles may have deleterious and beneficial effects, respectively. (5-8).

Since their identification, a number of observational studies have reported associations between these polymorphisms and coronary heart disease (CHD). These studies typically involve small numbers of participants and their interpretation has been complicated by studies investigating different polymorphisms, the variation in endpoints for CHD, population ethnicity (predominantly European Caucasians), the use of different genotyping methods (e.g. RFLP, SSCP, direct sequencing), and use of different sampling strategies. Three previous meta-analyses have however yielded similar findings (Table 1) (9-11).

Table 1

Previous meta-analyses/systematic reviews of LPL polymorphisms and CAD/MI

Reference (Year)	Number of studies	Number of participants	Clinical outcomes	Allele-specific Odds Ratio (95% CI)* [Number of participants]			
				<i>G188E</i>	<i>D9N</i>	<i>N291S</i>	<i>S447X</i>
Hokanson, J.E. (1997) <sup>§</sup>	14	15,708	Clinical coronary disease events Documented coronary disease based on angiography, or carotid artery thickness	<b>n.a.</b>	<b>1.59</b> (1.03-2.55) [2,881]	<b>0.93</b> (0.73-1.19) [5,998]	<b>0.81</b> (0.65-1.0) [2,075]
Hokanson, J.E. (1999)	16	17,630	Clinical coronary disease events Documented coronary diseases based on angiography, or intimal media thickness by B-mode ultrasonography	<b>5.25</b> (1.54-24.29) [n.a.]	<b>2.03</b> (1.3-3.18) [3,352]	<b>1.25</b> (0.99-1.6) [12,397]	<b>0.81</b> (0.65-1.0) [n.a.]
Wittrup, H.H., <i>et al.</i> (1999)	29	20,903	Ischemic heart disease	<b>4.9</b> (1.2-20.0) [12,207]	<b>1.4</b> (0.8-2.4) [1,961]	<b>1.2</b> (0.9-1.5) [12,487]	<b>0.8</b> (0.7-1.0) [3,438]

\* Wittrup et al. provide ORs for risk of IHD in heterozygous carriers of each mutation; <sup>§</sup> Study also provides analyses on *PvuII* (OR=0.90, 95% CI=0.8-1.01, [2,618]) and *HindIII* (OR=0.84, 95%CI=0.73-0.96, [2,259]).

## *Methods*

### **Criteria for considering studies**

#### *Polymorphisms*

Numerous variants in the LPL gene have been investigated for association with coronary heart disease of which seven have been most frequently investigated. Four of these are located at the N-terminal end of LPL and may influence the catalytic effect of LPL: a T-to-G change at bp –93 in the promoter region; an aspartic acid to asparagine exchange at codon 9, designated as the Asp and Asn alleles (Asp9Asn), respectively (alternatively, designated the D and N alleles using the single-letter amino acid code, D9N); a G-to-A mutation at nucleotide 818 in exon 5 resulting in a glycine to glutamic acid exchange at codon 188 (Gly188Glu/G188E); and an asparagine to serine exchange at codon 291 (Asn291Ser/N291S). The fourth coding variant, a nonsense mutation at codon 447 that alters a serine for a stop codon (Ser447Ter/S447X), is located at the C-terminal end and may influence the enzyme-mediated uptake of lipoproteins by cell-surface receptors. The final two variants, *PvuII* and *HindIII*, are located within introns 6 and 8, respectively.

#### *Clinical outcomes*

The clinical outcomes of interest are coronary stenosis (as defined by angiography of major coronary arteries), non-fatal MI (as defined by electrocardiogram, cardiac enzymes, and clinical features) and death by CHD.

#### *Lipid measurements*

The following measures of lipid metabolism are considered relevant: plasma triglyceride (TG) levels and plasma high-density lipoprotein (HDL) cholesterol levels.

#### *Types of studies*

Primary observational studies (case-control, cohort, and population-based studies) investigating associations between any of the seven LPL gene polymorphisms (either separately or in combination with other markers), and the clinical outcomes of interest (CAD and/or MI). Studies

of adults at any given risk of cardiovascular disease (with or without cardiovascular disease) will be accepted.

## Search methods

Electronic searches will be performed using MEDLINE, EMBASE, BIOSIS, Science Citation Index, Dissertation Abstracts, HuGE Pub Lit, and LocusLink. The search will not be limited to the English language, nor restricted to any age group, gender or publication type. Reference lists of all relevant studies, and meta-analyses, will be examined to identify any additional studies. Investigators in the field will also be contacted for references to studies not yet identified. Attempts will be made to obtain full-text translations of all relevant non-English articles.

The search strategy for assessing the association between the LPL polymorphisms and CHD is the following: (lipoprotein lipase\* OR lipoprotein lipase[mesh] OR lpl\* OR lipd\*) AND (gene\* OR polymorphi\* OR genetic\* OR mutation\* OR allele\* OR genotype\*) AND (coronary stenosis\* OR coronary stenosis[mesh] OR coronary artery disease\* OR coronary artery disease[mesh] OR cad\* OR coronary arteriosclerosis\* OR coronary arteriosclerosis[mesh] OR myocardial infarction\* OR myocardial infarction[mesh] OR mi OR coronary heart disease\* OR coronary heart disease[mesh] OR ischemic heart disease\* OR ischemic heart disease[mesh] OR myocardial ischemia\* OR myocardial ischemia[mesh]).

The search strategy for assessing the associations between the LPL polymorphisms and measures of lipid metabolism is the following: (lipoprotein lipase\* OR lipoprotein lipase[mesh] OR lpl\* OR lipd\*) AND (gene\* OR polymorphi\* OR genetic\* OR mutation\* OR allele\* OR genotype\*) AND (triglyceride\* OR triglycerides[mesh] OR tg OR high density lipoprotein OR high density lipoprotein[mesh] OR hdl OR intermediate density lipoprotein OR intermediate density lipoprotein[mesh] OR idl OR low density lipoprotein OR low density lipoprotein[mesh] OR ldl OR very low density lipoprotein OR very low density lipoprotein[mesh] OR vldl OR cholesterol OR cholesterol[mesh] OR hyperlipidemia OR hyperlipidemia[mesh] OR hypertriglyceridaemia OR hypertriglyceridaemia[mesh]).

## **Study selection**

All relevant articles identified by the search will be scanned on the basis of title, keywords and abstract (where available) by a single reviewer. Articles will only be rejected on the initial screen if the reviewer can determine that the article does not match the inclusion criteria outlined above. Where a title/abstract cannot be rejected with certainty, the full text of the article will be obtained for evaluation. If the reviewer is uncertain about whether to reject an article, the opinion of a second reviewer will be sought before making a decision. The full text of all relevant articles identified by reference searching will be obtained.

Two reviewers will then independently assess the eligibility of studies for inclusion in the review. If disagreements cannot be resolved by discussion a third reviewer will be consulted. If a consensus cannot be reached and further information cannot be obtained from the authors, the study will be excluded.

## **Quality assessment**

Methodological quality of each study to be included will be assessed according to the considerations described by Little *et al.* <sup>(12)</sup>.

## **Data extraction**

Data will be extracted independently by two reviewers using a pre-piloted data extraction form. Differences between reviewers' results will be resolved by discussion and, if necessary, through consultation with a third reviewer. All reviewers participating in the study will participate in a pilot of the data extraction form. Each pair of reviewers will pilot the data extraction form on ten, randomly selected articles. Additional information sheets detailing the genetic and clinical terminology will also be provided. Differences between reviewers' results will be resolved by discussion and any problems discussed at a meeting of all reviewers.

Descriptive data on the participants, study design, outcome measures and genetic polymorphisms, as outlined in the criteria for selecting studies (see above), will be extracted. In addition, data on the genetic methodology used in each article will be collected, including the genotyping assay, PCR

conditions, use of internal laboratory controls, and laboratory worker blinding. Attempts will be made to obtain any missing or unclear data by contacting the authors of each article.

## **Data synthesis**

Meta-analyses will be performed as inverse-variance weighted averages under assumptions of both a common effect and random effects. Random effects meta-analyses will be presented unless a strong relationship between effect size and precision is identified. Funnel plots will be used to investigate such relationships. Heterogeneity of results will be assessed visually in forest plots and using an  $I^2$  statistic that describes the proportion of total variation in observed results attributable to heterogeneity<sup>(13)</sup>.

We will implement the inheritance-model-free analysis of Minelli *et al.*<sup>(14)</sup> to identify evidence of any particular inheritance model. In the absence of evidence in favour of a particular model we will assume a co-dominant model or, if the allele frequency is very low (or if only collapsed carriers vs non-carriers data are available), a dominant model. We will implement alternative inheritance models as a sensitivity analysis. For association between each polymorphism and CAD/MI we will use unadjusted odds ratios; for association between each polymorphism and each measure of lipid metabolism we will use unadjusted differences in means. Where only adjusted odds ratios or adjusted differences in means are available we will use these instead.

## ***Potential conflict of interest***

None known

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