Analysis of gene-environment interaction in coronary artery disease: lipoprotein lipase and smoking as examples

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In a complex disorder such as coronary artery disease (CAD), both genetic and environmental factors influence the onset of disease. Such interactions imply that at the molecular level there is interplay between the gene product and the environmental insult, resulting in a greater than additive effect on risk; for example the synergy between variation in the lipoprotein lipase (LPL) gene and smoking on risk. LPL plays a dual role in lipid metabolism both in the hydrolysis of triglyceride-rich lipoproteins and also as a molecular bridge, enhancing the receptor-mediated uptake of lipoproteins both by the liver (anti-atherogenic) but also by receptors on the artery wall (pro-atherogenic). Smoking is associated with a 2-fold increase in CAD risk, and the mechanisms for this include endothelial damage and promotion of lipid oxidation. Results from a prospective study on CAD risk in healthy middle-aged men show that the risk associated with the LPL-D9N variant, which has a modest effect on plasma triglyceride levels, is enhanced up to 10 fold, but only in men who smoke. The proposed mechanism for this LPL:smoking interaction on CAD risk is the subject of this review.

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Risk factors for coronary artery disease

Studies over the last 20 years have lead to the identification of many coronary artery disease (CAD) "intermediate phenotypes" with high risk being associated with elevated plasma levels of lipids[1,2], fibrinogen[3], and with low levels of HDL cholesterol[4]. It is also now recognised that the inflammatory system occupies a key role in the atherosclerotic process[5], which probably explains why elevated levels of markers of the acute phase response system such as C-reactive protein[6], and cytokines such as interleukin-6[7] are also elevated in CAD subjects. It can thus be predicted that an environmental challenge which stresses any of these systems may be a "risk-environment" for CAD, including increasing age, male gender, dietary intake of fats or vitamins, use of cigarettes and alcohol, presence of hypertension, diabetes and obesity. Of these, smoking is particularly relevant, and will be the focus of this review.

Smoking as a risk factor for coronary artery disease

Smoking is known to roughly double life-time risk of CAD[8] and as shown in figure 1 is thought to increase cardiovascular risk by several different mechanisms. The products of tobacco combustion directly damage vascular endothelium, leading to increased secretion of adhesion molecules which enhance binding of platelets and monocytes to the vessel wall, thus promoting thrombosis and atherosclerosis[9-11]. Smoking disturbs lipoprotein metabolism by increasing insulin resistance and lipid intolerance, and is implicated in the production of small dense LDL. By stimulating catecholamines, smoking up-regulates hormone sensitive lipase, increasing circulating free fatty acid levels[12] thus causing atherogenic dyslipidaemia. In addition, smoking-induced lung damage may lead to an interleukin-6-mediated inflammatory response, causing hepatic up-regulation of fibrinogen expression[13] and increased risk of thrombosis[3]. Smokers have lower levels of antioxidants such as ascorbate and tocopherol and thus smoking may favour the oxidation of LDL[14].

The lipoprotein lipase gene and risk of hyperlipidaemia and coronary artery disease

The plasma level of any CAD intermediate phenotypes such as plasma triglyc-

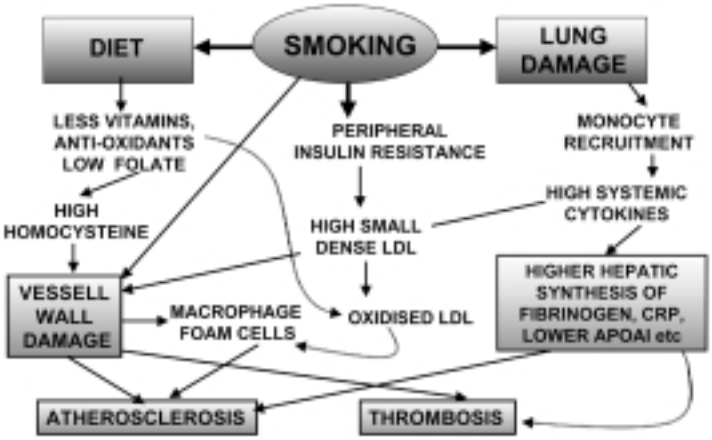

Figure 1. *Mechanisms for smoking effect on risk of coronary artery disease. Smoking leads to altered diet and disturbances in lipid metabolism resulting in greater plasma levels of small dense LDL which is prone to oxidation. It also increases inflammation leading to more monocytes, and endothelial wall damage. Taken together this leads to greater uptake of oxidised LDL in higher number of macrophages in lesions thus promoting atherosclerosis. CRP = C-reactive protein.*

erides will be due to the balance between the rate of production (in this case VLDL secretion from the liver) and rate of removal. Lipoprotein lipase (LPL) plays a central role in lipid metabolism, hydrolysing triglyceride-rich particles in muscle, adipose tissue and macrophages and generating free fatty acids and glycerol for energy utilization and storage[15]. It also has a key "bridging" role as a ligand in lipoprotein/cell surface interactions and receptor-mediated uptake of lipoproteins[16]. Any mutation that results in a partial deficiency of LPL would thus be predicted to result in a modest increase in plasma triglyceride levels, with the increase being proportional to the degree of deficiency caused by the mutation.

To date, two common mutations have been identified in the LPL gene[17], one that causes the substitution of the aspartic acid residue at codon 9 for asparagine (D9N) and the second that alters asparagine at 291 to serine (N291S), with carrier frequencies ranging from 1.6-6.7% in healthy subjects from different European populations[18]. It has been shown by *in vitro* mutagenesis and expression in COS cells that the first of these causes a modest 15-20% decrease in secreted LPL activity[19,20], probably because a larger proportion of the LPL-N9 is retained in the cells. However the LPL-N291S mutation has its effect by a different mechanism. It is known that LPL is only active as a "head-to-tail" dimer[17]. LPL-S291 constructs produce an LPL protein with significantly decreased dimer stability, which results in the levels of secreted LPL activity reduced by up to 50%[20]. In support of these *in vitro* effects, in healthy individuals it has been shown that carriers of either mutation have modestly higher triglyceride levels than non-carriers, with carriers of the more severe LPL-291S mutation having higher triglyceride levels and lower HDL than carriers of the milder LPL-N9 mutation[19,21], as well as a slower clearance of lipids from the blood after a fatty meal challenge[18]. Many case-control studies have reported higher frequencies of these variants in groups of patients with CAD or dyslipidaemias compared to groups of healthy subjects[17], although these results have not been entirely consistent.

Smoking and lipoprotein lipase genotype in coronary artery disease risk

We have now examined the effect of these mutations on CAD risk[22]. Over 2700 healthy men from the Second Northwick Park Heart Study[23] have been studied and for this analysis there have been 18 025 person-years of follow-up (on average 6.6 years/individual). Events included were fatal and non-fatal myocardial infarction, coronary artery surgery, and silent myocardial infarction on the follow-up ECG. Overall, the carrier frequencies of the S291 and N9 alleles were 3.9% (95% confidence interval-CI 3.2-4.7%) and 2.6% (95% CI 2.0-3.3%), respectively. As expected from previous studies, carriers of the S291 allele had a mean baseline triglyceride level that was 14.1% higher than that of non-carriers ($p = 0.014$). A similar trend was seen in N9 carriers who had on average 10.5% higher triglyceride level than non-carriers, although this difference was not statistically significant ($p = 0.15$), confirming the more modest effect of this mutation seen previously.

No evidence was found to suggest that the possession of S291 allele had any effect on the risk of CAD ($p = 0.73$), either in non-smokers or current smokers. By contrast, there was significant ($p = 0.05$) evidence for an increased risk of CAD in LPL-N9 carriers (hazard ratio adjusted for triglycerides 2.33; 95% CI 1.08-5.03). However as shown in figure 2[22], there was very strong evidence for an interaction between smoking and LPL genotype in determining risk ($p < 0.001$), that could not be explained by small differences in body mass index, cholesterol, or any other measured risk factor including triglyceride levels. Carrying the N9 allele appears to modify the effect of smoking such that car-

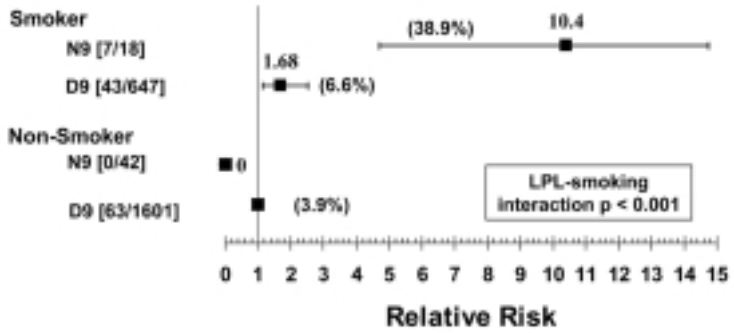

Figure 2. *Relative risk of coronary artery disease events by smoking and lipoprotein lipase (LPL)-D9N status. Graph of the estimated hazard ratio from the Cox's proportional hazard model, stratified by smoking and D9N genotype. Adjustment has been made for baseline triglycerides, age, clinic, body mass index, systolic blood pressure, cholesterol, and fibrinogen level (number of events/subjects in each group). The percentage of each group having an event is shown in brackets. Among non-carriers of N9, 6.6% (95% confidence interval-CI 4.9-8.8%) of smokers had a coronary artery disease event compared to 3.9% (95% CI 3.0-5.0%) of non-smokers, while in N9 carriers 38.9% (95% CI 17.3-64.3%) of smokers compared to 0% (95% CI 0-8.4%) of non-smokers had had an event. Data from Talmud et al.[22].*

riers who smoke were at significantly higher risk of having a CAD event compared to non-carriers who smoke. For the men who were non-carriers of N9, smoking increased the hazard of a CAD event by 1.68 (95% CI 1.1-2.4), similar to that reported earlier[8]. In the group of N9 carriers who were non-smokers there were no events (i.e. estimate of risk in this group was zero). The joint effect of smoking and carrying N9 gave a hazard ratio of 10.4 (95% CI 4.7-22.8) compared to non-carriers who did not smoke.

Mechanism of gene-environment effect

The results of this study suggest that one or more of the injurious effects of smoking are particularly poorly tolerated by subjects with the LPL-N9 variant. Since the enzymatic activity of the two LPLs (LPL-N9 and LPL-S291) are fairly similar, these data strongly suggest that the risk-mechanism of the LPL-N9 mutation is via its bridging function, and we can speculate on mechanisms by which smoking interacts with LPL-N9 to cause the apparent high risk of CAD. Under normal conditions, LPL is present on the endothelial luminal wall attached to heparan sulphate proteoglycans where triglyceride hydrolysis takes place. The local production of LPL is now well recognised as an important factor in the developing atherosclerotic lesion. Damage to the endothelium will promote the recruitment of monocyte/macrophages and smooth muscle cells into the lesion area, both of which synthesise LPL[24]. As well as its role in lipoprotein hydrolysis, LPL acts as a bridge between heparan sulphate proteoglycans and lipoproteins and initiates receptor-mediated catabolism of lipoproteins into cells[16]. The bridging function is also involved in endothelial cell-monocyte interactions, due to the ability of the LPL dimer to promote proteoglycan/proteoglycan interaction[25]. In addition, LPL has recently been shown to have a high affinity for oxidised LDL and to promote scavenger receptor uptake of oxidised LDL[26]. As discussed above, *in vitro* studies show LPL-N9 is poorly secreted into the medium, but these studies have not distinguished between secretion deficiency and retention on the cell surface. Based on these observations, we propose that the asparagine for aspartic acid amino acid substitution at residue 9 will increase the "stickiness" of LPL-N9. This effect, combined with the increased synthesis of LPL-N9 at the site of smoking-related endothelial damage, would lead to greater accumulation, retention or modification of lipoproteins on the endothelium and subendothelium. The enhanced recruitment of monocytes and/or oxidised LDL to the developing lesion thus stimulates foam cell formation leading to increased atherogenesis. Verification of this hypothesis requires additional *in vitro* and *in vivo* studies.

Conclusions and future avenues of research

Smoking is highly prevalent in most countries in Europe, and represents an increasing health burden worldwide. It is clear that although smoking increases risk in all subjects, some are particularly susceptible due to their genetic make-up. The high-risk estimate we have observed in N9 smokers in men from the Second Northwick Park Heart Study[23] requires confirmation by further prospective studies; because of the size of this group, the estimate lacks precision. However, since, for example in the UK there are a predicted 550 000 men over the age of 16 who are carriers for this LPL-N9 variant and roughly 25% of these are likely to be smokers, this represents an estimated 150 000 men who, if the data are confirmed, may be at considerable risk of CAD, and who would experience considerable benefit from cessation of smoking. The findings suggest that studies of ways and settings to implement the use of genetic risk information and targeted smoking cessation strategies would be useful. If this gene-environment interaction can be confirmed by repeat studies, it would be appropriate to include this as a genetic test in risk algorithms to advise healthy subjects how best to avoid CAD. This will enable us to give genotype-specific

life-style advice, and to tailor clinical and therapeutic decisions to an individual's genotype. The identification of the relationship between genetic variants in LPL and CAD points to the rate-limiting and thus key role in the pathological processes of this enzyme, with LPL being expressed in the atherosclerotic plaque in foam-cell macrophages. It also reveals potential novel therapeutic possibilities, for example to block LPL bridging functions on these cells, to prevent disease in a molecularly rational manner.

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