Letters

Increased frequency of the lipoprotein lipase 9N allele in adults with Type I (insulin-dependent) diabetes mellitus

Dear Sir,

Lipoprotein lipase (LPL) is a homodimeric lipolytic enzyme that hydrolyses triglycerides from triglyceride-rich lipoprotein particles at the luminal wall of capillaries, primarily in muscle and adipose tissue, to produce non-esterified fatty acids for energy or storage by adjacent tissues [1]. It is also involved in lipoprotein clearance through the binding of remnant lipoprotein particles to the LDL receptor-related protein. Further, LPL is expressed on macrophages, promotes LDL retention in the subendothelial matrix and is present in atherosclerotic lesions. Because of these roles, the LPL gene is considered a candidate gene for vascular disease, including coronary heart disease.

More than 50 single nucleotide polymorphisms have been described within the *LPL* gene. Some of these occur at relatively high frequencies in normal cohorts, have been shown to affect LPL function and have been associated with altered lipid phenotypes [1]. The *LPL D9N* polymorphism, producing an aspartic acid to asparagine substitution at amino acid position 9, occurs in exon 2 of the *LPL* gene. The *9N* allele has been found at carrier frequencies ranging from 2–4 % in various cohorts and causes a secretion deficiency of the LPL monomer, leading to decreased LPL activity [1, 2]. A recent meta-analysis of previous population and cohort based studies of *LPL* polymorphisms suggests that carriers of the *9N* allele have a mildly atherogenic lipid profile with raised triglyceride and decreased HDL cholesterol, decreased LPL activity and a possible increased risk of coronary heart disease [3].

Given that diabetic dyslipidaemia is associated with low LPL activity, we assessed the frequency of the *9N* variant in a cohort of Australian Caucasian subjects with Type I (insulin-dependent) diabetes mellitus attending the Royal Perth Hospital diabetic clinic ($n = 399$) and healthy community control subjects ($n = 635$). Approval for the study was granted by the Royal Perth Hospital Ethics Committee and all subjects provided written informed consent. The 236 male and 163 female diabetic subjects were 47 ± 15 (SD) years old with known diabetes for 21 ± 11 years and with a mean BMI of 26.8 ± 15.4 kg/m^2. The average HbA$_{1c}$ value for the cohort was $8.8 \pm 1.7\%$, and 77 % were treated with insulin injections four or more times daily. Macrovascular complications were evident in 12 % of subjects, peripheral vascular disease in 16 %, 56 % showed some degree of retinopathy, and 13 % and 6 % showed evidence of microalbuminuria and macroalbuminuria, respectively.

We found that 5.3 % (21/399) of the Type I diabetic subjects were heterozygous carriers of the *LPL 9N* allele compared with 2.5 % (16/635) of healthy control subjects, giving an odds ratio of 2.2 (95 % CI 1.1–4.2, $p < 0.025$). No subjects were homozygous for the polymorphism. For comparison, the frequency of *LPL 9N* was not statistically significantly different in our cohort of subjects with Type II (non-insulin-dependent) diabetes mellitus (3.5 %, 15/429) compared with the healthy control subjects. There was no sex imbalance in the carrier frequencies. The possibility that this result came about because of familial clustering or racial admixture has been explored and, based upon patient demographic records, is thought to be unlikely. The subjects were unrelated and generally descended from European families, the large majority originating from Anglo-Celtic ancestors in the UK and our control subjects were from the same racial background. Multivariate analyses of fasting serum lipids, when applied separately to the diabetic and the community control groups, showed no statistically significant associations between *LPL D9N* genotype and triglycerides, total cholesterol or HDL cholesterol concentrations using models adjusted for age, sex, BMI and apolipoprotein E genotype. There were no sex-specific associations or trends between the *LPL 9N* allele and fasting lipid profiles in the diabetic subjects. There were no associations evident between *LPL 9N* carrier status and coronary heart disease (myocardial infarction, angina pectoris, coronary angioplasty or coronary artery bypass surgery), cerebrovascular events, microvascular disease (retinopathy, nephropathy or peripheral vascular disease) or hypertension in models adjusted for age, sex, glycaemic control, duration of diabetes and apolipoprotein E genotype. Further subgroup analyses were not possible due to low *LPL 9N* carrier numbers upon cohort stratification.

The unexpectedly increased frequency of the *LPL 9N* allele in Australian Type I diabetic subjects suggests either an as-

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sociation between the disease state and a region at or near chromosome 8p22, for which *LPL 9N* is a marker, or that the inheritance of the *9N* allele has functional implications for diabetic metabolic processes. The *LPL 9N* allele occurs in the non-diabetic cohort, albeit at a lower frequency, and therefore it is not itself sufficient to cause Type I diabetes. If it were, genome-wide scans would have previously identified it as a strong risk factor. Rather, *LPL 9N* could be associated with another Type I diabetes-associated locus or *LPL 9N* might have an effect additive to those of the other diabetes-associated loci. At present, the association of loci on chromosome 8 with Type I diabetes is not clear [4, 5] but there could be a relation with the *IDDM2* locus. This locus involves a variable number of tandem repeats polymorphism of the insulin gene (INS VNTR) and is important to Type I diabetes. A recent study of a cohort with Type I diabetes in the United Kingdom showed an association between the chromosome region 8p24–21, which encompasses the LPL locus, and Type I diabetes in a subgroup of subjects who carried a high-risk form of the *INS VNTR*, an observation not seen in subjects with a low-risk *INS VNTR* or when all subjects were pooled [6].

By what other mechanisms could *LPL 9N* influence the development of Type I diabetes? In vivo murine studies provide new evidence which shows that LPL modulates islet cell insulin secretion by a process which involves the LPL-regulated supply of non-esterified fatty acids [7]. A deficiency of LPL can cause relative hypoglycaemia through hyperinsulinaemia, which is probably induced by the decreased supply of non-esterified fatty acids to islet cells [7]. Because it has been postulated that factors affecting intrauterine growth and fetal development can also affect the risk of development of Type I diabetes [8], it is possible that the effects of *LPL 9N* on pancreatic islet cells, working through this or other as yet unknown mechanisms, have implications for in utero organogenesis and pancreatic development. When *LPL 9N* acts, it is, however, almost certainly in concert with other predisposing factors such as the high-risk *IDDM2* locus.

Yours faithfully,
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