Lipoprotein Lipase Gene Variation: Association with Ishaemic Heart Disease in Type 2 Diabetes.

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Abstract

Lipoprotein lipase, the rate determining enzyme for the hydrolysis and removal of triglycerides from chylomicrons and very low density lipoproteins (VLDL), plays a central role in lipoprotein metabolism. By studying two restriction fragment length polymorphisms at the lipoprotein lipase gene locus, we examined whether variation at this gene may be associated with the presence of ischaemic heart disease in non-insulin dependent diabetic subjects. The frequency of 8.7 kb Hind III homozygous genotype was significantly increased in diabetic subjects with ischaemic heart disease (n=21) at 67% compared to diabetic subjects with out ischaemic heart disease (n=42) at 33% (p < 0.05) and non-diabetic control subjects (n=80) at 36% (p <0.05). There were no differences in the allele frequencies at Pvu II polymorphism between the three groups. There were no significant differences in the levels of triglyceride, cholesterol or high density lipoprotein (HDL) cholesterol between different Hind III genotypes within each of the three subject groups. We conclude that 8.7 kb Hind III homozygous genotype was associated with ischaemic heart disease in non-insulin-diabetic subjects with a risk ratio of 4.0 (95% confidence interval 1.3-12.1).

Introduction

Epidemiological studies show a considerable increase in the prevalence of ischaemic heart disease (IHD) in patients with non-insulin-dependent diabetes mellitus (NIDDM) compared with age and sex matched non-diabetic subjects. While some of this increased IHD prevalence may be accounted for by the clustering of hypertension, obesity and dyslipidaemia with NIDDM (syndrome X), it appears that even after correcting for these associated risk factors there is some independent effect of NIDDM which accelerates atheroma formation. The evidence that poor glycaemic control, as assessed by glycated haemoglobin levels, is an additional risk factor for IHD in NIDDM is scanty.

Additional mechanisms that may contribute to a NIDDM specific effect are hyperinsulinaemia, lipoprotein glycosylation, increased platelets aggregation and impaired fibrinolysis.

The role of dyslipidaemia as a risk factor for IHD in NIDDM has been extensively studied. The characteristic changes in lipoproteins in NIDDM subjects are moderate elevations of serum and VLDL triglyceride levels, which appear to correlate with
glycaemic control, and low HDL cholesterol.\textsuperscript{8,9} Epidemiological and prospective studies have shown that raised serum and VLDL triglyceride concentrations and reduced HDL cholesterol levels are associated with vascular disease in NIDDM subjects.\textsuperscript{1,10,11,12} The dyslipidaemia seen in NIDDM subjects is partly accounted for by reduced activity of lipoprotein lipase (LPL), the rate limiting enzyme for triglyceride removal from triglyceride-rich lipoproteins.\textsuperscript{13,14} The multitude of potentially atherogenic factors in NIDDM greatly complicates the task of identifying which factors predominate. It appears that genetic factors may be implicated in the development of IHD in NIDDM subjects, but it is not clear whether these genes are inherited independently or co-inherited together with genes responsible for NIDDM susceptibility. The study of DNA restriction fragment length polymorphism (RELP) markers around candidates genes in NIDDM subjects have previously shown association of markers at both the insulin gene\textsuperscript{15} and apoprotein AI- CIII-AIV gene complex\textsuperscript{16,17} with IHD, and of insulin gene polymorphisms with hypertriglyceridaemia.\textsuperscript{18} Previous studies in our laboratory have demonstrated an association in non-diabetic Caucasians of an RELP marker at the LPL gene locus with both hypertriglyceridaemia\textsuperscript{19} and IHD.\textsuperscript{20} To assess whether variation at the LPL locus may contribute to the development of IHD in NIDDM subjects we evaluate these RFLP markers at the LPL locus in a group of Caucasian NIDDM subjects with and without clinical evidence of IHD.

**Subjects and Method**

**Subjects**

Unrelated English Caucasian type 2 diabetic subjects (n=71) were recruited from the Diabetic Clinic, St Bartholomew’s Hospital. All NIDDM individuals met accepted criteria\textsuperscript{21} and had a disease duration of 1-30 years. Of the 4 subjects receiving insulin this was not commenced until at least five years after diagnosis. Fifteen diabetic subjects had a history of documented myocardial infarction confirmed by typical electrocardiogram and serum enzyme changes. A further 10 diabetic subjects gave a history of typical angina pectoris. The remaining diabetic subjects (n=46) gave no history of angina on a standard questionnaire, had palpable peripheral pulses and a normal resting electrocardiogram. The control subjects (n=85) of the same ethnic group were recruited from a health screening center, and had no personal or family history of diabetes or ischaemic heart disease, had a normal resting electrocardiogram and a fasting blood glucose below 6.0mM. The characteristics of the groups are given in Table 1

**Laboratory assays**

The analysis of cholesterol and triglyceride concentrations in serum and lipoprotein fractions was performed using enzymatic methods (Boehringer Mannheim, UK). HDL-cholesterol was assayed following heparin/manganese chloride precipitation. Blood glucose was assayed using an automated glucose oxidase method, and glycate haemoglobin by a specific ion-exchange chromatography technique (Bio-Rad, UK).

**Restriction endonuclease analysis**

DNA was extracted from leucocytes in whole blood by standard methods\textsuperscript{22} and
analysis of Hind III and Pvu II polymorphisms at the lipoprotein lipase locus performed as previously. Not all subjects were genotyped at both loci. The Hind III polymorphic site was detected by fragments of 17.5 Kb (H1 allele) or 8.7 Kb (H2 allele), and the Pvu II polymorphism identified by fragments of 7.0 Kb (P1 allele) or 4.4 Kb and 2.5 Kb (P2 allele).

**Statistical analysis**

The association of LPL polymorphisms with ischaemic heart disease in NIDDM subjects was assessed by Chi-squared analysis. The relative incidence (“risk ratio”)

**Table 1:** Clinical data of type 2 diabetic subjects with and without ischaemic heart disease and non-diabetic controls. (Mean values ± standard deviation)

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>NIDDMs without IHD</th>
<th>NIDDMs with IHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>85</td>
<td>46</td>
<td>25</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>85/0</td>
<td>19/27</td>
<td>18/7</td>
</tr>
<tr>
<td>Age (y)</td>
<td>55.4 ± 7.3</td>
<td>64.3 ± 10.1</td>
<td>67.1 ± 7.1</td>
</tr>
<tr>
<td>Duration (y)</td>
<td>-</td>
<td>8.1 ± 7.0</td>
<td>8.2 ± 6.3</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>25.6 ± 2.6</td>
<td>27.7 ± 5.8</td>
<td>26.4 ± 2.8</td>
</tr>
<tr>
<td>Blood Glucose (mM)</td>
<td>5.1 ± 0.4</td>
<td>12.4 ± 5.7</td>
<td>11.1 ± 6.0</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>-</td>
<td>7.9 ± 2.2</td>
<td>6.9 ± 2.1</td>
</tr>
<tr>
<td>Cholesterol (mM)</td>
<td>6.3 ± 1.0</td>
<td>5.8 ± 1.0</td>
<td>6.2 ± 1.4</td>
</tr>
<tr>
<td>Triglycerides (mM)</td>
<td>1.3 ± 0.6</td>
<td>1.9 ± 1.5</td>
<td>1.9 ± 1.0</td>
</tr>
<tr>
<td>HDL-Cholesterol (mM)</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.4</td>
<td>1.1 ± 0.3</td>
</tr>
</tbody>
</table>

Difference between diabetics with IHD and without IHD: HDL-cholesterol P < 0.02
Difference between diabetic and control groups: BMI P<0.01
Total cholesterol P<0.05
Triglycerides P<0.005

**Table 2:** Genotype and allele frequency distribution at lipoprotein lipase polymorphisms in control subjects and diabetic subjects with and without ischaemic heart disease.

<table>
<thead>
<tr>
<th>Hind III locus</th>
<th>Genotypes</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H1H1</td>
<td>H1H2</td>
</tr>
<tr>
<td>Controls</td>
<td>11 (0.14)</td>
<td>40 (0.50)</td>
</tr>
<tr>
<td>NIDDM without IHD</td>
<td>7 (0.17)</td>
<td>21 (0.50)</td>
</tr>
<tr>
<td>NIDDM with IHD</td>
<td>1 (0.05)</td>
<td>6 (0.29)</td>
</tr>
</tbody>
</table>

**Pvu II locus**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1P1</td>
</tr>
<tr>
<td>Controls</td>
<td>10(0.19)</td>
</tr>
<tr>
<td>NIDDM without IHD</td>
<td>7(0.24)</td>
</tr>
<tr>
<td>NIDDM with IHD</td>
<td>6(0.35)</td>
</tr>
</tbody>
</table>

Results are shown as genotype number (frequency) and allele frequency

At Hind III locus:
Genotype frequency
- NIDDM with IHD vs NIDDM without IHD P<0.05
- NIDDM with IHD vs Control Group P < 0.05

Allele frequency
- NIDDM with IHD vs NIDDM without IHD P < 0.02
- NIDDM with IHD vs Control group P < 0.02

Genotype and allele frequency differences at Pvu II locus not significant.
was calculated as $RI = hK/HK$, where $h$ is the number of patients with test genotype, $k$ the number of patients with the ‘normal’ genotype, $H$ the number of controls with the test genotype and $K$ is the number of controls with the ‘normal’ genotype.\textsuperscript{23} Comparison of demographic data between the two NIDDM groups was assessed by the Mann-Whitney test, and between different genotype groups by the non-parametric Kruskal-Wallis analysis of variance.

**Results**

Non-insulin dependent diabetic subjects with IHD had significantly lower HDL-Cholesterol concentrations than those without IHD ($p < 0.01$), but no difference was seen in total cholesterol or triglyceride levels despite the clear sex difference between the groups (Table 1). Duration of diabetes, body mass index, fasting blood glucose and glycated haemoglobin levels were not significantly different between diabetic subjects with and without evidence of IHD.

The distribution of genotypes at the $Hind$ III and $Pvu$ II loci in the non-diabetic population conformed to Hardy–Weinberg equilibrium. The frequency of the H2 allele was significantly increased in NIDDM subjects with IHD compared to both NIDDM subjects without IHD ($\chi^2 = 6.37$, df = 1, $P < 0.02$) Table 2. There was no difference in the frequencies of $Pvu$ II alleles between the three groups (Table 2).

In an attempt to elucidate the mechanism by which the H2 LPL allele may predispose to macroangiopathy in NIDDM subjects, we studied the relationship between LPL genotypes and serum lipid levels, body mass index and blood sugar. No significant difference between any of these risk factors was found between genotypic groups within each of the three clinical patient groups (data not shown)

**Discussion**

In this study we found an association of the H2 allele at the LPL locus with IHD in NIDDM subjects. Although the subgroup of NIDDM subjects with IHD genotyped at the $Hind$ III locus was small ($n=21$) the prevalence of H2H2 genotype (67%) was significantly increased in this group compared to both diabetic subjects without IHD (33%) and non-diabetic, non-IHD control subjects (36%). The risk ratio of the H2H2 genotype for the development of IHD in NIDDM subjects is 4.0 (95% confidence interval 1.3-12.1), similar to that of the non-diabetic Caucasian population of 2.73 (95% confidence interval 1.44-5.18).\textsuperscript{20} The genotype distribution in our Caucasian non-diabetic population does not differ from those reported previously.\textsuperscript{19,20} There was no difference in allele frequency at either of the RFLPs studied between the total diabetic and non-diabetic groups, suggesting that the LPL locus is unlikely to play a major part in the genetic susceptibility to NIDDM, but may predispose a subgroup of NIDDM subjects to IHD.

Various RFLP markers at candidate genes in the non-diabetic population have been shown to be associated with both atherosclerosis\textsuperscript{24} and hypertriglyceridaemia.\textsuperscript{19,22} Because of the increased prevalence of both dyslipidaemia and IHD in NIDDM subjects, great care must be exercised in interpreting the results of genetic marker association studies in NIDDM populations as any observed association could be due
to an association with NIDDM, IHD or hypertriglyceridaemia. When studying NIDDM subjects without ischaemic heart disease it is therefore important to exclude any evidence of IHD, especially with possibility of ‘silent’ myocardial ischaemia, and also those NIDDM subjects with a family history of IHD. We have attempted to address these problems in this study.

The human lipoprotein lipase gene, encoding a mature protein of 448 amino acids, has been localized to chromosome 8p22 and contains 10 exons spread over approximately 35kb. The polymorphic Hind III site appears to be within intron 8. The mutation giving rise to H2 allele is therefore unlikely to be functional but could be in linkage disequilibrium with an aetiological locus for IHD in NIDDM subjects, as well as in non-diabetic subjects, either within the LPL gene or an adjacent one. Lipoprotein lipase activity is low in untreated NIDDM subjects but increases after the treatment with sulphonylureas and insulin. The response of LPL to correction of hyperglycaemia in NIDDM subjects is variable, and it is therefore possible that variation in the LPL gene may predispose a subgroup of NIDDM subjects to IHD. The mechanism whereby the LPL H2 allele predisposes to IHD is unclear, as there was no clear difference in lipid levels in the three clinical subject groups between the different genotypic groups in this study, although within both NIDDM subject groups the number of subjects with each genotype may be too small for such an effect to be significant. However, Heinzmann et al. have shown in a population of non-diabetic Caucasian subjects undergoing coronary angiography and their spouses that a LPL-Hind III genotype (probably equivalent to our H1H1 genotype, although there is a small discrepancy in allele sizes) was associated with significantly decreased HDL-cholesterol levels and increased total cholesterol concentrations, confirming our previous report that variation in the LPL gene affects triglyceride and HDL-cholesterol levels. Analysis of a larger NIDDM population with and without clinical IHD will disclose whether the observation reported here can be confirmed or is a chance finding. Further studies comparing the activity of lipoprotein lipase, both in vitro and in vivo, in NIDDM subjects with and without IHD and between the different LPL genotype subsets may suggest the mechanism whereby variation at the LPL gene locus may predispose to IHD in NIDDM subjects.

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References


