Original article

Association of endothelial lipase Thr111Ile polymorphism with lipid metabolism and microvascular complications in type 2 diabetic patients

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Abstract

Aim. – Endothelial lipase (EL) is a key enzyme in lipid metabolism, and a polymorphism in the EL gene may be a candidate for modulating lipid parameters in type 2 diabetic (T2D) patients.

Methods. – In 396 T2D patients (age: 59.5 ± 10.7 years; BMI: 28.9 ± 5.3 kg/m2; HbA1c: 8.2 ± 1.9%), the c.584C > T polymorphism (rs2000813, p.Thr111Ile) was studied in 225 men (frequency of c.584T: 0.351) and 171 women (frequency of c.584T: 0.304). Patients’ metabolic parameters, and macrovascular and microvascular complications, were assessed at baseline and at follow-up (mean: 4.2 years).

Results. – Patients who were homozygous for the minor allele displayed modestly decreased low-density lipoprotein (LDL) cholesterol and raised apolipoprotein B at baseline, and raised systolic blood pressure and high-density lipoprotein (HDL) cholesterol on follow-up. Homozygosity for the minor allele was significantly associated with frequency of retinopathy (P = 0.025), with TT homozygous patients more likely to have diabetic retinopathy (OR: 3.505; 95% CI: 1.491–8.239) both initially and at follow-up.

Conclusion. – The c.584C > T EL polymorphism is associated with a higher risk of diabetic retinopathy that could be linked to modifications in HDL-cholesterol metabolism and blood pressure levels.

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Keywords: Endothelial lipase; Polymorphism of endothelial lipase gene; Type 2 diabetes mellitus; HDL cholesterol; Diabetic retinopathy; Genetics

Résumé

Association du polymorphisme Thr111Ile de la lipase endothéliale avec les paramètres lipidiques du sérum et les complications microvasculaires chez des patients diabétiques de type 2.

Objectif. – La lipase endothéliale est une enzyme-clé du métabolisme lipidique et certains des polymorphismes de son gène pourraient être de bons candidats pour moduler les paramètres lipidiques du sérum des diabétiques de type 2 (D2T).

Méthodes. – Dans un groupe de 396 D2T (âge 59,5 ± 10,7 ans, IMC 28,9 ± 5,3 kg/m2, HbA1c 8,2 ± 1,9 %), nous avons étudié le polymorphisme c.584C > T (rs2000813, p.Thr111Ile) chez 225 hommes (fréquence de l’allèle c.584T: 0,351) et 171 femmes (fréquence de l’allèle c.584T: 0,304). Les paramètres métaboliques ainsi que les complications micro et macro-vasculaires des patients ont été évalués initialement (t0), puis après une période de suivi moyen de 4,2 années (t1).

Résultats. – Les patients homozygotes pour l’allèle rare présentaient une diminution modeste du LDLc et une élévation de l’apolipoprotéine B à t0, une augmentation de la pression artérielle systolique et du HDLc à t1. L’homozygote pour l’allèle minoritaire était associé à une augmentation de fréquence de rétinopathie (P = 0,025), les sujets homozygotes TT présentant un risque plus élevé de rétinopathie (OR 3,505 ; 95% CI: 1,491–8,239) tant au temps t0 que t1.

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1. Introduction

Type 2 diabetes (T2D) patients are at high risk of early atherosclerosis, particularly coronary heart disease (CHD) and specific microvascular complications (such as nephropathy and retinopathy) [1]. This increased risk can be partly accounted for by lipoprotein disorders linked to insulin resistance, specifically, increases in very low-density lipoprotein (VLDL), triglycerides (TG) and cholesterol, and decreases in high-density lipoprotein cholesterol (HDL-C) [2,3]. Genetic factors modulating the concentrations of TGs and cholesterol are of particular interest in T2D patients. Of these, the genes encoding for the enzymes of the lipolytic system, such as lipoprotein lipase (LPL; OMIM ID: 238600), hepatic lipase (HL; OMIM ID: +151670) and endothelial lipase (EL; OMIM ID: *603684), may harbour common variations that can modulate the risk of CVD because of their central role in TG and HDL metabolism.

EL belongs to the same gene family as LPL and HL [4–6], and is produced by endothelial cells and macrophages, and by the liver, lungs, testes, ovaries and placenta. It is an amino-acid 482 protein encoded by 11 exons that share a 45% amino-acid homology with LPL and 40% with HL, and is primarily a phospholipase with little TG lipase activity. EL is more effective in hydrolyzing HDL lipids than are either LPL or HL, and appears to be a major determinant of HDL concentration, structure and metabolism, as demonstrated in various models of animal knockout and overexpression [7–10]. Although data in humans are less definitive [7,11], common genetic variants have been described [12]. Among them, the c.584C>T polymorphism (p.Ile111Thr) appears to influence HDL-C levels. In healthy Caucasians, those homozygous for the 111Ile (c.584T) allele had higher HDL-C and lower EL enzymatic activity [13]. In a community-based familial study of Japanese-Americans, the LIPG c.584C>T variant was strongly associated with HDL3 and apolipoprotein A1 (apoA1) plasma levels [14]. In a cohort study of coronary artery disease, TT homozygotes had 14% higher levels of HDL-C compared with CC homozygotes, although these polymorphisms had no impact on the risk of coronary disease [11,15]. TT has also been associated with acute myocardial infarction independent of HDL-C levels [16]. In addition, recent studies have suggested that EL may be an important determinant of HDL metabolism and inflammation acting at the level of the vessel wall [17,18].

Hypertriglyceridaemia and low HDL-C are both independent risk factors for CHD in the general population [19,20], and for macro- and microvascular complications of diabetes [3,21–25] in the context of low-grade inflammation. Our hypothesis was that the EL gene plays a direct or indirect role in the dyslipidaemic risk profile commonly found in T2D patients, and that it may also influence the development of macro- and microvascular complications. For this reason, the relationships between the p.Ile111Thr polymorphism and lipid profiles, and macro- and microvascular complications, were studied in a longitudinal cohort study of 396 T2D patients.

2. Methods

2.1. Study population

Starting in 1992, the present longitudinal cohort study included 396 unrelated French volunteers with T2D (171 women and 225 men), aged 59.5 ± 10.8 years (range: 27–83 years), attending the Diabetes Centre in Reims. The study protocol was approved by the local ethics committee, and the criteria for T2D were those defined by the National Diabetes Data Group [26]. Mean duration of diabetes was 11.7 ± 7.6 years, and mean body mass index (BMI) was 28.9 ± 5.3 kg/m². Arterial blood pressure was measured in the right arm after a 10-min rest in the supine position. Hypertension was diagnosed when systolic blood pressure (SBP) was ≥ 130 mmHg or diastolic blood pressure (DBP) was ≥ 80 mmHg, or antihypertensive treatment had been prescribed. Mean time to follow-up was 4 ± 1.6 years.

Patients were treated with diet and/or antidiabetic drugs (metformin [n = 253] and/or sulphonylureas [n = 280]) and/or insulin (n = 40). Lipid-lowering therapy was being taken by 20.8% of patients (n = 20 with statins and n = 64 with fibrates), and 48.8% of the patients were being treated for hypertension (50% with ACE inhibitors); all treatments were equally distributed in each genotype group. In addition, 30% were smokers, again with no genotype group differences. Most of the women were postmenopausal, but none were receiving hormonal replacement therapy (HRT).

2.2. Diabetic complications

CHD was diagnosed on the basis of previous myocardial infarction and/or angina and/or electrical signs on resting electrocardiography (ECG). Peripheral arterial disease (PAD) was diagnosed on the basis of intermittent claudication or the absence of peripheral pulse, as confirmed by ultrasonography. Diabetic retinopathy (DR) was described as either absent or present (simple, preproliferative, proliferative) by an ophthalmologist based on standard funduscopy examination and fluorescein angiography when necessary, using a grading scale derived from the Early Treatment Diabetic Retinopathy Study (ETDRS) classification system [27]. Nephropathy (NP) was diagnosed by creatinine clearance < 60 mL/min, using the Modification of Diet in Renal Disease (MDRD) study formula, and/or the presence of microalbuminuria (30–300 mg/24 h).
Table 1
Main characteristics of study patients (n = 396).

<table>
<thead>
<tr>
<th></th>
<th>Visit 1</th>
<th>Visit 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (n = 171)</td>
<td>Men (n = 225)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>59.5 ± 10.7</td>
<td>60.4 ± 11.09 (171)</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²)</strong></td>
<td>28.9 ± 5.3</td>
<td>29.8 ± 6.08 (171)</td>
</tr>
<tr>
<td><strong>Duration of diabetes (years)</strong></td>
<td>11.7 ± 7.6</td>
<td>11.7 ± 7.12 (171)</td>
</tr>
<tr>
<td><strong>Creatinine (µmol/L)</strong></td>
<td>93.6 ± 23.50</td>
<td>87.3 ± 26.13 (175)</td>
</tr>
<tr>
<td><strong>Creatinine clearance</strong></td>
<td>89.4 ± 24.4</td>
<td>108.5 ± 22.4</td>
</tr>
<tr>
<td><strong>Glucose (mmol/L)</strong></td>
<td>10.3 ± 3.8</td>
<td>10.7 ± 4.07 (171)</td>
</tr>
<tr>
<td><strong>Apolipoprotein B (mg/dL)</strong></td>
<td>1.45 (171)</td>
<td>1.77 (225)**</td>
</tr>
<tr>
<td><strong>Apolipoprotein A1 (mg/dL)</strong></td>
<td>2.03 (171)</td>
<td>2.2 (225)**</td>
</tr>
<tr>
<td><strong>LDL cholesterol (mmol/L)</strong></td>
<td>22.4 (75.0)</td>
<td>27.3 (160)</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td>3.2 (82)</td>
<td>3.16 (92)</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
<td>159 ± 27.3</td>
<td>160 ± 29.1 (170)</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mmHg)</strong></td>
<td>92 ± 14.8</td>
<td>91 ± 14.2 (169)</td>
</tr>
<tr>
<td><strong>Cholesterol (mmol/L)</strong></td>
<td>5.8 ± 1.24</td>
<td>5.9 ± 1.29 (171)</td>
</tr>
<tr>
<td><strong>LDL cholesterol (mmol/L)</strong></td>
<td>3.5 ± 1.02</td>
<td>3.5 ± 1.13 (168)</td>
</tr>
<tr>
<td><strong>HDL cholesterol (mmol/L)</strong></td>
<td>1.3 ± 0.43</td>
<td>1.4 ± 0.39 (168)</td>
</tr>
<tr>
<td><strong>Apolipoprotein Al (mg/dL)</strong></td>
<td>134 ± 22.9</td>
<td>143 ± 23.2 (170)</td>
</tr>
<tr>
<td><strong>Apolipoprotein B (mg/dL)</strong></td>
<td>130 ± 34.0</td>
<td>131 ± 35.0 (170)</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; numbers in parentheses are numbers of patients out of the total; *P < 0.05 vs women; **P < 0.001 vs women.

a Calculated by the Modification of Diet in Renal Disease (MDRD) study formula.

2.3. Laboratory measurements

Fasting blood glucose, cholesterol, TG and creatinine were measured, using a BM/Hitachi 747 analyzer (Boehringer Mannheim, Meylan, France), and apoA1 and apoB were measured with an Array 360 analyzer (Beckman, Gagny, France), according to the manufacturer’s instructions. HbA1c was assayed by high-performance liquid chromatography on a Diamat analyzer (Bio-Rad, Ivry-sur-Seine, France), according to the recommendations of the French Society of Clinical Biology (Société française de biologie clinique). HDL-C was determined after dextran sulphate–magnesium chloride precipitation.

2.4. Genetic analysis

Genomic DNA was extracted from white blood cells by phenol-chloroform. Fluorescence-polarization detection with template-directed dye-terminator incorporation (FD-TDI) was used to detect p.Thr111Ile (rs2000813), as described elsewhere [28]. Briefly, the template was amplified by polymerase chain reaction (PCR), and excess dNTP and unincorporated primers were removed, using ExoSAP-IT (USB Corporation, Cleveland, OH, USA). This was followed by single-base primer extension, using a polymorphic site-specific primer (Integrated DNA Technologies, Skokie, IL, USA), with incorporation of a fluorescent dye terminator (PerkinElmer, Boston, MA, USA) in the final extension step. The PCR primers used were ATGACGGGTATCTTTTTAGAAAA(C)(F), CTGAGAGATTTTGGTCTTGGAT(R) and CGTGTGACCCGGCGACCA alleles [C/T]. The assay was read with a microplate reader, using the fluorescence polarization (FP) technique, and the allele present in the target DNA was assigned, using algorithms and controls as previously described [28,29].

2.5. Statistical analysis

SPSS software for Windows (v11.0.1, 2001) was used for the statistical analyses. Allele and genotype frequencies were determined by gene-counting. Comparisons between groups were performed by chi-square tests ($\chi^2$) for categorical data. Comparisons of means of transformed or normally distributed variables used the independent samples t test, while comparisons of means of untransformed, non-normally distributed variables used the Wilcoxon test. The general linear model was used for linear and logistic regression models. Linear regression models for trait measurements included data-driven selection among the polymorphism category rare allele homozygotes (Ile/Ile) vs carriers of the common allele (Ile/Thr+Thr/Thr). Potential predictors included age, gender, time since diagnosis, HbA1c, BMI and presence of hypertension. For purposes of face validity of the regression models, age, time since diabetes diagnosis and hypertension status were retained in models of cross-sectional data, whereas age and time since diabetes diagnosis were retained in models of prospective data. Models in which a polymorphism effect did not achieve $P < 0.10$ are not reported here. To correct for multiple comparisons, bootstrap confidence intervals (CI) are presented for each predictor in a model. Model assumptions and goodness of fit were evaluated for each model. For transformed trait measures, estimated adjusted means on the original scale are presented.

3. Results

3.1. Clinical characteristics of the study population

Clinical and biological characteristics of the study participants are presented in Table 1. There were no differences in age, duration of diabetes, fasting blood glucose or C-peptide between genders. However, there were significant differences between
men and women in BMI, glycosylated haemoglobin and creatinine, and women had less favourable control of diabetes at baseline. Of the lipid parameters, only HDL-C and apoA1 were, as expected, significantly higher in women ($P < 0.001$). Total cholesterol was also raised in proportion to increases in HDL-C; lipid-lowering treatments did not significantly influence baseline values. On follow-up, gender-based differences in HbA1c were, as expected, significantly higher in women ($P < 0.0001$). Total cholesterol was also raised in proportion to increases in HDL-C; lipid-lowering treatments did not significantly influence baseline values. On follow-up, gender-based differences in HbA1c were, as expected, significantly higher in women ($P < 0.0001$).

3.2. Genotype and allele characteristics of the study population

The 111Ile minor allele frequency was similar to those reported in other populations (T allele = 0.308) [13], with frequencies of $T = 0.351$ in men and $T = 0.304$ in women (Table 2). Exploration of the role of inheritance mode on differences between trait means by EL genotype indicated that the effect of Ile111Thr was consistent with a recessive mode of inheritance. Thus, all subsequent analyses were conducted on genetic data collapsed into carriers of the major allele (Thr/Thr + Ile/Thr) and those homozygous for the minor allele (Ile/Ile).

Patients homozygous for the rare allele displayed modestly decreased low-density lipoprotein cholesterol (LDL-C) and raised apoB (Table 3). Linear regression analysis was used to examine the effects of covariates and independent variables on the traits measured. In the model fit for LDL-C ($n = 391$; $P < 0.008$), each 1 year increase in age was associated with a 0.01 mmol/L increase in LDL-C (95% CI: 0.002, 0.021; $P = 0.022$), controlling for time since diabetes diagnosis, hypertension and EL genotype. In addition, presence of the Ile/Ile genotype was associated with a 0.41 mmol/L decrease in LDL-C (95% CI: 0.71, 0.11; $P = 0.007$), controlling for age, time since diabetes diagnosis and hypertension. However, the model accounted for only 1.9% of the variance in LDL-C.

In the model fit for apoB ($n = 393$; $P < 0.001$), the presence of the Ile/Ile genotype was associated with a 0.13 mg/dL decrease in apoB (95% CI: 0.23, 0.027; $P = 0.013$), controlling for age, time since diabetes diagnosis, HbA1c and hypertension. The presence of hypertension was associated with a 0.07 mmol/L increase in apoB (95% CI: 0.003, 0.14; $P = 0.04$), controlling for age, time since diabetes diagnosis, HbA1c, and EL genotype. Each 1% increase in HbA1c was associated with a 0.039 mg/dL increase in apoB (95% CI: 0.022, 0.056; $P < 0.000$), controlling for age, time since diabetes diagnosis, hypertension and EL genotype. The model fit explained only a small portion of the variance in apoB (7.0%).

On follow-up, in the model fit for SBP ($n = 307$; $P < 0.0005$), the presence of the Ile/Ile genotype was associated with a 9.4 mmHg increase in SBP (95% CI: 2.0, 17.0; $P = 0.014$), controlling for age, time since diabetes diagnosis and gender, whereas being male was associated with a 7.1 mmHg increase in SBP (95% CI: 1.5, 12.7; $P = 0.013$), after controlling for EL genotype, time since diabetes diagnosis and age. Each 1 year increase in age was associated with a 0.5 mmHg increase in SBP (95% CI: 0.1, 0.7; $P = 0.001$), after controlling for time since diabetes diagnosis and hypertension. The 111Ile minor allele frequency was similar to those reported in other populations (T allele = 0.308) [13], with frequencies of $T = 0.351$ in men and $T = 0.304$ in women (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Allele/Gene</th>
<th>Women ($n = 171$)</th>
<th>Men ($n = 225$)</th>
<th>Total ($n = 396$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thr/Thr</td>
<td>76 (45.3%)</td>
<td>141 (62.6%)</td>
<td>217 (54.9%)</td>
</tr>
<tr>
<td>Ile/Thr</td>
<td>75 (44.7%)</td>
<td>84 (37.4%)</td>
<td>159 (39.1%)</td>
</tr>
<tr>
<td>Ile/Ile</td>
<td>9 (5.3%)</td>
<td>10 (4.4%)</td>
<td>19 (4.9%)</td>
</tr>
</tbody>
</table>

*Carriers of the p.111Ile (c.584T) allele (Thr/Ile + Ile/Ile = CT + TT).

### Table 3

Trait means grouped by genotype.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Visit 1 CC + CT</th>
<th>TT</th>
<th>Visit 2 CC + CT</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (Kg/m²)</td>
<td>28.9 ± 5.54 (354)</td>
<td>28.8 ± 4.66 (42)</td>
<td>29.0 ± 5.26 (269)</td>
<td>28.7 ± 4.31 (35)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>10.4 ± 3.76 (354)</td>
<td>9.6 ± 3.83 (42)</td>
<td>9.56 ± 3.31 (269)</td>
<td>8.6 ± 2.50 (33)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.3 ± 1.91 (354)</td>
<td>8.1 ± 1.83 (42)</td>
<td>8.2 ± 1.55 (276)</td>
<td>8.0 ± 1.46 (35)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>159 ± 27.9 (346)</td>
<td>158 ± 20.9 (41)</td>
<td>148 ± 24.5 (272)</td>
<td>159 ± 22.9 (35)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>92 ± 14.7 (344)</td>
<td>94 ± 14.8 (41)</td>
<td>84 ± 13.1 (271)</td>
<td>89 ± 13.4 (35)</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>2.2 ± 1.89 (354)</td>
<td>2.2 ± 1.56 (42)</td>
<td>1.9 ± 1.45 (272)</td>
<td>1.9 ± 1.48 (35)</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>5.8 ± 1.27 (354)</td>
<td>5.5 ± 0.98 (42)</td>
<td>5.5 ± 1.11 (272)</td>
<td>5.5 ± 1.14 (34)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.5 ± 1.03 (350)</td>
<td>3.2 ± 0.88 (41)</td>
<td>3.4 ± 0.86 (247)</td>
<td>3.3 ± 1.05 (30)</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.3 ± 0.43 (351)</td>
<td>1.3 ± 0.45 (42)</td>
<td>1.3 ± 0.38 (262)</td>
<td>1.4 ± 0.43 (34)</td>
</tr>
<tr>
<td>ApoA1 (mg/dL)</td>
<td>131 ± 34.3 (352)</td>
<td>118 ± 28.2 (42)</td>
<td>143 ± 24.1 (249)</td>
<td>151 ± 22.7 (32)</td>
</tr>
<tr>
<td>ApoB (mg/dL)</td>
<td>134 ± 22.5 (351)</td>
<td>136 ± 26.3 (42)</td>
<td>109 ± 27.5 (249)</td>
<td>99 ± 28.2 (32)</td>
</tr>
</tbody>
</table>

*Significantly different from values for females ($P < 0.05$).
since diabetes diagnosis, gender and EL genotype. The model fit explained only a modest portion of the variance in SBP (6.5%).

In the model fit for apoB on follow-up \( (n = 280; P < 0.0001) \), the presence of the Ile/Ile genotype was associated with a 0.097 mg/dL decrease in apoB \( (95\% \text{ CI: } -0.19, -0.001; P = 0.047) \), after controlling for age, time since diabetes diagnosis and HbaA1C, while every 1% increase in HbaA1C was associated with a 0.048 mg/dL increase in apoB \( (95\% \text{ CI: } 0.025, 0.071; P = 0.000) \), after controlling for EL genotype, time since diabetes diagnosis and age, and every 1 year increase in time since diabetes diagnosis was associated with a 0.006 mg/dL decrease in apoB \( (95\% \text{ CI: } -0.0098, -0.0022; P = 0.002) \), controlling for EL genotype, age and HbaA1C. Baseline apoB measurement was not included as a predictor to avoid overfitting the model selected for apoB level on follow-up. The model fit explained a modest portion of the variance in apoB \( (9.1\%) \).

In the model fit for HDL-C on follow-up \( (n = 291; P < 0.001) \), the presence of the Ile/Ile genotype was associated with a 0.14 mg/dL increase in HDL-C \( (95\% \text{ CI: } 0.008, 0.28; P = 0.038) \), after controlling for age, time since diabetes diagnosis, HbaA1C and BMI. Every 1% increase in HbaA1C was associated with a 0.03 mg/dL decrease in HDL-C \( (95\% \text{ CI: } -0.057, -0.0073; P = 0.011) \), controlling for EL genotype, time since diabetes diagnosis, age and BMI, and every 1-kg/m² increase in BMI was associated with a 0.02 mg/dL decrease in HDL-C \( (95\% \text{ CI: } -0.028, -0.0077; P = 0.001) \), controlling for time since diabetes diagnosis, age, HbaA1C and EL genotype. The model fit explained a modest portion of the variance in HDL-C \( (8.9\%) \).

### 3.3. Genotype association with diabetic complications

No relationship was found between macrovascular complications (CAD, PAD) or nephropathy. The EL genotype was associated with the frequency of retinopathy \( (Table \; 4; \chi^2: 3.733; 1\text{df}; P = 0.053; \text{OR: } 2.458; 95\% \text{ CI: } 1.047, 4.112) \). In the model fit for DR on follow-up \( (n = 307; P = 0.029) \), the presence of the Ile/Ile genotype was associated with 2.06 greater odds of DR \( (95\% \text{ CI: } 1.02, 4.17; P = 0.002) \), controlling for age, time since diabetes diagnosis, hypertension and HbaA1C. Each 1% increase in HbaA1C was associated with 1.07 greater odds of DR \( (95\% \text{ CI: } 1.02, 1.11; P = 0.002) \).

On follow-up, these findings persisted. The EL genotype was significantly associated with the frequency of DR \( (Table \; 4; \chi^2: 9.60; 1\text{df}; P = 0.002; \text{OR: } 3.51; 95\% \text{ CI: } 1.49, 8.24) \). In the model fit for DR at visit 1 \( (n = 395; P < 0.001) \), the presence of the Ile/Ile genotype was associated with 2.74 greater odds of DR \( (95\% \text{ CI: } 1.32, 5.67; P = 0.007) \), after controlling for age, time since diabetes diagnosis, hypertension and HbaA1C. Although retinopathy was not associated with LDL-C levels—as LDL-C and EL genotype are related—LDL-C was also considered in the multivariate analysis, with no significant change in model fit. However, when LDL-C was included in the same models, retinopathy was found to be associated with the Ile/Ile genotype \( (OR: 2.40; 95\% \text{ CI: } 1.05, 5.55) \).

The presence of hypertension was associated with 2.28 greater odds of DR \( (95\% \text{ CI: } 1.04, 5.02; P = 0.04) \), controlling for EL genotype, time since diabetes diagnosis, age and HbaA1C. Each 1% increase in HbaA1C was associated with 1.38 greater odds of DR \( (95\% \text{ CI: } 1.19, 1.60; P < 0.001) \), controlling for time since diabetes diagnosis, age, EL genotype and hypertension, and every 1 year increase in time since diabetes diagnosis was associated with 1.07 greater odds of DR \( (95\% \text{ CI: } 1.02, 1.11; P = 0.002) \).

### 4. Discussion

The role of dyslipidaemia in the risk of macro- and microvascular complications in T2D is related in part to genetic factors. It was hypothesized that the EL gene was one such factor and, for this reason, the Thr111Ile missense polymorphism and its relationship to lipid profile, macrovascular and microvascular complications, were studied in 396 T2D patients and followed longitudinally. The impact of the 111Ile minor allele was consistent with a recessive mode of inheritance, with homozygotes for the rare allele differing from carriers of the major allele. The polymorphism was associated with modest changes in LDL-C and apoB levels at baseline, and with SBP and HDL-C at follow-up. More important, homozygotes for the rare allele were more likely to be diagnosed with DR both at baseline and on follow-up.
Exploration of the complex pathophysiology of DR in T2D patients has led to the evaluation of several candidate genes. In a study of Chinese T2D patients, promoter polymorphism of the aldose reductase gene was found to be associated with early onset of DR [30–32], while the Gln192Arg missense polymorphism of the paraoxonase-1 gene was associated with the prevalence of DR and with nephropathy in Japanese patients [24]. Also, the vascular endothelial growth factor (VEGF) g.−634C > G polymorphism was a risk factor for macular oedema as well as DR in an independent sample of Japanese patients [33].

An important consideration in human genetic studies is the influence of race and ethnicity on gene variation and phenotype. In a recent review [34] of candidate gene studies of DR, 20 genes and 34 variants were studied in multiple cohorts. The aldose reductase gene (AKR1B1) was found to have the greatest number of polymorphisms significantly associated with DR. The z−2 microsatellite was found to confer risk for (in both type 1 diabetes and T2D), and the z + 2 to confer protection against, DR in T2D, regardless of ethnicity. Polymorphisms in NOS3, VEGF, ITGA2 and ICAM1 were also associated with DR. Taken together, these findings are intriguing but can only be considered preliminary in nature since their replication in independent studies has not been reported. Furthermore, the majority of these studies were of small samples of patients, and case-selection criteria were not standardized.

DR is a specific complication of diabetes that is present in 21% of T2D patients at the time of diagnosis [25,35], and can be a cause of vision loss if left untreated. Its development depends mostly on the duration of diabetes and on glycaemic control, and the evidence supports the contribution of both genetic and environmental factors [36]. The risk of severe DR in siblings of affected individuals is substantially higher, and analysis of the Diabetes Control and Complications Trial (DCCT) data provides evidence that DR tends to cluster in families with type 1 diabetes. In addition, differences in the frequency of DR occurrence in different ethnic groups further support the presence of a genetic component [37].

The present study found only modest influences of the LIPG c.584C>T polymorphism on lipid parameters. The HDL-C-raising effect of the minor 111Ile allele observed here is in accordance with the Lipoprotein and Coronary Atherosclerosis Study (LCAS) in CHD patients [11]. There was also a modest, yet consistent, effect of the c.584C>T variation on SBP over time in our study population. Other recent studies have pointed out a possible link between EL and hypertension, as shown in stroke-prone hypertensive rat models [38], and Badellino et al. [39] reported positive correlations between both pre- and postheparin EL concentrations and blood pressure (r = 0.18 and r = 0.23, respectively; P < 0.001) in humans, with a link to coronary artery calcification scores. Whether the link between hypertension, itself a risk factor for DR [40], is direct or indirect remains open to speculation. However, our present data suggest that, even after controlling for the presence of hypertension, c.584C>T remains an independent predictor of DR. The association between c.584C>T and DR was not confounded by the presence of hypertension; the lack of association between the polymorphism and macrovascular complications is also in agreement with the LCAS study, and should be considered confirmation. Although the polymorphism does not appear to be a strong predictor of either macrovascular complications or diabetic nephropathy, it appears to be influential on the expression of DR. Homozygosity for the p.111Ile allele is a risk factor for DR, and is independent of duration of disease, age, presence of hypertension and glycaemic control.

Although involvement of lipid-metabolism candidate genes in the risk of diabetic microvascular disease is still a matter of debate, a review of several recent reports allows us to speculate on the role of lipid metabolism and, more specifically, of EL in the process. The role of apoE, recently demonstrated by Ishida et al. [41], in the lipid-containing deposits (drusen) that accumulate in Bruch’s membrane and are implicated in early age-related macular degeneration highlights the importance of lipids in the development of microvascular complications, and may share common pathophysiological patterns with DR. Furthermore, the same authors have also demonstrated a direct role for HDL efflux of hydroperoxidized lipid from retinal pigment epithelial cells [42]. It is tempting to consider functional abnormalities of HDL particles in this genotype subset. EL is primarily a phospholipase that plays a central role in HDL metabolism and reverse cholesterol transport [41,43,44], and acts at the level of endothelium. Retinal pigment epithelium (RPE) accepts lipids from the retinal photoreceptor outer segments (POS) and, every day, each RPE cell phagocytoses and degrades more than 1000 POS discs, which are particularly rich in phospholipids and contain the photoreactive pigment rhodopsin. The T111I variant may possess an altered enzymatic activity or property that could bring about lipid efflux from RPE into the circulation via HDL particles.

The effects of the peroxisome proliferator-activated receptor-alpha (PPAR-α) agonist fenofibrate in lowering TG concentrations and modestly raising HDL-C concentrations in the large T2D cohort of the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study [45–47] resulted in a reduced incidence of microvascular complications (DR and diabetic nephropathy); more recently, the results of the Action to Control Cardiovascular Risk in Diabetes (ACCORD) Eye Study confirmed these findings, and also found that intensive combination treatment (fenofibrate + simvastatin) of diabetic dyslipidaemia reduced the rate of DR progression by 40% [48]. Although there is, as yet, no clear explanation of this phenomenon, it is possible to speculate on an ameliorating role of fenofibrate, which is known to activate the lipolytic cascade, increase circulating levels of HDL, and lead to both a quantitative and qualitative reduction in HDL liperoxidation.

To our knowledge, this is the first report of LIPG g.584C>T as a risk factor for DR acting through perturbation of both blood pressure and reverse lipid transport in the retina that may be facilitated by EL activity. Considering the gravity of DR, these present findings merit further exploration in functional studies of HDL metabolism, as well as validation by larger-scale, independent population studies. Ultimately, such data may lead to new insights regarding potential therapeutic targets for this debilitating complication of diabetes.
Conflict of interest statement

The authors have no conflict of interest to declare with respect to this manuscript.

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