Allelic variations of the vitamin D receptor (VDR) gene are associated with increased risk of coronary artery disease in type 2 diabetics: The DIABHYCAR prospective study

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Abstract

Aim. – Vitamin D deficiency is associated with coronary artery disease (CAD), and the actions of vitamin D are mediated by binding to a specific nuclear vitamin D receptor (VDR). This study investigated the associations of VDR gene variants with CAD in two cohorts of type 2 diabetes patients.

Methods. – A cohort of 3137 subjects from the prospective DIABHYCAR study (CAD incidence: 14.8%; follow-up: 4.4 ± 1.3 years) and an independent, hospital-based population of 713 subjects, 32.3% of whom had CAD, were assessed. Three SNPs in the VDR gene were genotyped: rs1544410 (BsmI); rs7975232 (ApaI); and rs731236 (TaqI).

Results. – In the DIABHYCAR cohort, an association was observed between the A allele of BsmI and incident cases of CAD (HR: 1.16, 95% CI: 1.05–1.29; P = 0.002). Associations were also observed between BsmI (P = 0.01) and TaqI (P = 0.04) alleles and baseline cases of CAD. The AAC haplotype (BsmI/ApaI/TaqI) was significantly associated with an increased CAD prevalence at the end of the study compared with the GCT haplotype (OR: 1.12, 95% CI: 1.02–1.28; P = 0.04). In a cross-sectional study of the independent hospital-based cohort, associations of ApaI (P = 0.009) and TaqI (P = 0.03) alleles with CAD were observed, with similar haplotype results (OR: 1.33, 95% CI: 1.03–1.73; P = 0.03).

Conclusion. – The haplotype comprising the minor allele of BsmI, major allele of ApaI and minor allele of TaqI of VDR (AAC) was associated with an increased risk of CAD in type 2 diabetes patients. This effect was independent of the effects of other known cardiovascular risk factors.

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Keywords: Coronary artery disease; Type 2 diabetes; VDR gene

Résumé

Des variations alléliques du gène codant pour le récepteur de la vitamine D (VDR) sont associées à la maladie coronarienne chez des diabétiques de type 2. L’étude prospective DIABHYCAR.

Objectif. – Des taux circulants bas de vitamine D sont associés à une augmentation du risque de maladie coronarienne. Les actions de la 1,25-(OH)2D3 dépendent de sa liaison à un récepteur intracellulaire spécifique (VDR). Nous avons évalué l’impact des variations alléliques du gène codant pour VDR dans la maladie coronarienne chez des diabétiques de type 2 à fort risque vasculaire.

Méthodes. – Nous avons étudié 3137 sujets de l’étude prospective DIABHYCAR (incidence de maladie coronarienne de 14,8 % ; suivi de 4,4 ± 1,3 ans). Une population cas–témoin indépendante a été également étudiée (cohorte NCH, 713 sujets, prévalence de maladie coronarienne de 32,3 %). Trois SNPs ont été génotypés : rs1544410 (BsmI), rs7975232 (ApaI) et rs731236 (TaqI).

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1. Introduction

Cardiovascular disease is the leading cause of mortality and morbidity in patients with diabetes, and accounts for up to 80% of deaths among patients with type 2 diabetes (T2D) [1]. Such patients have a threefold greater risk than non-diabetic individuals of developing atherosclerosis and its clinical complications [2]. Known risk factors for atherosclerosis, such as arterial hypertension, central obesity and dyslipidaemia, frequently coexist with diabetes and contribute to the increased prevalence of cardiovascular disease in diabetic patients. However, T2D is an independent risk factor for cardiovascular disease [3].

The vitamin D endocrine system regulates multiple aspects of cellular metabolism, differentiation and replication in many target organs in addition to those directly involved in calcium homeostasis [4]. It also plays an important role in glucose homeostasis, most notably in the mechanism of insulin release. Epidemiological studies have suggested that low serum levels of vitamin D are associated with increased risk of atherosclerosis, endothelial dysfunction, coronary artery disease (CAD), heart failure, and cardiovascular and all-cause mortality [5–8]. Inactivation of the vitamin D system in animal models has confirmed these findings [9,10].

The actions of vitamin D are mediated by binding of 1,25(OH)2D3 to a specific vitamin D receptor (VDR), a member of the steroid/thyroid hormone receptor superfamily [11]. Several frequent polymorphisms in the VDR gene have been reported to be associated with a variety of physiological and pathological phenotypes, including intrauterine and early postnatal growth, body weight and body height, as well as insulin secretion, insulin sensitivity, glucose tolerance and susceptibility to both type 1 and type 2 diabetes [12–19]. The present study investigated the associations of VDR gene variants with CAD risk in a prospective cohort of T2D patients. Replication analysis was performed in a separate cross-sectional study.

2. Methods

2.1. Prospective study subjects

DIABHYCAR was a multinational trial conducted in 4912 subjects with T2D, aged 50 years or older at baseline, and selected on the basis of persistent micro- or macroalbuminuria (urinary albumin excretion $\geq 20$ mg/L) without renal failure (serum creatinine $\leq 150$ μmol/L). The trial looked at whether a low dose of ramipril (1.25 mg/day) able to reduce urinary albumin excretion would also reduce cardiovascular and/or renal events such as myocardial infarction, stroke, acute heart failure, end-stage renal failure and cardiovascular death. Study design and results (which were negative regarding the drug effect) have been published elsewhere [20,21]. For the purposes of the trial, myocardial infarction was defined as the occurrence of at least two of the three following criteria: constrictive chest pain lasting 20 min or more; increased serum creatinine phosphokinase and/or troponin levels; and typical electrocardiography (ECG) changes. Sudden death was defined as death occurring instantaneously or within 1 h of the onset of new cardiac symptoms (arrhythmia, myocardial infarction), or as a non-witnessed death when no cause of death could be determined; fatal stroke was not included in this definition.

An independent committee adjudicated all study events, and all participants gave their written informed consent. The study protocol had the approval of the Angers University Hospital Ethics Committee. For the present investigation, 3137 French participants from DIABHYCAR were included. At baseline, 172 of them (5.5%) had a previous history of myocardial infarction and 311 (9.9%) had a diagnosis of angina pectoris (Table 1). Duration of follow-up was 4.4 ± 1.3 years. During the follow-up, 95 cases of myocardial infarction (3%), 295 cases of coronary revascularization (coronary artery bypass grafting, angioplasty or stenting; 9.4%) and 137 cases of sudden death (4.4%) were reported in 465 subjects (Table 1).

2.2. Cross-sectional study subjects

The NCH study included 713 French Caucasian men and women with T2D recruited at the departments of diabetology of the Necker and Cochin Hospitals in Paris. For the purposes of the present investigation, 230 subjects (32.3%) were considered to have CAD (Table 2). The presence of CAD was documented by either a history of myocardial infarction or coronary revascularization in 144 patients. Screening for silent myocardial ischaemia was performed in 347 patients deemed at high risk for CAD [22]. These patients underwent a stress test [stress ECG, stress thallium-201 single-photon emission computed tomography (SPECT) or dobutamine echocardiography], and those with
Table 1
DIABHYCAR cohort characteristics at baseline by coronary artery disease (CAD) status and incidence of CAD events during follow-up.

<table>
<thead>
<tr>
<th></th>
<th>CAD at baseline</th>
<th>CAD events during follow-up (incidence)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Subjects (n)</td>
<td>2654</td>
<td>483</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>72%</td>
<td>80%</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65 ± 8</td>
<td>68 ± 8</td>
</tr>
<tr>
<td>Known duration of diabetes (years)</td>
<td>55 ± 10</td>
<td>56 ± 10</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>171 ± 54</td>
<td>169 ± 54</td>
</tr>
<tr>
<td>Haemoglobin A1c (%)</td>
<td>7.9 ± 1.8</td>
<td>7.9 ± 1.6</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>29.4 ± 4.6</td>
<td>29.2 ± 4.4</td>
</tr>
<tr>
<td>Arterial hypertension (%)*</td>
<td>54%</td>
<td>66%</td>
</tr>
<tr>
<td>Tobacco-smoking (%)</td>
<td>18%</td>
<td>12%</td>
</tr>
<tr>
<td>Randomization group: ramipril (%)†</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Previous myocardial infarction (%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Previous angina pectoris (%)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; LDL/HDL: low-density lipoprotein/high-density lipoprotein; UAE: urinary albumin excretion; statistics of quantitative parameters are by Student’s t tests using log-transformed data:
* 367 subjects with CAD and 2143 subjects without CAD at baseline, 354 incident cases of CAD and 2156 subjects without CAD during follow-up.
† Systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg, or below either of these values with antihypertensive medication and a history of hypertension.
‡ Original DIABHYCAR study treatment group: ramipril vs placebo.
§ Cases of myocardial infarction or angina pectoris at baseline, or incident cases of myocardial infarction, coronary revascularization or sudden death during follow-up.

Table 2
NCH cohort characteristics according to coronary artery disease (CAD) status.

<table>
<thead>
<tr>
<th></th>
<th>Without CAD</th>
<th>With CAD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>483</td>
<td>230</td>
<td>–</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>59%</td>
<td>72%</td>
<td>0.0007</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61 ± 11</td>
<td>67 ± 10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Known duration of diabetes (years)</td>
<td>51 ± 12</td>
<td>53 ± 12</td>
<td>0.05</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>169 ± 67</td>
<td>160 ± 67</td>
<td>0.06</td>
</tr>
<tr>
<td>Haemoglobin A1c (%)</td>
<td>8.2 ± 2.1</td>
<td>8.2 ± 1.8</td>
<td>0.65</td>
</tr>
<tr>
<td>Treatment (diet/OHAs/insulin)</td>
<td>20%/54%/26%</td>
<td>13%/47%/40%</td>
<td>0.0003</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.8 ± 5.5</td>
<td>29.0 ± 5.2</td>
<td>0.52</td>
</tr>
<tr>
<td>Arterial hypertension (%)</td>
<td>55%</td>
<td>53%</td>
<td>0.80</td>
</tr>
<tr>
<td>Total cholesterol ≥ 230 mg/dL (%)</td>
<td>38%</td>
<td>59%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL cholesterol ≥ 130 mg/dL (%)</td>
<td>55%</td>
<td>65%</td>
<td>0.02</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>87 ± 34</td>
<td>72 ± 31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>UAE: normal/microalbuminuria/proteinuria (%)</td>
<td>69%/21%/10%</td>
<td>53%/29%/18%</td>
<td>0.0008</td>
</tr>
<tr>
<td>Tobacco-smoking (%)</td>
<td>44%</td>
<td>59%</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD unless otherwise stated; OHAs: oral hypoglycaemic agents; LDL/HDL: low-density lipoprotein/high-density lipoprotein; UAE: urinary albumin excretion; statistics of quantitative parameters are by Student’s t test using log-transformed data:
* Present or pretreatment (statins, fibrates) values.
† 389 subjects without CAD and 215 with CAD.
‡ Normal: <20 mg/24 h; microalbuminuria: ≥ 20 mg/24 h but <300 mg/24 h; proteinuria: ≥ 300 mg/24 h.
§ Systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, or below either of these values with antihypertensive medication and a history of hypertension.
ε Current or past.
a positive test result underwent coronary angiography. CAD was considered present if significant stenosis (≥ 50%) was observed in at least one major vessel or branch. CAD was considered absent in patients with a normal stress test or no significant coronary stenosis on angiography. Details of the stress tests and angiography are reported elsewhere [22].

Significant coronary stenosis was observed in 86 patients. The control group consisted of 261 subjects with a normal stress test or no significant coronary stenosis on angiography plus 222 subjects in whom screening for myocardial ischaemia was not performed as they were thought to be at low risk for CAD. Subjects from the latter group had no clinical signs, symptoms or past history of CAD, presented with fewer than two cardiovascular risk factors and had normal ECGs. All of these participants gave their written informed consent, and the study had the approval of the ethics committee of AP-HP Necker Hospital in Paris.

2.3. VDR genotyping

DNA was extracted from peripheral blood samples using standard procedures. There are at least five haplotype blocks with high linkage disequilibrium (LD) and areas with very low LD in the VDR gene (chr. 12q12-q14) in Caucasians. Blocks 1–4 are found in the promoter region while block 5 encompasses exons 4–9 and 3’ UTR [23]. For the present study, three single nucleotide polymorphisms (SNPs) located in haplotype block 5 were genotyped: rs15444410 (intron 8, G > A); rs7975232 (intron 8, A > C); and rs731236 (exon 9 ATT > ATC, Ile352Ile). These SNPs are known by the names of their associated restriction enzymes (BsmI, ApgI and TaqI, respectively) and this nomenclature is used throughout this report. Genotyping was performed using Assay by Design (ABD) kits from Applied Biosystems (Life Technologies Corp., Carlsbad, CA, USA).

2.4. Statistical analyses

Results are expressed as means ± SD unless otherwise stated. Differences between groups were assessed by Student’s t, contingency table chi-square and Fisher’s exact tests. Genotypes were in Hardy–Weinberg equilibrium in all groups of subjects. Genotype associations with CAD were assessed using regression models, and Kaplan–Meier curves were used to plot survival (disease-free) rates over time according to genotype. Cox proportional-hazards survival regression analyses were used to examine the effects of explanatory variables on time-related survival (disease-free) rates in prospective analyses, and logistic-regression analyses were used for the cross-sectional analyses. Hazard ratios (HRs) and odds ratios (ORs) with their 95% confidence interval (CI) were computed for the risk alleles. Adjustments for clinical and biological parameters were carried out by including these parameters as covariates in the regression model. Data were log-transformed for the analyses when the normality of distribution was rejected by the Shapiro–Wilks W test. P < 0.05 was considered statistically significant. Given the strong LD between the SNPs (see below), no P-value correction for multiple SNP testing was performed, as it was considered that they were not independent analyses. The power to detect associations of the SNPs with baseline prevalence, incidence during follow-up and total prevalence of CAD at the end of the study in the DIABHYCAR cohort was 0.93, 0.89 and 0.98, respectively, for ORs and HRs ≥ 1.2 and alpha = 0.05. Statistical analyses were performed with JMP software (SAS Institute Inc., Carey, NC, USA), and LD and haplotype analyses were performed with Thesias v3.1 software [24].

3. Results

3.1. DIABHYCAR study: baseline prevalence of CAD

At baseline, the prevalence of CAD (cases of myocardial infarction and/or angina pectoris) was 15.4%. Characteristics of the subjects with or without CAD at baseline are shown in Table 1. Prevalences of CAD at baseline according to genotype were 13.7% (GG), 16.1% (GA) and 16.9% (AA) for BsmI, 16.8% (AA), 14.6% (AC) and 15.5% (CC) for ApgI, and 14.2% (TT), 15.9% (TC) and 16.6% (CC) for TaqI, suggesting a possible dominant effect of the minor allele for BsmI and TaqI. Genotype frequencies in subjects with or without CAD at baseline are shown in Table 3. Logistic-regression analyses confirmed associations of the A allele of BsmI (OR: 1.30, 95% CI: 1.05–1.62; P = 0.01) and C allele of TaqI (OR: 1.24, 95% CI: 1.02–1.54; P = 0.04) with CAD at baseline in a dominant model, whereas no significant association with ApgI was observed.

3.2. DIABHYCAR study: incidence of CAD during follow-up

The incidence of CAD events (myocardial infarction, sudden death or coronary revascularization) was 14.8%. Known cardiovascular risk factors such as male sex, dyslipidaemia, arterial hypertension, decreased renal function and albuminuria were either more frequent or more severe in incident cases of CAD compared with subjects without CAD during follow-up (Table 1). Incidences of CAD according to genotype were 12.3% (GG), 16.5% (GA), 15.9% (AA) for BsmI, 13.5% (AA), 15.5% (AC) and 14.7% (CC) for ApgI, and 13.3% (TT), 16.4% (TC) and 13.8% (CC) for TaqI, suggesting a possible dominant effect of the A allele for BsmI (Fig. 1). Genotype frequencies in subjects with or without a CAD event during follow-up are shown in Table 3. Cox proportional-hazards survival regression analyses showed an association of the A allele of BsmI with the incidence of CAD in a dominant model (HR: 1.16, 95% CI: 1.05–1.29; P = 0.002), adjusted for allocation group in the original DIABHYCAR trial (drug or placebo), sex, age and duration of diabetes (Table 3). There was no interaction between treatment group in the original DIABHYCAR study and the effect of genotype on CAD (data not shown). The association remained significant following adjustment for body mass index (BMI), HbA1c, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, urinary albumin excretion (UAЕ), creatinine clearance and arterial hypertension. Nominal association of the C allele of TaqI with the incidence of CAD during follow-up was also observed, whereas no association with ApgI was noted.
Table 3
DIABHYCAR and NCH cohorts: VDR genotype frequency by coronary artery disease (CAD) status.

<table>
<thead>
<tr>
<th>CAD status</th>
<th>n</th>
<th>rs1544410 (BsmI)</th>
<th>rs7975232 (ApaI)</th>
<th>rs731236 (TaqI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>GA</td>
<td>AA</td>
</tr>
<tr>
<td>DIABHYCAR: baseline</td>
<td></td>
<td>0.365 0.475 0.160</td>
<td>0.256 0.516 0.228</td>
<td>0.370 0.477 0.153</td>
</tr>
<tr>
<td>Without CAD</td>
<td>2654</td>
<td>0.319 0.502 0.179</td>
<td>0.283 0.486 0.231</td>
<td>0.337 0.496 0.167</td>
</tr>
<tr>
<td>With CAD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>483</td>
<td>1.30 (1.05–1.62)</td>
<td>1.16 (0.93–1.35)</td>
<td>1.24 (1.02–1.54)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>0.01</td>
<td>0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIABHYCAR: follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CAD event</td>
<td>2672</td>
<td>0.369 0.470 0.161</td>
<td>0.261 0.507 0.232</td>
<td>0.372 0.470 0.158</td>
</tr>
<tr>
<td>Incident cases of CAD&lt;sup&gt;b&lt;/sup&gt;</td>
<td>465</td>
<td>0.297 0.529 0.174</td>
<td>0.258 0.534 0.208</td>
<td>0.328 0.531 0.141</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td></td>
<td>1.16 (1.05–1.29)</td>
<td>1.08 (0.97–1.12)</td>
<td>1.10 (0.99–1.21)</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.002</td>
<td>0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>DIABHYCAR: study end</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without CAD</td>
<td>2332</td>
<td>0.375 0.465 0.160</td>
<td>0.258 0.510 0.232</td>
<td>0.378 0.467 0.155</td>
</tr>
<tr>
<td>With CAD&lt;sup&gt;c&lt;/sup&gt;</td>
<td>805</td>
<td>0.308 0.519 0.173</td>
<td>0.267 0.514 0.219</td>
<td>0.329 0.515 0.156</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>1.42 (1.19–1.69)</td>
<td>1.13 (0.97–1.38)</td>
<td>1.31 (1.10–1.56)</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.0001</td>
<td>0.23</td>
<td>0.002</td>
</tr>
<tr>
<td>NCH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without CAD</td>
<td>483</td>
<td>0.312 0.520 0.168</td>
<td>0.280 0.475 0.245</td>
<td>0.411 0.438 0.151</td>
</tr>
<tr>
<td>With CAD&lt;sup&gt;d&lt;/sup&gt;</td>
<td>230</td>
<td>0.279 0.517 0.204</td>
<td>0.376 0.440 0.184</td>
<td>0.348 0.478 0.174</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>1.40 (0.83–2.36)</td>
<td>1.47 (1.14–1.77)</td>
<td>1.75 (1.04–3.05)</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.21</td>
<td>0.099</td>
<td>0.03</td>
</tr>
</tbody>
</table>

DIABHYCAR cohort: ORs for BsmI (XA vs GG), ApaI (AA vs XC) and TaqI (XC vs TT) genotypes adjusted for sex, age and duration of diabetes; HRs for BsmI (XA vs GG), ApaI (AX vs CC) and TaqI (XC vs TT) genotypes adjusted for sex, age, duration of diabetes and allocation group in the original DIABHYCAR trial (drug or placebo); NCH study: ORs for BsmI (A allele), ApaI (A allele) and TaqI (C allele) genotypes in a codominant model adjusted for sex, age and duration of diabetes.

<sup>a</sup> History of myocardial infarction or angina pectoris.
<sup>b</sup> Myocardial infarction, coronary revascularization or sudden death during follow-up.
<sup>c</sup> Baseline plus incident cases.
<sup>d</sup> History of myocardial infarction, coronary revascularization or significant stenosis (≥50%) on coronary angiography.

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Fig. 1. Kaplan–Meier survival (disease-free) curves for the DIABHYCAR cohort during follow-up according to BsmI genotype. The survival (y) axis represents the absence of a CAD event, defined as myocardial infarction, coronary revascularization or sudden death. The cumulative incidence of CAD events at each time point can be computed as 100% minus survival.

3.3. DIABHYCAR study: CAD prevalence at study end

Total CAD prevalences at the end of the study (baseline plus incident cases) according to genotype were 22.1% (GG), 27.8% (GA) and 27.3% (AA) for BsmI, 26.3% (AA), 25.7% (AC) and 24.5% (CC) for ApaI, and 23.1% (TT), 27.6% (TC) and 26.1% (CC) for TaqI. Allelic associations were confirmed, and presented more robust statistical significance (Table 3) – OR: 1.42, 95% CI: 1.19–1.69, \( P = 0.0001 \) for the A allele of BsmI, and OR: 1.31, 95% CI: 1.10–1.56, \( P = 0.002 \) for the C allele of TaqI. There was a strong LD between the three SNPs (D’: 0.90–0.94) and the two more common haplotypes, GCT and AAC (BsmI/ApaI/TaqI), represented ~82% of all haplotypes. GCT frequency was 46.6% in CAD cases at the end of the study and 44.0% in those without CAD, while AAC frequencies were 38.8% and 36.0%, respectively. The AAC haplotype was significantly associated with an increased CAD prevalence compared with the GCT haplotype (OR: 1.12, 95% CI: 1.02–1.28; \( P = 0.04 \)) after adjusting for sex, age and duration of diabetes.

3.4. NCH cross-sectional study: CAD prevalence according to VDR genotypes and haplotypes

Characteristics of subjects of the NCH cohort according to CAD status are shown in Table 2. Cardiovascular risk factors such as male sex, dyslipidaemia, arterial hypertension, decreased renal function, albuminuria and a history of cigarette-smoking were either more frequent or more severe in those with CAD. Logistic-regression analyses were performed to assess associations of genotype with CAD (Table 3). Association of
the A allele of ApolI was observed with CAD in a codominant model (OR: 1.47, 95% CI: 1.14–1.77; P = 0.009, adjusted for sex, age and duration of diabetes), and remained significant following adjustment for total and HDL cholesterol, arterial hypertension, creatinine clearance, albuminuria, use of insulin and tobacco-smoking. These results are in keeping with genotype-related prevalences of CAD: 39% vs 30% vs 26% in A-allele homozygous, heterozygous and C-allele homozygous subjects, respectively. Also observed was an association of the C allele of TaqI with CAD (OR: 1.75, 95% CI: 1.04–3.05; P = 0.03). The two more common haplotypes of BsmI/ApolI/TaqI variants, GCT and AAC, represented ~81% of all haplotypes. GCT frequency was 45.0% in CAD cases and 38.2% in those with no CAD, while AAC frequencies were 40.1% and 36.3%, respectively. The AAC haplotype was significantly associated with CAD compared with the GCT haplotype (OR: 1.33, 95% CI: 1.03–1.73; P = 0.03, adjusted for sex, age and duration of diabetes).

4. Discussion

It was observed in two independent cohorts of French Caucasians that common variants of the VDR gene modulate the risk of CAD in T2D patients. One cohort was originally recruited for a multicentre prospective clinical trial: associations of the A allele of BsmI with baseline and incident cases of CAD were observed in a dominant model with a 12–48% increase in CAD risk for A-allele carriers. Less robust associations were also observed for the C allele of TaqI. In the smaller, hospital-based cohort, recruited from a single diabetes department, the A allele of ApolI and C allele of TaqI were associated with increased prevalences of CAD. Interestingly, the stronger association with CAD was observed for a different variant in the two cohorts. This observation might be related to differences in the two cohorts of LD between the SNPs and the putative functional variant in the 3′ untranslated region of the VDR gene (see below). It may also be related to phenotype differences due to the different study designs and ascertainment methods used in each cohort. In this regard, it is noteworthy that DIABHYCAR participants, but not those in the NCH cohort, were selected on the basis of persistent micro- or macroalbuminuria at baseline. Also, the possibility that these findings only reflect a type I error (false-positive results) cannot be excluded. Nevertheless, in both cohorts the SNPs were in LD, and the most frequent haplotype comprised the major allele of BsmI, the minor allele of ApolI and the major allele of TaqI (GCT). In both cohorts, the haplotype comprising the complementary alleles (AAC) was associated with an increased risk of CAD.

A series of clinical studies found associations between VDR polymorphisms and phenotypes related to obesity, diabetes, insulin sensitivity and insulin secretion [12,14–18]. However, data for associations with CAD in studies of sufficient statistical power are scarce. Ortlepp et al. [25] studied 3441 German subjects, of whom 19% had diabetes, referred for diagnostic coronary angiography. They reported no association of BsmI with CAD, but the results are difficult to interpret because BsmI was not in Hardy–Weinberg equilibrium in the subset of subjects with CAD. More recently, Monraats et al. [26] investigated the contribution of 15 haplotype tagging SNPs to the risk of coronary restenosis after percutaneous coronary intervention in 3104 Dutch subjects. They observed associations between several SNPs and the risk of restenosis, including rs4516035 (exon 1a -1012A > G; haplotype block 2), rs17883984 (exon 1b -25C > A; haplotype block 3) and rs2238135 (exon 1c -1633G > C; haplotype block 4). All these SNPs were located outside of the haplotype block examined in the present study.

Genetic factors contribute substantially to the variability of circulating levels of 25(OH)D, with heritability estimated to be 30–40% [27,28]. However, genome-wide association (GWA) studies have suggested that the VDR gene is not a major contributor to the variability of circulating levels of vitamin D [29,30]. Instead, it is clear that VDR plays a major role in the pleiotropic actions of 1,25(OH)2D3 [31]. Ramagopalan et al. [32] identified 229 genes with significant changes in expression in response to vitamin D through VDR binding. The pathophysiological basis of the association of low vitamin D levels with atherosclerosis and CAD can be attributed to both direct vascular effects and indirect effects on the cardiovascular system. Vascular smooth muscle cells, endothelial cells and cardiomyocytes express 1-alpha-hydroxylase as well as VDR, and have the ability to convert circulating 25-hydroxyvitamin D into the hormonally active form of 1,25-dihydroxyvitamin D [33]. Vascular effects of vitamin D include modulation of smooth muscle cell proliferation, vascular calcification, inflammation and thrombosis [34–36]. In addition, vitamin D is a negative regulator of the renin–angiotensin system by directly suppressing renin gene expression [37]. Inactivation of either 1-alpha-hydroxylase or VDR in mice resulted in increased renin–angiotensin system activity, arterial hypertension and cardiac hypertrophy [10]. Clinical studies have also shown that vitamin D supplementation reduces blood pressure, plasma renin activity and angiotensin II levels [36,38].

It is noteworthy that the allelic associations with CAD observed in our present study were independent of the effects of other known cardiovascular risk factors, including obesity, dyslipidaemia, renal dysfunction and arterial hypertension. As for the renin–angiotensin system, no interaction was observed between the functional insertion/deletion polymorphism of the angiotensin-converting enzyme gene and VDR polymorphisms on the risk of CAD (data not shown).

It is postulated that the observed associations of these SNPs of haplotype block 5 with complex traits are due to LD with a functional variant in the 3′ untranslated region of the VDR gene affecting the regulation of stability and degradation of mRNA [11]. The most frequent haplotypes found in subjects of European descent, GCT and AAC, are in LD with the long and short alleles, respectively, of the poly(A) sequence in the 3′ untranslated region [39]. In some studies, these haplotypes were associated with variable VDR gene transcription, mRNA stability, and mRNA and protein levels [23,40].

In conclusion, our present study found that the AAC haplotype composed of the minor allele of BsmI, the major allele of ApolI and the minor allele of TaqI polymorphisms of the VDR gene is associated with an increased risk of CAD in T2D patients.
This effect was independent of the effects of other known cardiovascular risk factors. The pathophysiological mechanisms underlying this allelic association need to be investigated in further studies. They might be related to modulation of the antiatherogenic effects of vitamin D. More studies are also needed to clarify the genetic mechanisms underlying the association of the VDR variants with CAD. Such studies need to be based on extended haplotypes that take into account the five haplotype blocks in the VDR gene [26], including the promoter and 3′ untranslated regions. Interaction with circulating levels of vitamin D, not available in our present study, also needs to be investigated.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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