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Taq I polymorphism of the vitamin D receptor and risk of severe diabetic retinopathy

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Abstract

Aims/hypothesis. Vitamin D, a molecule with antiproliferative, antiangiogenic, antioxidant and immunosuppressive effects, could play a role in the pathogenesis of severe diabetic retinopathy. We examined whether *Taq I* polymorphism of the vitamin D receptor is involved in the development of severe diabetic retinopathy.
Methods. 200 unrelated C-peptide-negative French Type I diabetic patients were randomly selected (male:female, 103:97, age 44.4 ± 12.4 years, diabetes duration: 27.7 ± 10.0 years, BMI: 24.3 ± 3.4 kg/m^2, HbA_{1c}: 8.6 ± 1.3 %). The *Taq I* site was analysed by PCR followed by digestion with Taq I enzyme. Diabetic retinopathy was assessed by retinal angiography and classified as presence ($n = 101$) or absence ($n = 99$) of severe (preproliferative or proliferative) diabetic retinopathy.
Results. Frequency of wild-type genotype *TT* was lower in patients with severe diabetic retinopathy ($n = 27$) when compared with control subjects ($n = 42$, OR = 0.5, $p = 0.028$). Allele frequencies were not different between patients (*T*: $n = 112$ and *t*: $n = 90$) and control subjects (*T*: $n = 128$, and *t*: $n = 70$, $p = 0.075$). Global χ^2 ($df = 2$): $p = 0.064$. In subjects with diabetes duration of more than 25 years, *TT* was lower in severe diabetic retinopathy ($n = 14$) than control subjects ($n = 18$, OR = 0.3, $p = 0.01$). Allele frequencies were different between patients (*T*: $n = 68$ and *t*: $n = 66$) and control subjects (*T*: $n = 52$, OR = 0.5, and *t*: $n = 26$, OR = 1.9, $p = 0.034$). Global χ^2 ($df = 2$): $p = 0.024$. In subjects with HbA_{1c} over 9 %, *Tt* was higher in patients ($n = 28$) than control subjects ($n = 15$, OR = 3.1, $p = 0.019$). Allele frequencies were not different between patients (*T*: $n = 52$ and *t*: $n = 38$) and control subjects (*T*: $n = 57$, and *t*: $n = 29$, $p = 0.31$). Global χ^2 ($df = 2$): $p = 0.035$.
Conclusion/interpretation. In French Type I (insulin-dependent) diabetic patients, we demonstrate an association between *TT* form (VDR) and low risk for severe diabetic retinopathy, especially in patients with long duration, and between *Tt* variant and high risk for severe diabetic retinopathy in subjects with poor glycaemic control. [Diabetologia (2002) 45: 436–442]

Keywords Diabetic retinopathy, *Taq I* polymorphism, genetic, vitamin D receptor, diabetes.

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Abbreviations: DR, Diabetic retinopathy; VDR, vitamin D receptor; VEGF, Vascular endothelial growth factor; IGF-1, insulin-like growth factor-1.

Neovascularization plays a role in determining the severity of diabetic retinopathy (DR) due to the effects of several angiogenic and growth factors, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and insulin-like growth factor (IGF-1) [1].

Chronic hyperglycaemia induces non-severe diabetic retinopathy (DR) in most patients of long duration, but not severe DR in the majority of them, sug-

gesting genetic factors play a role [2]. A sub-analysis of the Diabetes Control and Complications Trial (DCCT) showed a strong retinopathy transmission in families of patients with severe DR but not in those with non-severe DR [3]. Therefore, finding a genetic marker for this disease would be important in identifying patients who could benefit from preventive treatment.

It is well known that 1,25-dihydroxyvitamin D3 (calcitriol) is involved in the maintenance of mineral homeostasis. In addition, it has non-calciotropic effects, including antiproliferative, antiangiogenic, antioxidant and immunosupressive properties [4]. Recently, an inverse strong association between serum vitamin D concentrations and severity of DR was found [5], suggesting a protective role of active vitamin D forms for risk of severe DR.

The vitamin D receptor (VDR) is expressed in most cells of the retina including the endothelial cells, in animals and human beings [6–8]. Several polymorphisms of VDR have been associated with many conditions [9–18] including Type I (insulin-dependent) diabetes [11–14], Type II (non-insulin-dependent) diabetes mellitus [15], obesity [16] and insulin resistance [17].

Because of the relation between *t* allele of *Taq I* polymorphism of the VDR and reduced steady state VDR mRNA [19], we hypothesized that *Taq I* polymorphism could be involved in the pathogenesis of severe DR through a reduction of the non-calciotropic effects of 1,25-dihydroxyvitamin D3.

To examine whether the *Taq I* polymorphism is involved in the development of severe DR in a Caucasian French cohort with Type I diabetes, a case-control study was carried out.

Subjects and methods

From a group of 1000 Type I diabetic patients who regularly attended the diabetes outpatient clinic at the Hôtel-Dieu Hospital of Paris, two groups of unrelated patients were recruited at random during 1999 and 2000. Patients were classified into the following categories: study group ($n = 101$) with severe DR including proliferative ($n = 73$) or preproliferative ($n = 28$) DR, and control group ($n = 99$) with absent ($n = 32$) or background ($n = 67$) DR. All patients enrolled in this study gave informed consent. The protocol was in agreement with the Declaration of Helsinki. All patients fulfilled the following criteria: Caucasian with Type I diabetes diagnosis before 35 years of age (WHO criteria), C-peptide-negative and medical follow-up by our medical staff for at least 2 years. To reduce a chance of diabetic patients being wrongly classified as having non-severe DR because of a short duration of diabetes, we selected only patients with a diabetes duration of more than 15 years. Stages of retinopathy were defined according to the EURODIAB IDDM Complications Study, by trained ophthalmologists, using fluorescein angiograms, except in cases where there was no DR (fundoscopy after pupillary dilatation). Our classification was as follows: control group, no DR (level 0) and background DR (levels 1 and 2); severe DR group, preproliferative (level 3) and proliferative DR (levels 4 and 5) [20]. DN was assessed by AER: normal AER (< 30 mg/24 h; $n = 114$); microalbuminuria (30–300 mg/24 h; $n = 40$); albuminuria (> 300 mg/24 h; $n = 46$). Urinary albumin concentration was determined by enzyme immunoassay from a 24-h urine collections. LDL-cholesterol concentrations were taken from medical records. High blood pressure (HBP) was defined by a systolic blood pressure greater than 139 mmHg or a diastolic blood pressure greater than 89 mmHg (measured with a sphygmomanometer after 5 min at rest) or by a pharmacological treatment for arterial hypertension. The patients underwent a physical examination and completed a standard questionnaire on personal medical history regarding diabetes diagnosis, treatment, occurrence of acute and chronic complications, family history of diabetes and its complications. Each individual provided a blood sample for biochemical analysis (HbA_{1C} by HPLC method, Diamat, Bio-Rad, Hercules, Calif., USA and serum creatinine) and DNA extraction.

Genomic DNA was extracted from peripheral blood leukocytes of each patient using standard methods (Puregene, Gentra Systems, Minn., USA). The extracted DNA was stored at 4 °C until analysed. Genotypes were scored blindly. Ambiguous and homozygous samples were reanalysed. Polymerase chain reaction (PCR) of the DNA sequence flanking the *Taq I* restriction site of the VDR gene was carried out using forward primer 5'-CCA AGA CTA CAA GTA CCG CG-3' and reverse primer 5'-TGA GGA GGG CTG CTG AGT-3' (Sigma-Genosys, Pampisford, Cambridgeshire, England). The PCR product was digested using restriction enzymes, *Taq I* (Life Technologies, Paisley, Scotland). Digestion reactions were set according to the manufacturers instructions. The digested product was fractionated using 2 % agarose gel electrophoresis. Gels were stained with ethidium bromide and visualized under UV light and photographed. Absence or presence of T/C nucleotide substitution (ATT to ATC) leading to a synonymous change at codon 352 (isoleucine) in exon 9 was detected when *Taq I* restriction site was absent (*T* allele) or present (*t* allele), respectively [21].

The statistical difference in genotype distribution and allele frequencies among the groups and the subgroups (sorted by $HbA_{1c} > 9.0\,\%$ and duration of diabetes > 25 years) was assessed by χ^2 (chi-square) analysis. When appropriate, the odds ratio and 95 %-CI estimating the relative risks of severe DR associated with *Taq I* polymorphism was calculated. Allele frequencies were used to calculate genotype frequencies according to the Hardy-Weinberg equilibrium. Comparisons between the clinical characteristics of the two groups were made by unpaired *t* tests. A forward stepwise regression analysis was done to assess the influence of independent variables on severe DR (eg. sex, age, diabetes duration, HbA_{1c}, hypertension, LDL-cholesterol, albuminuria and *Taq I* polymorphism). A *p* value of less than 0.05 was considered to be statistically significant.

Results

The two groups had a similar distribution of sex, age, BMI, smoking, LDL-cholesterol and microalbuminuria. Patients had a longer diabetes duration (30.0 ± 10.1 vs 25.2 ± 9.4 years, $p < 0.0001$), higher HbA_{1c} concentrations (8.9 ± 1.4 vs $8.3 \pm 1.1\,\%$, $p < 0.0001$), higher serum creatinine concentration (104.2 ± 62.5 vs 86.9 ± 20 mg/dl, $p < 0.01$) and, more frequent occurrence of arterial hypertension (73.3 vs 41 %, $p < 0.02$) and albuminuria (37.6 vs 8.1 %,

Table 1. Genotype distribution and allele frequencies

	Study group ($n = 101$)	Control group ($n = 99$)	OR (95%-CI)	p value (Yates corrected p)
Genotypes				
TT	27 (26.7)	42 (42.4)	0.50 (0.26–0.94)	0.0196 (0.028)
Tt	58 (57.4)	44 (44.4)	1.69 (0.93–3.07)	0.066 (0.090)
tt	16 (15.8)	13 (13.1)	1.25 (0.53–2.95)	0.58 (0.73)
Alleles				
T	112 (55.4)	128 (64.6)	0.68 (0.45–1.04)	0.060 (0.075)
t	90 (44.6)	70 (35.4)	1.47 (0.96–2.24)	0.060 (0.075)

Data are expressed as n (%). *TT*, *Tt* and *tt* are the wild-type, heterozygous and homozygous genotypes, respectively. Global χ^2 ($df = 2$) = 5.47, $p = 0.064$

$p < 0.0001$), than control subjects. Normoalbuminuria was more frequent in control subjects than patients (74.7 vs 39.6%, $p < 0.001$).

Genotypes in whole sample and in both study and control groups were in Hardy-Weinberg equilibrium ($p = 0.783$, $p = 0.103$ and $p = 0.377$, respectively). The genotype distribution was *TT* 34.5%, *Tt* 51% and *tt* 14.5%. The frequency of wild-type genotype *TT* was lower in patients with severe DR ($n = 27$, 26.7%) when compared with control subjects ($n = 42$, 42.4%, $\chi^2 = 5.45$, OR = 0.5 [95%-CI = 0.26–0.94], $p = 0.019$, Yates corrected $p = 0.028$). The frequency of *Tt* was not different between patients ($n = 58$, 57.4%) and control subjects ($n = 44$, 44.4%, $\chi^2 = 3.37$, OR = 1.69 [95%-CI = 0.93–3.07], $p = 0.066$, Yates corrected $p = 0.090$). The frequency of *tt* variant was not different between patients ($n = 16$, 15.8%) and control subjects ($n = 13$, 13.1%, $\chi^2 = 0.30$, OR = 1.25 [95%-CI = 0.53–2.95], $p = 0.58$, Yates corrected $p = 0.73$). Allele frequencies showed a non significant difference between patients (*T* allele: $n = 112$, 55.4% and *t* allele: $n = 90$, 44.6%) and control subjects (*T* allele: $n = 128$, 64.6%, $\chi^2 = 3.53$, OR = 0.68 [95%-CI = 0.45–1.04], and *t* allele: $n = 70$, 35.4%, OR = 1.47 [95%-CI = 0.96–2.24], $p = 0.060$, Yates corrected $p = 0.075$). Global χ^2 ($df = 2$) was not significant ($\chi^2 = 5.47$, $p = 0.064$). The genotype and alleles frequencies are summarized in Table 1.

When we analysed only those subjects who had had diabetes for more than 25 years (patients: $n = 67$ and control subjects: $n = 39$), the frequency of *TT* was lower in patients with severe DR ($n = 14$, 20.9%) when compared with control subjects ($n = 18$, 46.2%, $\chi^2 = 6.31$, OR = 0.3 [95%-CI = 0.11–0.79], $p = 0.006$, Yates corrected $p = 0.01$). The frequency of *Tt* was not different between patients ($n = 40$, 59.7%) and control subjects ($n = 16$, 41.0%, $\chi^2 = 2.74$, OR = 2.1 [95%-CI = 0.88–5.15], $p = 0.06$, Yates corrected $p = 0.097$). The frequency of *tt* variant was not different between patients ($n = 13$, 19.4%) and control subjects ($n = 5$, 12.8%, $\chi^2 = 0.36$, OR = 1.6, [95%-CI = 0.48–5.83], $p = 0.38$, Yates corrected $p = 0.54$). Allele frequencies showed a difference between patients (*T* allele: $n = 68$, 50.7% and *t* allele: $n = 66$, 49.3%) and control subjects (*T* allele: $n = 52$, 66.7%, $\chi^2 = 4.46$, OR = 0.5 [95%-CI = 0.27–0.95], and *t* allele: $n = 26$, 33.3%, OR = 1.9 [95%-CI = 1.04–3.61], $p = 0.02$, Yates corrected $p = 0.034$). Global χ^2 ($df = 2$) was significant ($\chi^2 = 7.47$, $p = 0.024$).

When we analysed only those subjects with HbA_{1c} concentration over 9.0% (patients: $n = 45$ and control subjects: $n = 43$), the frequency of *TT* was not different between patients with severe DR ($n = 12$, 26.7%) and control subjects ($n = 21$, 48.8%, $\chi^2 = 3.71$, OR = 0.4 [95%-CI = 0.14–1.01], $p = 0.031$, Yates corrected $p = 0.054$). The frequency of *Tt* was higher in patients with severe DR ($n = 28$, 62.2%) when compared with control subjects ($n = 15$, 34.9%, $\chi^2 = 5.53$, OR = 3.1 [95%-CI = 1.18–8.09], $p = 0.01$, Yates corrected $p = 0.019$). The frequency of *tt* variant was not different between patients ($n = 5$, 11.1%) and control subjects ($n = 7$, 16.3%, $\chi^2 = 0.16$, OR = 0.6 [95%-CI = 0.15–2.53], $p = 0.48$, Yates corrected $p = 0.69$). Allele frequencies were not different between patients (*T* allele: $n = 52$, 57.8% and *t* allele: $n = 38$, 42.2%) and control subjects (*T* allele: $n = 57$, 66.3%, $\chi^2 = 1.01$, OR = 0.7 [95%-CI = 0.36–1.34], and *t* allele: $n = 29$, 33.7%, OR = 1.4 [95%-CI = 0.74–2.77], $p = 0.24$, Yates corrected $p = 0.31$). Global χ^2 ($df = 2$) was significant ($\chi^2 = 6.67$, $p = 0.035$).

We compared clinical parameters between carriers (*Tt* and *tt*) and non-carriers (*TT*). Only BMI was higher in carriers (24.7 ± 3.1 kg/m2) when compared with non-carriers (23.1 ± 3.2 kg/m2, $p = 0.03$) at the study group. All data are summarized in Table 2.

The forward stepwise regression analysis confirmed that albuminuria ($F = 16.5$, $p = 0.00007$), *TT* genotype ($F = 5.05$, $p = 0.026$) and diabetes duration ($F = 4.42$, $p = 0.037$), were the only significant independent variables for severe DR.

The distribution for *TT*, *Tt*, and *tt* genotypes and, *T* and *t* alleles was not different between normoalbuminuric ($n = 41$, 36.0%, $n = 57$, 50.0%, $n = 16$, 14.0%, $n = 139$, 61.0% and $n = 89$, 39.0%, respectively), microalbuminuric ($n = 16$, 40.0%, $n = 18$, 45.0%, $n = 6$, 15%, $n = 50$, 62.5%, $n = 30$, 37.5%, respectively, NS) and albuminuric patients ($n = 12$, 26.1%, $n = 27$, 58.7%, $n = 7$, 15.2%, $n = 51$, 55.4%, $n = 41$, 44.6%, respectively, NS). Global χ^2 ($df = 4$) was not significant ($\chi^2 = 2.24$, $p = 0.69$).

Table 2. Comparison of clinical parameters according to *Taq I* genotypes

	Study group ($n = 101$) (Proliferative, $n = 73$; preproliferative DR, $n = 28$)		Control group ($n = 99$) (Absent, $n = 32$; background DR, $n = 67$)	
	TT vs ($n = 27$)	*Tt* ($n = 58$) + *tt* ($n = 16$)	*TT* ($n = 42$) vs	*Tt* ($n = 44$) + *t t* ($n = 13$)
Sex (M/F)	8/19	37/37	24/18	34/23
Age (years)	46.3 ± 12.0	46.0 ± 11.8	43.0 ± 12.7	42.9 ± 13.5
Diabetes duration (years)	29.5 ± 11.3	30.5 ± 9.9	25.5 ± 8.5	25.2 ± 10.0
Age of diagnostic (years)	16.0 ± 5.9	15.7 ± 7.5	17.5 ± 8.2	17.8 ± 8.1
Weight (kg)	65.2 ± 10.9	69.5 ± 11.7	72.5 ± 9.3	70.8 ± 12.4
Height (cm)	167.6 ± 8.7	167.4 ± 9.3	171.2 ± 8.5	171.6 ± 8.7
BMI (kg/m^2)	23.1 ± 3.2	24.7 ± 3.1*	24.7 ± 3.2	24.0 ± 3.7
HbA_{1c} (%)	9.29 ± 1.44	8.79 ± 1.31	8.08 ± 1.19	8.51 ± 1.19
LDL cholesterol (mg/dl)	1.23 ± 0.59	1.27 ± 0.41	1.26 ± 0.43	1.19 ± 0.31
Hypertension (n, %)	20 (74.1)	54 (73.0)	18 (42.9)	23 (40.4)
Systolic pressure (mmHg)	134.5 ± 18.6	140.2 ± 21.1	137.1 ± 16.9	133.9 ± 16.8
Diastolic pressure (mmHg)	73.2 ± 9.6	77.9 ± 11.2	75.6 ± 10.1	74.7 ± 8.4
Smoking (n, %)	7 (26.0)	28 (37.8)	18 (42.9)	21 (36.8)
Serum creatinine (mg/dl)	92.6 ± 32.0	108.7 ± 70.8	84.6 ± 17.6	89.6 ± 22.0
Normoalbuminuria (n, %)	11 (40.7)	29 (39.2)	30 (71.4)	44 (77.2)
Microalbuminuria (n, %)	6 (22.2)	17 (23.0)	10 (23.8)	7 (12.3)
Albuminuria (n, %)	10 (37.0)	28 (37.8)	2 (4.8)	6 (10.5)

*$p = 0.03$ between carriers and non-carriers from the study group. The other comparisons intragroup are NS

Discussion

We have shown that *TT* variant of *Taq I* polymorphism of VDR is associated with non-severe DR (absent or background DR) in a French Caucasian cohort with Type I diabetes, especially in patients who had diabetes longer than 25 years, and also that the *Tt* variant is associated with high risk of severe DR in patients with HbA_{1c} concentrations of more than 9.0%. Although the frequency of *T* allele showed no statistical difference at the whole population, it was slightly lower in retinopathic patients (55.4%) when compared with control subjects (64.6%, $p = 0.075$), a finding reinforced by a reduced odds ratio and the narrow bounds of the 95%-CI (OR = 0.7 [95%-CI = 0.45–1.04]). It is not surprising that interactions between *Tt* genotype and HbA_{1c} of more than 9.0% and, especially, between *TT* genotype and diabetes duration of more than 25 years amplified the effect of *Taq I* polymorphism found at whole population. Most case-control studies have found that analysis of the association between VDR polymorphisms, including *Taq I* site, and other different conditions (eg. osteoporosis, cancer) requires the inclusion of other potentially relevant variables (eg. calcium intake, ageing) [9, 21, 22]. Our study of these interactions was based on the fact that a long duration of diabetes and poor glycaemic control are involved in DR [23]; a long duration of diabetes has been significantly correlated with low serum vitamin D concentration ($p < 0.05$) in Caucasian individuals with Type II diabetes [5], as has poor glycaemic control [24, 25]. In addition, hyperglycaemia has been associated with reduced vitamin D effects on bone cells [26, 27] and low VDR expression in intestinal and renal cells [28].

Although diabetes duration, HbA_{1c} concentration, hypertension and albuminuria were different between the study and control groups ($p < 0.0001$, $p < 0.001$, $p < 0.02$ and $p < 0.001$, respectively), forward stepwise regression showed that *TT* genotype effect on DR risk was not affected by those variables. Updated Hb*A1 c* concentration was not significant ($p = 0.053$). Unfortunately, baseline HbA_{1c} concentrations, a better parameter of chronic glycaemic exposure, [23], were not available for analysis. Furthermore, non-glycaemic factors could be important in the progression of diabetic complications [3, 23]. Even though our study did not include non-diabetic control subjects, the distribution of *Taq I* polymorphism among our cohort was similar to that observed in other French cohorts [16, 29], thus excluding a selection bias.

We hypothesized that antiproliferative, antiangiogenic, antioxidant and immunosuppressive properties of 1,25-dihydroxyvitamin D3 protect against severe DR, and this effect must requires a normal function of VDR. Recently, a strong inverse association was found between serum vitamin D concentration and severity of DR [5], suggesting a protective role of active vitamin D for risk of severe DR. Treatment with calcitriol inhibits angiogenesis and reduces the size of tumors in transgenic murine retinoblastoma, in a dose-dependent manner, an effect well recognized in other tumours [30] including in human cells of retinoblastoma [8]. Moreover, it was found that topical administration of calcitriol can strongly reduce corneal neovascularization inhibiting leucocyte migration, and could also inhibit IL-1 alpha, IL-1 beta and IL-8 production by human corneal cells in vitro [31]. This could be partly because of its capacity to downregulate nuclear factor-kappa B [32]. Recently, one report

showed that calcitriol could inhibit VEGF [33] although other studies found the opposite effect [34]. On the other hand, calcitriol strongly represses the IGF-1 pathway because of its ability to stimulate insulin-like growth factor binding protein-3 secretion [35]. Finally, functional genetic studies support our hypothesis. For example, investigators found in three different types of human cells, that steady state concentrations of VDR mRNA with *T* allele were 30% higher than *t* allele [19]. Moreover, in gene constructs containing the 3'untranslated region (3'UTR) from individuals homozygous for *baT* haplotype, activity of cells transfected was found to be increased 1.5-fold to fivefold when compared with 3'UTR from individuals homozygous for *BAt* haplotype, an effect of the increase VDR mRNA stability [36]. However, previous studies had suggested the opposite effect [37, 38].

Although most of the above-mentioned effects have been shown in a dose-dependent manner, and especially at pharmacological concentrations, some reports have found, after administration of low calcitriol doses, and in different types of animals and human cells, significant growth cell inhibition, antioxidant activity and immunosuppressive effects, despite physiologic concentrations of calcitriol [39–43].

Because a risk of DR has been associated with insulin resistance [44] and low beta-cell function [45, 46], active vitamin D could induce retinal protection indirectly by inducing beta-cell protection [47], insulin secretion [48–50] and insulin sensitivity [49–51]. Interestingly, at least one report associated the *b* allele of *Bsm I*, which shows strong linkage disequilibrium with *T* allele of *Taq I* site, with significantly lower serum calcitriol concentrations than control subjects [52], although other studies found the opposite effect [21]. In addition, VDR genotype combination *BAt* confers high risk for Type I diabetes in German people [11].

We cannot exclude the possibility that the *TT* variant of the *Taq I* site is a marker of a nearby functional polymorphism within the VDR gene or a nearby gene. Interestingly, the G-protein beta 3 subunit gene (GNB3) is located very near to the VDR locus (12q12-q13 and 12q12-q14, respectively), and this family of proteins has been associated with many regulatory functions including cell growth and angiogenesis [53]. One report has showed an association between diabetic nephropathy and a genetic variant in the G-protein beta 3 subunit, *C825T* polymorphism [54]. However, no association between the latter and DR exists, and, the majority of reports on diabetic nephropathy have not found any association [55].

Because the functional effects of *Taq I* genotypes could be tissue-specific as with other VDR polymorphisms [21, 22], and because the physiopathology of DR is quite different from that of diabetic nephropathy it not surprising that there is no association between *Taq I* polymorphism and the latter. Polymorphisms clearly involved in DR could have no role to play in diabetic nephropathy (eg. microsatellite 5' of aldose reductase), and vice versa (eg. polymorphism I/D of the angiotensin-1 converting enzyme) [56, 57]. However, the aim of our study was not to investigate diabetic nephropathy and the existence of many different VDR polymorphisms which we did not study, means that there could be an association between VDR polymorphisms and this complication. Recently, a small observational study in Italian subjects found no transmission of *BB* genotype – wild-type form of the BsmI polymorphism of VDR – among 15 diabetic individuals, from 86 uremic patients [58].

In conclusion, we have shown an association between the *TT* variant of the *Taq I* polymorphism of VDR and low risk for severe DR in French Type I diabetic patients, especially in patients who had diabetes for a long time, and between the *Tt* variant and a high risk of severe DR in patients with poor glycaemic control.

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