Gene 566 (2015) 212–216

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Effects of vitamin D receptor gene polymorphism and clinical characteristics on risk of diabetic retinopathy in Han Chinese type 2 diabetes patients

Xing Zhong [a,1], Yijun Du [a,1], Yuan Lei [b], Nina Liu [a], Yanyun Guo [a], Tianrong Pan [a,*]

[a] Department of Endocrinology, The Second Affiliated Hospital of Anhui Medical University, Hefei 230061, PR China
[b] Department of Endocrinology, The Fourth Affiliated Hospital of Anhui Medical University, Hefei 230022, PR China

ARTICLE INFO

Article history:
Received 30 October 2014
Received in revised form 9 April 2015
Accepted 17 April 2015
Available online 18 April 2015

Keywords:
Diabetic retinopathy
Vitamin D receptor
Polymorphism
Risk factors

ABSTRACT

Purpose: The purpose of this study was to investigate the association of *VDR* polymorphism with development of retinopathy in a Han Chinese population with type 2 diabetes mellitus.
Materials and methods: A total of 204 T2DM patients were subdivided into groups without diabetic retinopathy (NDR, $n = 110$) and those with DR ($n = 94$). *VDR* rs2228570 (FokI:C > T), rs1544410 (BsmI:G > A), and rs7975232 (ApaI:A > C) polymorphism was assayed by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP).
Results: Diabetes duration (10.0 vs. 5.0 years, $P < 0.01$) was longer, systolic blood pressure (143.98 ± 24.31 vs. 135.11 ± 15.23, $P < 0.01$), and HbA1c (9.2 ± 2.06 vs. 8.35 ± 1.62, $P < 0.01$) were higher in DR than in NDR patients. Distribution frequencies of the rs2228570, rs1544410, and rs7975232 genotypes followed the Hardy–Weinberg equilibrium. *VDR* rs2228570 TT genotype frequency was significantly higher in DR ($n = 30$; 31.9%) than in NDR patients ($n = 14$; 12.7%; $P < 0.01$). DR patients carried more rs2228570 T alleles ($n = 113$; 60.1%) than did NDR patients ($n = 89$; 40.5%; $P < 0.01$). Genotype frequencies of rs1544410 and rs7975232 in NDR and DR patients were not different. Logistic analysis confirmed that diabetes duration (odds ratio (OR) 1.108, $P < 0.01$), SBP (OR 1.022, $P < 0.05$), HbA1c (OR 1.267, $P < 0.05$), and the *VDR* rs2228570 T allele (OR 1.467, $P < 0.01$) were independently associated with DR risk. TAA haplotype frequency was significantly higher in DR (24.0%) than in NDR (16.1%) patients ($P < 0.05$).
Conclusions: Diabetes duration, SBP, HbA1c, and the rs2228570 T allele were associated with increased risk of DR. *VDR* rs2228570 might be good candidate biomarker of DR in Han Chinese T2DM patients.

1. Introduction

Diabetic retinopathy (DR) is a frequent complication of diabetes mellitus, and is a leading cause of blindness in the working age population (Fong et al., 2004). More than 60% of adults with type 2 diabetes mellitus (T2DM) develop DR within 20 years of disease onset (Morello, 2007), but early detection can prevent severe loss of vision and blindness. The worldwide prevalence of DR in people with diabetes has been estimated at 34.6% and that of proliferative diabetic retinopathy (PDR) at 7.0% (Yau et al., 2012). According to the World Health Organization, DR causes 4.8% of the 37 million annual cases of blindness worldwide (Resnikoff et al., 2004).

Epidemiological data from the Diabetes Control and Complications Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS) have identified hyperglycemia, high blood pressure, and duration of diabetes as major risk factors of development and progression of DR (DCCT Group, 1993; Stratton et al., 2001). Other DR risk factors include age at onset of diabetes, hyperlipidemia, gender and ethnicity (Chistiakov, 2011). There is strong evidence that good diabetes control helps to prevent DR, but some patients develop DR despite good control and others avoid retinopathy despite poor control or diabetes of long duration. This suggests that genetic factors may influence susceptibility to retinopathy (S. Patel et al., 2008). Candidate genes for increased risk of developing DR include those coding for aldose reductase (ALR), nitric oxide synthase (NOS), receptor for advanced glycation end products (RAGE), angiotensin converting enzyme (ACE), human leucocyte antigen (HLA), vascular endothelial growth factor (VEGF), and vitamin D receptor (VDR) (Radha et al., 2002).

Abbreviations: ACE, angiotensin converting enzyme; ALR, aldose reductase; BMI, body mass index; DR, diabetic retinopathy; DCCT, Diabetes Control and Complications Trial; HLA, human leucocyte antigen; NOS, nitric oxide synthase; PCR, polymerase chain reaction; RAGE, receptor for advanced glycation end products; RFLP, restriction fragment length polymorphism; T2DM, type 2 diabetes mellitus; UKPDS, UK Prospective Diabetes Study; VDR, Vitamin D receptor; VEGF, vascular endothelial growth factors.

* Corresponding author.
E-mail address: pantianrong1968@163.com (T. Pan).
[1] Xing Zhong and Yijun Du contributed equally to this article.

http://dx.doi.org/10.1016/j.gene.2015.04.045
0378-1119/

Vitamin D affects not only calcium metabolism, but also has antiproliferative and antiangiogenic activities (Lin and White, 2004). Hedlund et al. (1996) reported a correlation between cellular expression of VDR and sensitivity to growth inhibition by vitamin D secosteroids. The active form of this steroid acts through specific VDR binding. VDR is extensively expressed in most cells of the retina including the vascular endothelial cells in humans (Johnson et al., 1995). Several polymorphisms, including rs2228570, rs1544410 and rs7975232, have been described for the *VDR* gene. *VDR* rs2228570 (*FokI*: C > T) in exon 2, and rs1544410 (*BsmI*: G > A) and rs7975232 (*ApaI*: A > C) in intron 8 lead to an alternative transcription initiation site and result in a VDR protein with three additional amino acids (Uitterlinden et al., 2004). Taverna et al. (2005) found a novel association between functional *VDR* gene rs2228570 polymorphism and risk of advanced DR in patients with C-peptide-negative type 1 diabetes. However, Cyganek et al. (2006) found that *VDR* gene polymorphisms did not increase DR risk in a population of T2DM patients in Poland. It is believed that various genetic factors are responsible for interracial differences. Available data thus supports a hypothesis that *VDR* polymorphisms interact with known clinical risk factors of DR in the Han Chinese population.

The purpose of this study was to investigate the association of *VDR* polymorphism with development of retinopathy in a Han Chinese population with T2DM.

2. Subjects and methods

2.1. Subjects

T2DM patients at the Department of Endocrinology at the Second Affiliated Hospital of Anhui Medical University were enrolled in this study. They were diagnosed according to the World Health Organization criteria and had a fasting plasma glucose (FPG) level ≥7.0 mmol/L or a 2-h post-load glucose concentration ≥11.1 mmol/L (Alberti and Zimmet, 1998). Patients with T2DM were eligible for inclusion if 1) the duration of diabetes was >1 year, and 2) they were treated with insulin or oral antidiabetic drugs. Patients with 1) type 1 diabetes, 2) acute or chronic inflammatory disease, and 3) impaired kidney function or overt nephropathy were excluded. DR was evaluated by trained ophthalmologists in the specialty unit of our hospital. The diagnosis of DR was confirmed by the presence of any of the following: microaneurysms, intraretinal hemorrhages, definite venous beading, prominent intraretinal microvascular abnormalities, neovascularization, or vitreous/preretinal hemorrhage (Wilkinson et al., 2003). The 204 T2DM patients who were enrolled were divided into a group without DR (NDR; 51 men, 59 women, with a mean age of 57.08 ± 11.96 years) and a group with DR (44 men, 50 women with a mean age of 58.71 ± 10.58 years). A control (CON) group of 116 healthy, age and gender-matched individuals who did not smoke and drink was also included. Control participants had no history of DM, normal FPG, normal findings on ophthalmoscopic examination, and without any other inflammatory or chronic diseases. All patients gave informed consent before enrolling in this study. The study was conducted following the ethical principles of the Declaration of Helsinki, and was approved by the Medical Ethics Committee of Anhui Medical University.

All participants were unrelated and were of the Han nationality. The patient demographic and clinical data included age, gender, diabetes duration, body mass index (BMI), blood pressure (BP), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and glycated hemoglobin (HbA1c). HbA1c was measured by high performance liquid chromatography (HLPC). FPG was determined by the glucose oxidase method, TC, TG and HDL-C by an enzymatic colorimetric method, and LDL-C was calculated using the Friedewald formula.

2.2. Determination of genotypes

The rs2228570, rs1544410, and rs7975232 polymorphisms of the *VDR* gene were assayed by restriction fragment length polymorphism analysis of polymerase chain reaction-amplified DNA fragments (PCR–RFLP). In brief, approximately 3 mL specimens of whole blood were collected from each participant into EDTA tubes and stored at −20 °C until use. Genomic DNA was extracted from peripheral blood leukocytes using a commercial kit according to the manufacturer's instructions (Promega, Madison, WI, USA). The extracted DNA was stored at 4 °C until assayed. Genotypes were scored blindly. Primers and PCR conditions are shown in Table 1. PCR was carried out in a total volume of 25 μL containing 0.5 μL of each primer (20 pM), 0.5 μL dNTP (10 pM), 3 mM $MgCl_2$, 5% dimethyl sulfoxide, 4.5 U Taq polymerase, 50 mM KCl, 10 mM Tris–HCl pH 8.3, and 20 ng of genomic DNA. The PCR product was digested using the restriction enzymes listed in Table 1, which included FokI (Takara), BsmI (Fermentas) and ApaI (Takara). Digestion reactions were run according to the manufacturer's instructions, and the digested product was fractionated by 2% agarose gel electrophoresis. Gels were stained with ethidium bromide, examined under UV light, and photographed.

2.3. Statistics

Measurement data with normal or nonnormal distributions were expressed as means ± standard deviation (SD) or medians (25th–75th percentile), respectively. Student's *t* test was used to compare between-group differences in clinical characteristics. Hardy–Weinberg equilibria were calculated to evaluate expected and observed gene and genotype frequencies. A power analysis performed using Quanto software, version 1.2 (http://biostats.usc.edu/Quanto.html) confirmed that the sample size had a minimum power of 83% at a small effect size (0.1) and alpha level (0.05). The haplotype frequencies in each study group were analyzed using SHEsis Online haplotype analysis software (http://analysis.bio-x.cn/myAnalysis.php). The significance of between-group differences in genotype distribution and allele frequencies was determined using the chi-square test. A forward, stepwise regression analysis was carried out to assess the independence of the effect of experimental variables (including demographic data and VDR gene polymorphisms) on DR. $P < 0.05$ was considered significant. The

Table 1
Primer sequences and restriction fragment length polymorphism conditions for the identification of *VDR* gene polymorphisms.

SNPs	Primers	PCR conditions	Restriction enzymes	Annealing temp.(°C)	Incubation time (h)	Restriction fragment lengths (bp)
rs2228570 (C > T)	F: AGCTGGCCCTGGCACTGACTCTGCTCT R: ATGGAAACACCTTGCTTCTCCTCCCTC	94 °C for 5 min, followed by 32 cycles of 94 °C for 30 s, 60 °C for 50 s, and 72 °C for 1 min, and a final elongation step at 72 °C for 4 min.	FokI	37	1	CC: 265 CT: 265,196, and 69 TT: 196 and 69
rs1544410 (G > A)	F: GCGAGACGTAGTAAAAGG R: AGAGGTCAAGGGTCACTG	94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 62 °C for 50 s, 72 °C for 1 min, and a final elongation step at 72 °C for 4 min.	BsmI	37	8	GG: 359 GA: 359,190, and 160 AA: 190 and 169
rs7975232 (A > C)	F: CAGAGCATGGACAGGGAGCAA R: GCAATCCCTCATGGCTGAGGTCTC	94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 66 °C for 50 s, 72 °C for 1 min, and a final elongation step at 72 °C for 4 min.	ApaI	37	1	AA: 740 AC: 740, 520, and 220 CC: 520 and 220