Research Article

Gender-Specific Association of Vitamin D Receptor Polymorphism Bsm-I with Type 1 Diabetes Mellitus

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ABSTRACT

Type 1 diabetes mellitus is recognized as a T-cell-mediated autoimmune disease. Vitamin D is known to suppress T-cell activation by binding to the vitamin D receptor (VDR); and thus, VDR gene polymorphisms may be related to T-cell-mediated autoimmune diseases. We, therefore, investigated a VDR gene polymorphism in type 1 diabetes. We examined the VDR gene *Bsm* –I polymorphism in 55 type 1 diabetic patients and 55 controls, and the association between the VDR gene polymorphism and type 1 diabetes. We found a significantly higher frequency of bb genotype in type 1 diabetics overall, compared with controls (P = 0.027). Moreover, there was a significant difference in bb genotype frequency between type 1 diabetic females and control females (P = 0.011), whereas this difference was not observed between type 1 diabetics and controls within males group (P = 0.688). In conclusion, we found an association between a VDR gene polymorphism and type 1 diabetes in Syrian population.

Keywords: Diabetes mellitus, Polymorphism, Vitamin D.

INTRODUCTION

Type 1 diabetes is considered to be T-helper 1 (Th1) type autoimmune disease[1]. Because this disease usually affects relatively younger ages, many patients suffer from diabetic complications that reduce their quality of life. Therefore, prevention of this disease is extremely important[2]. Both genetic and environmental factors are involved in causing type 1 diabetes[3], and viral infection[4,5], Gluten and other cereal-derived proteins[6], cow's milk[7,8], BCG vaccination[9] and vitamin D deficiency[10] are candidates for environmental factors. Among these environmental factors, vitamin D administration to an animal model of type 1 diabetes has been shown to prevent the disease[11] , therefore an intervention trial of vitamin D administration to high risk subjects for type 1 diabetes should be considered.

This approach is attractive because the vitamin D receptor is expressed on CD4 T cells, which are a key immune cell in the development of type 1 diabetes. In addition, vitamin D compounds are known to suppress T-cell activation by binding to the VDR[12] and thus, VDR gene polymorphisms are likely to be related to T-cell mediated autoimmune disease. Vitamin D exerts its genomic action via the nuclear vitamin D receptor (VDR), The VDR belongs to the steroid receptor super-family of ligand-activated transcription factors[13], and is widely expressed in many cell types, including lymphocytes and macrophages [14], and the importance of vitamin D is further supported by the finding that pancreatic b-cells express VDR[15]. The VDR gene is located on chromosome 12q (12-12q14) and is highly polymorphic[16].

In humans, epidemiological studies indicated that dietary vitamin D supplementation during early childhood decreases the risk of type 1 diabetes [17, 18] and that maternal intake of vitamin D during pregnancy may have a protective effect on the appearance of islet autoantibodies in offspring [19,20]. However, because the genetic background of subjects varies, we should focus on those with a genetically higher risk of type 1 diabetes that is related to vitamin D[10].

MATERIALS AND METHODS

Patients and controls

Fifty five unrelated Syrian patients already diagnosed with T1DM, age 7-58 years, who were attending diabetic clinics in Damascus and Dara'a were enrolled in the study. The male to female ratio of T1DM patients was 25/30. To characterize our diabetic population, we recorded date of birth, age at the onset of diabetes, the season of the onset, and body mass index (BMI).

Age at the onset of diabetes was between 15 days and 26 years in the diabetic group, with an average of 13.75 years (SD=6.91), and patients with older age of the onset were excluded. The average BMI of all patients was 22.4 kg/m2 (SD=3.2). Reference values for the prevalence of each genetic variant were obtained from 50 healthy subjects (24 males and 26 females) which don't have any autoimmune disease and don't have T1D family history with age 26-66 years (average=39.86, SD=11.66).

The advantage of this population is the extremely low risk of development of T1DM. However, each subject filled out a detailed questionnaire concerning health risk factors before blood donation and none of them indicated the presence of T1DM in first-degree relatives. At the beginning of sample collection the informed consent of patients or parents of diabetic children was obtained for the use of blood samples for diagnostic and scientific purposes.

DNA isolation

Blood was collected in EDTA–K3 tubes and stored at -20 C° until DNA extraction. DNA was purified by using QIAamp Blood minikit (Qiagen, Germany)

Genotyping

Referring to the VDR gene sequence, PCR amplification of the region containing the polymorphism was performed using the forward primer in exon 7 (5'-CAACCAAGACTACAAGTACCGCGTCAGTGA-3') and the reverse primer in intron 8 (5'-AACCAGCGGGAAGAGGTCAAGGG-3').

The PCR conditions used in this study were as follows: 95 °C for 5 min, followed by 30 cycles using the following temperature profile: 95 °C for 1 min, 56°C for 1 min, and 72 °C for 1 min, and final elongation for 10 min. The PCR products were 825-bp long (B allele) and were digested with BsmI at 65 °C for 1 h, and then subjected to electrophoresis in 2% agarose gel stained with ethidium bromide. The lengths of the restriction fragments were 649 and 176 bp (b allele). Genotype was determined from the lengths of fragments, i.e. BB, Bb, and bb[10].

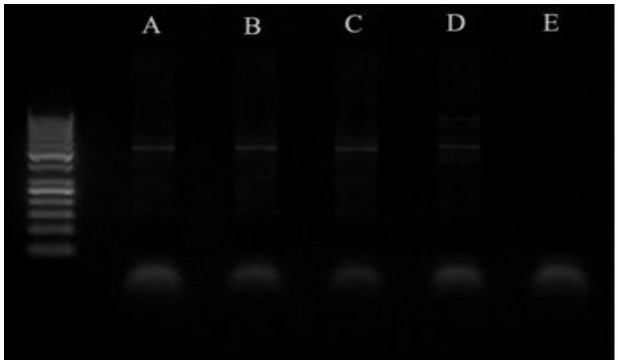

Figure 1: Ethidium bromide-stained 2% agarose gel showing 825-bp PCR products of the VDR gene, first lane is for marker, lanes A and B represents patients samples, lanes C and D are for control samples, and lane E is negative control.

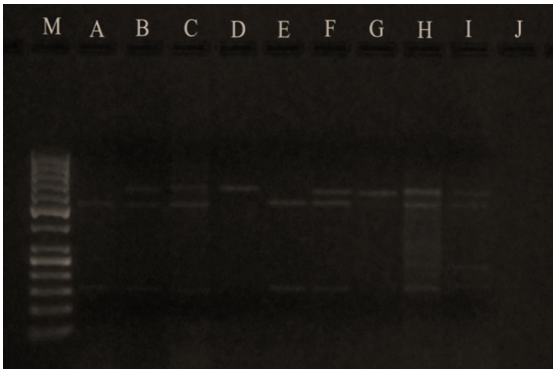

Figure 2: Detection of *Bsm I* polymorphism by PCR-RFLP. The upper bands represent allele B, the lower bands allele b. Lanes B, C,D,F,H and I Bb heterozygotes; lanes D and G BB homozygotes; and lanes A and E bb homozygote. Lane J is negative control. Samples A-D are diabetic and lanes E-I are control.

Statistical analysis

Hardy–Weinberg equilibrium was calculated to evaluate the relationship between gene frequencies and genotype frequencies. Comparisons of genotype frequency between groups were performed using chi-squared test and IBM SPSS statistics 20 software was used for the statistical calculations.

RESULTS AND DISCUSSION

Results

Higher frequency of bb genotype in type 1 diabetics

The Bsm I polymorphisms in the VDR gene was investigated by PCR-RFLP analysis in type 1 diabetes patients. We also genotyped 50 healthy individuals as the control group. The frequencies of the alleles and the genotypes were in Hardy-Weinberg equilibrium among the patients and the controls. 45.5% of the patients were heterozygous for the Bsm I polymorphism, 12.7% were homozygous BB and 41.8 were homozygous bb.

The respective frequencies in the control group were 52%, 28% and 20%. These frequencies are significantly different from those in the diabetic patients ($\chi2=7.253$, P = 0.027, df = 2). The allelic and genotypic frequencies in the patients and controls for the Bsm I polymorphism are shown in Table 1.

Table 1: Genotype frequencies of VDR BsmI gene polymorphism

	Patients N (%)	Controls N (%)
BB	7 (12.7)	14 (28.0)
Bb	25 (45.5)	26 (52.0)
bb	23 (41.8)	10 (20.0)
B allele	39 (35.5)	54 (54.0)
b allele	71 (64.5)	46 (46.0)

Higher frequency of bb genotype in type 1 diabetes females

The number of females having the bb genotype was higher in T1DM than in controls (12/3), the females having Bb genotype were equal in the two groups (15), and the number of females having BB genotype was lower in T1D than in controls (3/10). This difference is significant between female patients and controls ($\chi2=9.111$, P = 0.011, df = 2).

This difference was not exist when comparing genotypes within the males group. There were 11/7 (patient/control) carrying bb genotype, 10/11 carrying the Bb genotype, and the BB genotype was equal in both groups (4), ($\chi2=0.748$, P = 0.688, df = 2).

Discussion

The VDR is a member of the steroid hormone receptor superfamily[21], and regulates gene transcription through interaction with hormone response elements in the

promoter region of target genes[22]. In the immune system, for example, vitamin D promotes monocyte differentiation and inhibits lymphocyte proliferation and secretion of cytokines, such as interleukin 2 (IL2), interferon-g, and IL12[23].

Clear effects of 1,25(OH)2D3 and its analogues on the different major players in the pathogenesis of type 1diabetes mellitus have been described. A modest stimulation of insulin synthesis and insulin secretion by 1,25(OH)2D3 is observed *in vivo* as well as *in vitro*. Moreover, a direct β-cell protection by 1,25(OH)2D3 and its analogues against metabolic and inflammatory stress has been demonstrated. On the other hand, major effects on the immune system, involved in the pathogenesis of type 1 diabetes have been described *in vitro* as well as *in vivo*, and prevention of type 1 diabetes and its recurrence after islet transplantation can be achieved by 1,25(OH)2D3 and its analogues (alone or in combination with other immune modulators) [12,24].

Table 2: Genotype frequencies of VDR BsmI gene polymorphism according to sex

Sex		Polymorphism			Total
		Bb	Bb	Bb	
Female	Patient	12	15	3	30
	Control	3	15	10	28
	Total	15	30	13	58
Male	Patient	11	10	4	25
	Control	7	11	4	22
	Total	18	21	8	47

Environmental factor(s), specific to some groups of type 1diabetic patients, may alter the risk associated with particular SNPs in the VDR gene[25]. For instance, VDR functions together with 1α,25-dihydroxyvitamin D3, the level of which is dependent on various environmental factors. These factors include vitamin D intake in the diet or as a supplement and its synthesis from precursors in skin under ultraviolet light exposure[26]. Such environmental factors may modulate risk associated with the sequence variation in the VDR gene, e.g., certain variants may only be functionally important among a subpopulation of subjects with vitamin D insufficiency. While it is difficult to design a study to evaluate such potential interaction directly, it may manifest itself as regional or temporal heterogeneity in association between patients, who would have developed type 1 diabetes in different environments[27].

It has been shown that uncut alleles of the VDRB polymorphism increase receptor function and upregulate vitamin D-induced protein expression[28], over more strong LD was observed between the BsmI RFLP and the polyA variable number of tandem repeat (VNTR) in the 3'UTR. This latter polymorphism follows a bimodal distribution and that subjects can be classified as having alleles with short or long polyA stretches. Ingles et al. reported strong linkage between the ''b'' allele and a long polyA stretch and the ''B''allele and a short polyA stretch. There seems to be a trend for the haplotypes linked to short polyA VNTR alleles in the 3'UTR to display somewhat better responses than the haplotypes linked to long polyA VNTR alleles. It is tempting to speculate that perhaps this is due to a slightly better mRNA stability and half-life. This would theoretically result in higher numbers of VDR being present in the target cell and thus giving this target cell a better response to vitamin D [23].

Other publications also report association between gender and the consequences of VDR gene polymorphisms in T1D[20], in another study VDR polymorphisms were associated with sex-dependent growth [30]. However, the significance of this finding is hard to appreciate since the disease generally affects males and females equally[20], raising the question as to whether this finding is related to a specific feature of the selected population rather than a true association with the disease. Confirmation of the association with other genetic approaches, for example using family studies, would provide more convincing evidence.

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