Vitamin D receptor polymorphism and susceptibility to type 1 diabetes in chilean subjects: A case-parent study

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Abstract. Several reports have found a relation between polymorphisms of the vitamin D receptor gene (VDR) and the development of type 1 diabetes. We have examined the association of three VDR polymorphism with type 1 diabetes in 59 Chilean case-parents trios. Genotyping for Bsm1, Apa1 and Taq1 polymorphism were performed. Transmission/disequiibrium tests were used to assess gene-disease associations through the evaluation of allelic transmission to affected offspring. Non-significant increased transmissions of B allele (probability of transmission = 52.5%, $p = 0.69$), A allele (probability of transmission = 58.4%, $p = 0.17$) and T allele (probability of transmission = 52.0%, $p = 0.77$) were estimated in Bsm1, Apa1 and Taq1 sites, respectively. Haplotype-based analyses showed non-significant preferential transmissions (global $p = 0.52$). The present study does not support the hypothesis of a significant contribution of VDR alleles in the etiology of type 1 diabetes of Chilean cases.

Key words: Polymorphism, TDT analysis, Type 1 diabetes, VRD

Introduction

Type 1 diabetes is a chronic disease resulting from the autoimmune destruction of the insulin producing beta cells. More than 20 years ago, the HLA region was found to contain a major locus that influences predisposition to type 1 diabetes [1]. It is apparent that population frequencies of HLA alleles and haplotypes vary dramatically between ethnic groups. The incidence of the disease has large geographical variations, which might be caused by differences in frequencies of HLA or other genetic markers encoded genetic susceptibility factors among different populations [2].

The Chilean population is a mixed group with European genes (mainly from Spain) and Amerindian genes (mainly from the Mapuche population). The incidence of type 1 diabetes in Chile is low, but has been increasing during the last 10 years (2,49/100,000 in 1989 to 4,58/100,000 in 2000), which suggests that environmental factors are contributing to this increase [3].

Several genetic markers (different from HLA alleles) have been proposed as susceptibility factors in type 1 diabetes. Among them, vitamin D receptor (VDR) gene has been suggested to play a role in the pathogenesis of type 1 diabetes mellitus [4]. Several major polymorphic sites have been described within the VDR gene. Among them, polymorphic Bsm1 and Apa1 sites are present in intron 8, and a silent T to C substitution creates a Taq1 restriction site in exon 9 [5]. The meaning of this observation remains unclear and its relevance must be checked the allele and haplotype distribution in different population samples [6–8]. Inconsistent gene-disease results previously reported in association studies [6–8] have encouraged us to check the relevance of VDR polymorphisms in different populations. Therefore, We have investigated three polymorphic sites of VDR gene on chromosome 12q12–14 as candidate for type 1 diabetes susceptibility locus for the first time in Chilean population.

Patients and methodology

A total of 59 unrelated cases and their parents (177 individuals) were genotyped. Type 1 diabetes was diagnosed according to the World Health Organization criteria. Age at onset ranged from 1 to 14 years (24 girls and 35 boys) with a mean of 8.5 ± 3.2 years at the time of diagnosis. All patients and their parents provided written consent to their participation in the study which was approved by the local ethics committee. Genomic DNA was extracted from peripheral blood leucocytes using standard techniques. All type 1 diabetic patients and parents were genotyped using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP), for three restriction sites in

Table 1. Transmissión of vitamin D receptor alleles and haplotypes in 59 Chilean case-parent trios

	Bsm1 (b allele)		Apa1 (a allele)		Taq1 (t allele)	
Transmitted/ non-transmitted	28/59		27/65		23/48	
p	0.69		0.17		0.77	
Haplotypes	bat	bAt	BAt	bAT	BAT	Bat
p	0.50	0.26	0.61	0.42	0.85	0.45
p value (global)			0.52			

the VDR gene, Bsm1(B/b), Apa1(A/a) and Taq1(T/t) in concordance with previous protocols [6]. By sampling unrelated affected children as probands, associations between the VDR polymorphisms and disease would cause the probability of transmission to differ from the expected value under the hypothesis of no-association (probability of transmission = 50%). The transmission/disequilibrium test (TDT) was used to detect preferential transmission from heterozygous parents to affected offspring [9]. In addition, we performed haplotype-based TDT analyses implemented in the TRANSMIT program [10]. Empirical p-values for observed vs expected transmissions were obtained for each haplotype and for the whole set of haplotypes.

Results

We found non-significant increased transmissions of the B allele (probability of transmission = 52.5%, $p = 0.69$), A allele (probability of transmission = 58.4%, $p = 0.17$) and T allele (probability of transmission = 52.0%, $p = 0.77$). Haplotype-based analysis based on three RLFPs also showed non-significant preferential transmissions (global $p = 0.52$), "bat" haplotype ($p = 0.50$), "bAt" haplotype ($p = 0.26$), "BAt" haplotype ($p = 0.61$) and "BAT" haplotype (p = 0.85) (Table 1).

In accordiance with our results, it is not possible to split the population in ethnically homogeneous subpopulations in order to carry out separate association analysis. This is because Chilean population have been mixing since 14th century and it is very difficult or impossible to assign individuals to specific ethnic groups.

Discussion

The VDR locus has been associated in several studies with susceptibility to osteoporosis [11], primary hyperparathyroidism [12] and a considerable number of pathologies with autoimmune basis such as: Lupus [13], Addison's disease [14], Graves' disease [15], and Hashimoto's thyroiditis [16]. The role of VDR variants in type 1 diabetes has also been examined in several populations. RFLP analysis of VDR revealed that the BAt haplotype is relatively common in Caucasian population (16.7%) and rare in Japanese (1.4%) [17]. In 1997, McDermott et al. [18] reported a strong association between VDR alleles and type 1 diabetes in Indians Asians. In this latter study, the haplotype bAT conferred an increased risk in Indians while this combination seems to be protective in Germans [6]. In a recent study, Skrabic et al. [19], reported an association between the BAt VDR haplotype and type 1 diabetes in a case-control study conducted in Dalmatian population from South Croatia. These findings are concordant with the German study (case-parent design) regarding to the potential role of the BAt haplotype as risk factor to develop type 1 diabetes. In our study, we have not found any statistical evidences supporting an association between VDR alleles or haplotypes with type 1 diabetes. However, it is worthnoting that the bAt haplotype showed a non-significant preferential transmission from parents to affected child. The present family-based study has the advantage that avoids the confounding effect of population stratification by ethnicity [20]. Case-parents studies were specifically designed to avoid spurious association due to population stratification that may affect, for example, the case-control study. Consequently, population stratification is not a reason for explaining significant or non-significant results from case-parents studies.

Although we cannot completely exclude the possibility of a minor role of these or others VDR alleles, for example as recently described Fok1 polymorphism [21], in our type 1 diabetics children, the present study does not suggest a major contribution of these VDR alleles to the disease in Chilean patients.

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