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CREM variant rs17583959 conferred susceptibility to T1D risk in the Tunisian families

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ABSTRACT

Type 1 diabetes mellitus (T1D) is a chronic autoimmune disease caused by the destruction of insulin-producing pancreatic β-cells by autoreactive T cells. Studies in animal models, such as the non-obese diabetic (NOD) mouse reveal that this disease is under the control of several genes that encode molecules implicated in regulation of transcription factors and in T cell activation. In order to underline the role of the genes involved in this regulation pathways, we investigated, using the Sequenom MassARRAY platform, 13 single-nucleotide polymorphisms (SNPs) belonging to *CREM*, *IRF5*, *STAT4*, and *STAT5a/b* genes in 59 T1D Tunisian families.

In the current study, we identified an association with rs17583959 (allele G; Z score = 2.27; p = 0.02; Genotype GG: score = 1.96; p = 0.04) of CREM gene. In LD analysis a strong LD between the 3 CREM variants (Block 1) was detected; rs2384352 was in complete LD with rs1148247. When haplotypes were constructed between CREM polymorphisms (rs1148247, rs17583959, rs2384352), AGA haplotype (H2) was significantly over-transmitted from parents to affected offspring (Z score = 2.988; P = 0.002) and may confer a risk for T1D disease. Whereas, AAG haplotype (H5) (Z score = −2.000; p = 0.045) was less transmitted than expected to affected children suggesting its protective effect against T1D pathology. No significant association in *IRF5*, *STAT4*, and *STAT5a/b* genes were observed.

In conclusion, this study shows an eventually involvement of CREM gene in the development of T1D pathology in Tunisian families. These facts are consistent with a major role for transcription factor genes involved in the immune pathways in the control of autoimmunity. Further researches of association and functional analysis across populations are needed to confirm these findings.

1. Introduction

Type 1 diabetes (T1D) is a disease characterized by a T-cell-mediated autoimmune destruction of the insulin-secreting β-cells in the pancreas. T1D is multifactorial with strong genetic and environmental components [1]. In addition to the Human leukocyte antigen (*HLA*) class II alleles which accounts for up to 30%–50% of genetic T1D risk, multiple non-*HLA* loci contribute to disease risk: *INS*, *PTPN22*, *CTLA-4*, *IL-2RA* [1–3]. Most of the recently identified autoimmunity loci are shared among multiple autoimmune diseases (AIDs) [4]: the HLA region, the cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene in Graves' disease (GD), Hashimoto thyroiditis and type 1 diabetes (T1D) or the PTPN22 gene in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), GD and T1D [5–7]. The recent WTCCC GWA study reported several loci implicated in more than one disease. By grouping three auto-immune diseases (RA, CD and T1D), four regions on chromosomes 4, 10, 12 and 18 showed high association signals [8]. Mutations in genes implicated in regulation of transcription factors and inflammatory cytokines could lead to an immune dysregulation that predisposes to immune diseases.

These transcription factors play a pivotal role in both innate and adaptive immunity, and play an important role in the initiation and progression of AIDs [9]. In fact, among the diverse transcriptional factors whose alterations have been proven to be of relevance in the pathogenesis of AIDs, recent studies have established the impor-

Abbreviations: T1D, Type 1 diabetes; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium.

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tance of the cAMP-responsive elements members, such as c-AMP responsive element binding protein (CREB) and cAMP-responsive element modulator alpha (CREMα) [10,11]. CREMα acts as a transcriptional repressor and was proposed to be a key mechanism for decreased IL-2 production in SLE T cells [12–14]. CREMα can form homoduplexes or heteroduplexes with CREB and bind to the −180 site of the IL-2 promoter. This regulatory site is critical for IL-2 production because mutations of this site almost completely abolish IL-2 transcription [12,15].

On the other hand, IFN regulatory factors (IRF 5), one family member of type I interferon genes that encodes for a transcription factor have been reported to play an important role in the initiation and progression of AIDs [9]. *IRF5* is expressed in dendritic cells, monocytes and B cells and is involved in various activities, specifically the activation of proinflammatory cytokines such as tumor necrosis factor-α (*TNF-α*), interleukin *(IL)-6* and *IL-12* [16]. Indeed, the role of proinflammatory cytokines in the autoimmune destruction of pancreatic β-cells is well established [17].

Furthermore, the Signal Transducer and Activator of Transcription protein 4 (STAT4) plays pivotal roles in the differentiation and proliferation of both T helper 1 (Th1) and Th17 cells [18]. STAT5a/b have been reported to influence the development and maintenance of T regulatory (Treg) cells [19,20], and differentiation of Th17 cells [21]. The Th17 cells are the T helper cells which produce interleukin 17 (IL-17), an inflammatory cytokine implicated in various AIDs [22].

In view of the importance of *CREM, IRF5, STAT4* and *STAT5a/b* in the initiation or repression of several genes implicated in different immune pathways, we proposed to determine whether polymorphisms of these genes contribute to the development of T1D in 59 Tunisian families.

2. Subjects and methods

2.1. Study populations

A total of 255 individuals from 59 families (23 multiplex families and 36 simplex families), including 86 children with T1D (mean age, 12 ± 6.36 years with a range of 2–45 years) and 169 of their biological first degree relatives (mean age, 30 ± 10.60 years with a range of 3–57 years) were collected at the pediatric and endocrinology departments of Hedi Chaker University Hospital (Sfax, Tunisia). The inclusion criteria for the recruitment of T1D patients were the presence of diabetic ketosis at onset, dependence on insulin therapy for controlling hyperglycemia, and testing positive for at least one of the anti-islet auto-antibodies (ICA, IAA, GADA, IA2A). Patients with other forms of diabetes were excluded. All patients were notobese, were free of concomitant complications, and did not receive any additional medication. T1D patients and their first degree relatives are originated from the South of Tunisia and were asked to sign a consent form according to the study protocol, and all institutional ethics requirements were met.

2.2. Methods

2.2.1. DNA extraction and genotyping

Genomic DNA was extracted from whole blood samples using a standard salting-out and phenol-chloroform methods according to a previously described protocol [23]. Genotyping was performed using the Sequenom MassARRAY platform according to manufacturer's instructions (iPlex assay®, Sequenom, San Diego, CA) at the Instituto Gulbenkian de Ciência (Oeiras, Portugal). Thirteen variants from five genes were investigated: *CREM* (rs1148247, rs17583959 and rs2384352), *IRF5* (rs2004640, rs2070197, rs729302, rs2280714, rs752637 and rs10954213), *STAT4* (rs7574865), *STAT5a* (rs3198502) and *STAT5b* (rs16967620 and rs4029774). The selected reference SNPs were retrieved from the HapMap database and the mapping information obtained from the db SNP built 126 found at http://www.ensembl.org (Table 1). Genotypes of all SNPs were determined using our previously described protocol [24] and we excluded all SNPs with call rate lower than 85% in all samples.

Table 1
Investigated polymorphisms in the candidate genes.

Gene/SNP	Chromosome	Position	Allele	HWE
CREM/rs1148247	10	35496946	G/A	0.97
CREM/rs17583959	10	35422103	G/A	0.75
CREM/rs2384352	10	35492832	A/G	0.74
IRF5/rs2004640	7	128578301	G/T	0.92
IRF5/rs2070197	7	128589000	C/T	0.74
IRF5/rs2280714	7	128594725	C/T	0.45
IRF5/rs729302	7	128568960	A/C	0.81
IRF5/rs752637	7	128579420	A/G	0.97
IRF5/rs10954213	7	128589427	A/G	0.44
STAT5b/rs16967620	17	40422341	G/T	0.67
STAT5b/rs4029774	17	40428961	A/G	0.87
STAT5a/rs3198502	17	40462994	G/T	0.99
STAT4/rs7574865	2	191964633	G/T	0.54

A total of 16 SNPs studied were retrieved from the HapMap database and the mapping information obtained from the db SNP built 126 found at http://www.ensembl.org.

2.2.2. Data analysis

The family-based association test (FBAT) was performed with FBAT program v2.0.4. This framework uses generalized score statistics to perform a variety of transmission disequilibrium tests (TDT), including haplotype analyses. Moreover, the FBAT program provides estimates of haplotype frequencies. Unaffected members of the pedigrees were included in the study, their genotype information contributing to increase statistical power of the FBAT analysis, especially for families with missing parents. The allele/genotype and haplotype transmission tests were conducted under "biallelic" mode. For all statistical tests, differences were considered to be statistically significant if the p-value was ≤0.05. FBAT were performed under an additive model in the present study. The pairwise linkage disequilibrium (LD) analysis was applied to detect the inter-marker relationship with Haploview program version 4.2 using D' values. The Hardy–Weinberg equilibrium (HWE) was checked in healthy parents prior to analysis.

3. Results

3.1. CREM

From all 3 variants spanning CREM, the risk allele G of rs17583959 showed evidence for over-transmission from parents to their T1D offspring (p = 0.02; Z = 2.27), while the A allele was under-transmitted than expected (p = 0.02; Z = −2.27) (Table 2). In the genotype analysis, the homozygous genotype GG was transmitted more frequently to affect children's than what would be expected by chance (p = 0.04; Z = 1.96) and conferred disease association for T1D (Table 2).

On the other hand, one block (Block 1) was detected in LD analysis across 3 CREM variants; rs2384352 was in complete LD with rs1148247 (Fig. 1). FBAT-based haplotypic analysis including the 3 SNPs (Block 1), showed that AGA haplotype (H2) was significantly over-transmitted from parents to affected offspring (p = 0.002; Z = 2.988). Whereas, AAG haplotype (H5) was less transmitted than expected to affected children, suggesting it's protective effect against T1D (p = 0.045; Z = −2.000) (Table 3).