Interleukin 18 (IL18) gene promoter polymorphisms are associated with type 1 diabetes mellitus in Brazilian patients

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A B S T R A C T

Interleukin 18 (IL-18) is a cytokine that plays an important role in the Th1 response, by its ability to induce IFN-γ production in T cells and natural killer cells. Functional variants of IL18 gene have been reported as associated with type 1 diabetes (T1D). In the present study were analyzed three promoter single nucleotide polymorphisms (SNPs), at −656 (rs1946519), −607 (rs1946518) and −137 (rs187238) position, in 181 children and adolescents with T1D and 122 healthy individuals, both from metropolitan area of Recife, Northeast of Brazil. T1D patients were stratified according to the presence autoimmune thyroiditis and celiac disease. Allele and genotype frequencies of IL18 SNPs were Hardy–Weinberg equilibrium in patients and controls. The allele −137G and the haplotype −656G/−607C/−137G were more frequent in T1D patients (OR = 1.82 and 1.97, respectively) then in healthy controls. However, those SNPs were not associated with the age of T1D onset as well as with the insurgence of AITD and/or CD in concomitant with T1D patients. Our findings suggest an association between IL18 promoter SNPs and susceptibility to T1D in Brazilian patients.

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1. Introduction

Type 1 diabetes mellitus (T1D) is a complex multifactorial disorder where pancreatic insulin producer beta cells are immunologically destroyed through a genetic and environmental interplay [1]. Moreover, during the chronic disease evolution of T1D, some patients could develop other organ-specific autoimmune events including the insurgence of autoimmune thyroiditis (AITD) and/or celiac disease (CD) [2–4], suggesting a shared pathogenesis pathway for these disorders.

The inflammatory pathway has an important role in the development and complications of T1D [5]. In inflammations events, several interleukins (ILs) could be produced in order to activate specific immune components and present itself as a potential factor in association to T1D pathogenesis [6–8].

The pro-inflammatory interleukin 18 (IL-18) is directly involved in the innate and specific immune response, due to its ability to activate monocytes, macrophages, natural killer (NK) and in synergy with IL-12, to promote Th1 type response [8,9].

Single nucleotide polymorphisms (SNPs) located at promoter position −607 (dbSNP: rs1946518) and −137 (dbSNP: rs187238) of IL18 gene (11q22.2–q23.3), involved in the decrease of IL18 expression [10–12], have been studied and associated with the development of T1D [11–13]. However, divergent results of association have been found in different populations, being necessary replications studies in population with different genetic background.

The aim of this work is to investigate the association between IL18 promoter polymorphisms, namely-656 (dbSNP: rs1946519), −607 (rs1946518) and −137 (rs187238) and T1D in a population from Northeast of Brazil. This genetic association was never performed in an admixture population, such the Brazilian one, additionally, we hypothesize that patient carrying the IL18 SNPs possess a lower risk to the insurgence of AITD and/or CD in concomitance with T1D, since low levels of IL-18 could diminish the Th1 response and the production of INF-γ.

2. Materials and methods

2.1. Patients

T1D patients (n = 181) were enrolled and followed up at the three major pediatric endocrinology centers of health public...
service from Recife, Brazil (Instituto de Medicina Integral Professor Fernando Figueira, Hospital da Restauração and Hospital das Clínicas – UFPE). The diagnosis of T1D followed the criteria described by the American Diabetes Association [14]. The mean age of T1D patients was 13.2 years (SD ± 4.1), with 101/181 (56%) females, a gender ratio equals to 1.26:1. The average age at the onset of T1D was 7.3 years (SD ± 3.4) and the mean duration of the disease was 5.9 years (SD ± 3.8).

2.2. Autoimmune thyroid and celiac disease diagnosis

Antibodies to thyroperoxidase (Anti-TPO) diagnosis were performed by chemio-luminescence (Innulite anti-TPO Ab, Diagnostic products Co., Los Angeles, USA). Patients with positive anti-TPO (titer exceeding 35 IU/ml, accordingly to manufacturer’s suggestion) were considered as having AITD. Anti-transglutaminase antibodies (anti-tTG) were determined by using the ELISA Eu-tTG kit (Eurospital, Trieste, Italy) following manufactures instructions. Patients presenting 10 AU (absorbance units) for anti-tTG antibodies were considered positive and subsequently screened for the presence of HLA DQ2 and/or DQ8 HLA haplotypes by using the Eu-DQ kit (Eurospital, Trieste, Italy). After gluten-free diet in all CD patients, the tTG autoantibodies levels declined, remaining under the cutoff value of 7 AU.

The frequency of AITD in the T1D patients (T1D + AIDT + DC+) was 20.9% (38/181), the percentage of patients with CD (T1D + AIDT + DC+) was 5.5% (10/181), while patients characterized by both AITD and CD (T1D + AIDT + DC+) were 3.3% (6/181).

2.3. Healthy subjects

Healthy individuals from the same geographical region of patients group with no clinical evidence or family history of autoimmune diseases were included as control group. We enrolled 142 healthy subjects, mean age 21.4 years (SD ± 2.4), 93/142 (65%) females, a gender ratio 1.89:1. In the choice of the control population, the presence of any HLA possibly associated with celiac disease, AIDT, T1D and a titer exceeding 35 IU/ml for anti-TPO, 10 AU for anti-tTG and 40 nU/ml for anti-IAA were an exclusion criterion.

A free and informed consent from each person responsible for the patient and healthy individuals were obtained. The local ethical committee (IMIP number 1717/2010) approved the study.

2.4. DNA extraction

Genomic DNA was extracted from peripheral whole blood using the Wizard genomic DNA purification kit (Promega, Madison, MA) according to the standard laboratory protocols.

2.5. SNP genotyping and haplotypes reconstruction

The SNPs −656 C/A (rs1946519), −607 G/T (rs1946518) and −137 G/C (rs187238) located at promoter region of IL18 gene were genotyped using fluorescent allele-specific probes (TaqMan® , Life) with ABI-7500 Real-Time as platform. Linkage disequilibrium and haplotypes were computed using the Arlequin software (version 3.1) and SNPsstats (http://bioinfo.iconcologia.net/SNPsstats).

2.6. Statistical analysis

Allele and genotype frequencies of IL18 promoter SNPs were obtained by direct counting. Fisher’s exact test was used to correlate SNPs distribution and the increased susceptibility, clinical aspects of T1D and Hardy–Weinberg equilibrium were obtained using the R program (2005; version 2.1.1; http://www.r-project.org/).

Kruskal Wallis test was used in the age at diagnoses analyses. The power test analysis was performed through the G-Power (version 3.1). Odds Ratio (OR) and 95% Confidence Intervals (CI) were also calculated. The p-values < 0.0015 was considered as statistically significant after Bonferroni correction for multiple tests.

3. Results

In this work we analyzed three functional polymorphisms, −656 C/A, −607 G/T and −137 G/C, at promoter region of the IL18 gene in 181 T1D patients and 122 healthy subjects from the metropolitan region of Recife (Brazil). SNPs allele and genotype distribution are listed in Table 1. Genotype and allele SNPs frequencies in the studied groups were in Hardy–Weinberg equilibrium.

Thirty-three analyses were done and six statistical differences were found. However, after Bonferroni Multiple Test correction only two associations for the −137 SNP remained statistically significant (p < 0.0015). The −137G allele was less frequent in healthy subjects than in T1D + AITD + CD− (OR = 1.82, p = 0.0015; 95%CI = 1.24–2.69) and when compared with all T1D + patients (p = 0.0001; OR = 1.96 95%CI = 1.37–2.80). In addition, the −137G/G genotype was also statistically less frequent in healthy individuals than in patients T1D + AITD + CD− (p = 0.0005 OR = 4.55 95%CI = 1.77–12.9) and in T1D+ (p = 0.00004 OR = 5.00 95%CI = 2.13–12.4).

No association was found with the IL18 promoter polymorphism and the age at diagnosis of T1D.

Eight haplotypes were found in our population as showed in Table 2. The haplotypes −656G/−607C/−137G, −656T/−607A/−137C and −656T/−607A/−137G represent more than 80% of all haplotypes in the studied populations, the other five ones were rare. All SNPs were in linkage disequilibrium (D' > 0.8) in all groups, excluding the −656 and −137 SNPs in the T1D + AITD + DC + and healthy groups (D' = 0.6).

The haplotype −656G/−607C/−137G and −656T/−607A/−137C were differentially distributed in case subjects when compared with healthy individuals (p < 0.000007).

4. Discussions

T1D is an autoimmune disorder resulted by initial infiltration of Th1 and Th2 lymphocytes in pancreatic islet followed by progressive destruction of beta-cells [15,16]. The predominance of Th1 response is widely associated with T1D onset due to a higher production of interferon-γ (INF-γ) [17]. However, genetic studies on INF-γ listed some rare variants with low phenotypic impact [18], suggesting other genetic approaches on the regulation of INF-γ production by the IL-18 pathway in synergy with IL-12. In fact, IL-18 was originally named as an INF-γ-inducing factor with a correlation between genetic SNP at promoter site of IL18 and INF-γ production and T1D development [10].

The −607A and −137 SNPs are known to down-regulate the gene expression of IL18 at the transcriptional level by disrupting the CAMP-responsive element-binding site and by changing the H4TF-1 nuclear factor binding at IL18 promoter [10]. Both events could influence, at least in part, the INF-γ production.

In the present report, the allele −137G and genotype −137GG were associated with the susceptibility to T1D. In addition, the haplotype −656G/−607C/−137G was more frequent in case subjects. Both results supported the hypothesis that lower levels of IL-18 are protective to T1D onset. Several findings have been supporting that increased levels of IL-18 are associated with the susceptibility to T1D. The plasma levels of IL-18 in T1D patients are increased in subclinical stage and involved with disease
As well, no association was found with the use of several autoimmune disorders, reported no correlation with inflammatory pathway triggered by IL-18 are not shared in these disorders. Moreover, a recent meta-analysis study of the SNP IL18 promoter SNPs with the susceptibility to T1D [21–24].

In summary, our results showed an association of the −137G SNP at promoter of IL18 with the susceptibility to develop T1D in Brazilian patients. Brazilian individuals from Northeast who carry this allele are more than 1.8 times more susceptible to T1D onset (OR = 1.82 95%CI = 1.24–2.69) and almost 2.0 times more if they carry the haplotype −656G/−607A/−137G (OR = 1.97 IC95% = 1.31–2.97). However, those SNPs are not involved with the age at T1D diagnose and the insurgen ce of AITD and/or CD in concomitan t patients. It is important to note that after T1D patients' progression [17,19]. Moreover, NOD mice with IL-18 deficiency are protected of T1D progression, since they exhibit reduced T cell turnover and fewer effectors T cell [11,20].

The pathogenesis of autoimmune diabetes is complex and not influenced only by genetic profile, but environmental factors could play an important role. As Dong et al. [12] observed the genetic pool diversity and different environmental conditions might contribute to achieve opposite results in different populations of IL18 association.

Table 1

<table>
<thead>
<tr>
<th>IL18 polymorphism</th>
<th>T1D + AITD + CD+</th>
<th>Healthy</th>
<th>T1D + AITD + CD−</th>
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<tbody>
<tr>
<td>−656 G</td>
<td>8 (67)</td>
<td>214 (59)</td>
<td>13 (65)</td>
<td>153 (60)</td>
</tr>
<tr>
<td>T</td>
<td>4 (33)</td>
<td>148 (41)</td>
<td>7 (35)</td>
<td>101 (40)</td>
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<tr>
<td>G/G</td>
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<td>9 (33)</td>
<td>5 (30)</td>
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<tr>
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<td>2 (30)</td>
<td>69 (54)</td>
</tr>
<tr>
<td>T/T</td>
<td>1 (17)</td>
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Table 2

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Associations according to SNP position:

Comparison | Associated allele | Associated genotype
--- | --- | ---
T1D + AITD + CD− vs healthy | T: p = 0.00 OR = 0.66 95%CI = 0.45–0.95 | T: p = 0.00 OR = 0.66 95%CI = 0.45–0.95
T1D + AITD + CD− vs healthy | T: p = 0.03 OR = 0.69 95%CI = 0.49–0.97 | T: p = 0.03 OR = 0.69 95%CI = 0.49–0.97
T1D + AITD + CD− vs healthy | G: p = 0.01 OR = 2.04 95%CI = 1.13–3.78 | G: p = 0.01 OR = 2.04 95%CI = 1.13–3.78
T1D + AITD + CD− vs healthy | G: p = 0.0015 OR = 2.42 95%CI = 1.24–2.69 | G: p = 0.0015 OR = 2.42 95%CI = 1.24–2.69
T1D + AITD + CD− vs healthy | G: p = 0.00012 OR = 1.96 95%CI = 1.37–2.80 | G: p = 0.00012 OR = 1.96 95%CI = 1.37–2.80

Haplotypes | T1D + AITD + CD+ | Healthy |
--- | --- | ---
-656G/−607C/−137G | 7 (58.4) | 95 (38.9)
-656T/−607A/−137C | 3 (25) | 84 (34.4)
-656T/−607A/−137G | 1 (8.3) | 57 (24.1)
Rare haplotypes | 1 (8.3) | 19 (8.3)

Associations found:

Comparison | Haplotype
--- | ---
T1D + AITD + CD− vs healthy | −656G/−607A/−137G OR = 1
T1D + AITD + CD− vs healthy | −656G/−607C/−137G OR = 0.01 OR = 5.70 IC95% = 2.33–15.35
T1D + AITD + CD− vs healthy | −656G/−607A/−137G OR = 0.001 OR = 2.02 IC95% = 1.3–3.15
T1D + AITD + CD− vs healthy | −656G/−607A/−137G OR = 0.0005 OR = 1.97 IC95% = 1.31–2.97
ple size in order to expand the knowledge of IL18 variations on T1D development and the insurgence of others autoimmune disorders.

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