Brief Genetics Report

Interleukin-18 Promoter Polymorphisms in Type 1 Diabetes

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Type 1 diabetes is believed to be a Th1 lymphocyte–mediated disease, and both environmental and genetic factors play a role in its pathogenesis. It was recently found that interleukin (IL)-18 acts as a proinflammatory cytokine and, in synergy with IL-12, promotes development of Th1 lymphocyte response by induction of γ-interferon production. The aim of our study was to evaluate the frequency of known polymorphisms in the IL-18 promoter in patients with type 1 diabetes in comparison with healthy control subjects, since higher levels of IL-18 were recently reported in the subclinical stage of type 1 diabetes. We studied two recently described single-nucleotide polymorphisms of the promoter of IL-18 gene at the position −137 and −607, which have been suggested to cause differences in transcription factor binding and have an impact on IL-18 gene activity. The genotype distribution differed significantly between patients with type 1 diabetes and control subjects. The difference reflected an increase in the GC genotypes and a decrease in GG genotypes at position −137 in the promoter of IL-18 gene. AA genotype at position −607 was found only in the control group. The results also demonstrated that the contribution of −137GC genotypes to genetic susceptibility to type 1 diabetes differs depending on the combination of IL-18 promotor gene haplotypes. Our study suggests the first evidence of an association between type 1 diabetes and polymorphisms in the promoter of IL-18 gene. *Diabetes* 51:3347–3349, 2002

It was recently reported by Nicoletti et al. (1) that interleukin (IL)-18 serum levels are increased in the subclinical stage of type 1 diabetes in first-degree relatives of type 1 diabetic patients.

IL-18, which is predominantly secreted by activated monocytes/macrophages, is a pleiotropic cytokine involved in the regulation of innate and acquired immune response, playing a key role in autoimmune, inflammatory, and infectious diseases (2). IL-18 acts as a proinflammatory factor and, in synergy with IL-12, promotes development of Th1 lymphocyte response by induction of γ-interferon (IFN-γ) production, modulates activity of NK cells, increases tumor necrosis factor-α and IL-1 production by macrophages, upregulates the expression of adhesion molecules, and induces nitric oxide production in the area of chronic inflammation (2,3).

The role of IL-18 in the animal model of autoimmune diabetes was first reported by Rothe and colleagues (4,5), who found that increased IL-18 mRNA production by macrophages followed by increased IFN-γ levels is associated with an active stage of autoimmune diabetes in NOD mice. It was recently shown that in the course of insulitis, IL-18 is also produced by pancreatic B-cells, which induce the exacerbation of inflammation (6,7). On the other hand, it was found that systemic administration of exogenous IL-18 to NOD mice suppresses the development of diabetes, probably due to downregulation of the proinflammmatory activities of the innate immune system (8).

Type 1 diabetes in humans is also believed to be a Th1 lymphocyte–mediated disease, and both environmental and genetic factors play a role in its pathogenesis (9–11). To our knowledge, there have been no published studies that have estimated the role of IL-18 promoter polymorphisms in the predisposition to type 1 diabetes in humans. However, IL-18 gene locus on chromosome 11q22.2–q23.3 has not been mapped by whole-gene scan studies as a region conferring major susceptibility to type 1 diabetes; therefore, we decided to study its role as a candidate for type 1 susceptibility gene, since the genetic association between IL-18 and destructive insulitis has been suggested in the animal model of autoimmune diabetes (5,12,13). In the NOD mouse, which spontaneously develops autoimmune diabetes, IL-18 gene position is located within the Idd2 interval on mouse chromosone 9 and has been suggested as a candidate gene for the Idd2 susceptibility gene (4). Moreover, it was recently shown that IL-18 acts in synergy with IL-12 in enhancing IFN-γ mRNA transcription and also that IL12p40 gene locus is associated with type 1 diabetes (IDDM18) (3,14).

The aim of our study was to evaluate the frequency of known polymorphisms in the IL-18 promoter in patients with type 1 diabetes in comparison with healthy control subjects. We studied two recently described single-nucleotide polymorphisms of the promoter of IL-18 gene at the position −137 and −607, which were suggested to cause

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IFN-γ, γ-interferon; IL, interleukin.

TABLE 1
Frequency of alleles and distribution of genotypes of IL-18 promoter polymorphisms in type 1 diabetic and control subjects

Loci		Alleles: Type 1 diabetes	Alleles: Control subjects	*P*		Genotypes: Type 1 diabetes	Genotypes: Control subjects	*P*
n		402	388			201	194	
	G	251 (62.4)	282 (72.7)		GG	63 (31.3)	94 (48.5)	
−137				0.002	GC	125 (62.2)	94 (48.5)	0.0015
	C	151 (37.6)	106 (27.3)		CC	13 (6.5)	6 (3.0)	
	C	273 (67.9)	256 (66.0)		CC	72 (35.8)	73 (37.6)	
−607				0.564	CA	129 (64.2)	110 (56.7)	0.001
	A	129 (32.1)	132 (34.0)		AA	0 (0)	11 (5.7)	

Data are *n* (%) unless otherwise indicated.

the differences in transcription factor binding and have an impact on IL-18 gene activity. A change at position −137 from G to C changes the H4TF-1 nuclear factor binding site and a change from C to A at position −607 disrupts a potential cAMP-responsive element–binding protein binding site (15).

Table 1 shows the distribution of alleles and genotypes in the promoter of IL-18 gene in type 1 diabetic and healthy control subjects. The genotype distribution differed significantly between patients with type 1 diabetes and control subjects. The difference reflected an increase in the GC genotypes and a decrease in GG genotypes at position −137 in the promoter of IL-18 gene. An increase in the frequency of the C allele at −137 locus was also noted. Moreover, AA genotype at position −607 was found in the control group only (Table 1).

The results demonstrated that the contribution of −137GC genotypes to genetic susceptibility to type 1 diabetes differs depending on the combination of IL-18 promotor gene haplotypes (Table 2). Although −137GC/−607AC and −137CC/−607AC haplotypes are positively associated with type 1 diabetes, the combination of GC or CC genotypes at position −137 with protective genotype AA at position −607 resulted in a neutral association of −137CC/−607AA or −137GC/−607AA haplotypes with type 1 diabetes. These data suggest the interaction of the −137GC genotype with −607AA genotype in conferring susceptibility to type 1 diabetes.

Our results suggest the first evidence for the association between type 1 diabetes and polymorphisms in the promoter of IL-18 gene. To our knowledge, there has only been one study published concerning the role of the promoter of IL-18 in other autoimmune disease mediated by Th1-derived cytokines (15). Giedriatis et al. (15) speculated that there may be a possible link between G→C polymorphism at position −137 of the promoter of IL-18 gene and the development of multiple sclerosis, but the higher frequency of allele C at position −137 observed in their study in subjects with sclerosis multiplex did not achieve statistical significance. However, they have shown that polymorphism at positions −137 and −607 in the promoter region of IL-18 gene appears to be functional, with increased transcriptional activity attributed to the variant alleles.

Taking into consideration our findings and those of Giedriatis et al., one could suggest that the increased levels of IL-18 in the preclinical stage of type 1 diabetes, in comparison to the healthy control subjects found previously by Nicoletti et al. (1), are the result of a genetic predisposition for the upregulated expression of the promoter of IL-18 gene, resulting in Th1-directed immune response rather than only the secondary result of monocyte/macrophage activation during the autoimmunity.

In summary, we have shown for the first time that the presence of the C allele at position −137 of the IL-18 promoter could have a role in the predisposition to type 1 diabetes. Moreover, our study suggests that in the Polish population, subjects carrying AA genotype at position −607 of the promoter of IL-18 gene have a low risk of type 1 diabetes development.

TABLE 2
Frequency of haplotypes of IL-18 promoter polymorphisms in type 1 diabetic and control subjects

Haplotypes	Type 1 diabetes	Control subjects	*P*	*Pc*
n	201	194		
−137CC/−607AC	13 (6.5)	1 (0.5)	0.0016	0.014
−137CC/−607AA	0 (0.0)	5 (2.6)	0.0278	NS
−137CC/−607CC	0 (0.0)	0 (0.0)	—	—
−137GC/−607AC	101 (50.3)	86 (44.3)	NS	NS
−137GC/−607AA	0 (0.0)	6 (3.1)	0.0135	NS
−137GC/−607CC	24 (11.9)	2 (1.0)	0.0000	0.0000
−137GG/−607AC	15 (7.4)	23 (11.9)	NS	NS
−137GG/−607AA	0 (0.0)	0 (0.0)	—	—
−137GG/−607CC	48 (23.9)	71 (36.6)	0.0062	0.056

Data are *n* (%) unless otherwise indicated.

RESEARCH DESIGN AND METHODS

The study was carried out in 201 type 1 diabetic patients from the Bialystok region of Poland (96 women and 105 men, aged 23.2 ± 12.6 years). Subjects were selected on the basis of the data from the prospective register of new cases of type 1 diabetes in the Bialystok region, which was established in 1994 as part of the EURODIAB TIGER program (16). Between January and April 2002, the patients were invited to the Department of Endocrinology, Diabetology and Internal Medicine, Medical Academy of Bialystok for blood collection. Diagnosis of type 1 diabetes was made according to the criteria defined by the World Health Organization in 1985, the presence of ketosis, low BMI, and the need for insulin therapy (mean age of diabetes diagnosis was 11.4 ± 5.8 years). The control group consisted of a sample of 194 unrelated healthy volunteer subjects from the medical staff of our hospital and medical students living in the Bialystok region (mean age 24.2 ± 6.3 years), who had no family history of diabetes or other autoimmune diseases. All patients and control subjects were informed of the purpose of the study, and their consent were obtained.

DNA was extracted from peripheral blood leukocytes, and polymorphisms were detected by using PCR sequence-specific primers, in the position −607 and −137 in the promoter of IL-18 gene. For the position −607 forward primers, two sequence-specific primers (5′-GTTGCAGAAAGTGTAAAAATTATTAC-3′ or 5′-GTTGCAGAAAGTGTAAAAATTATTAA-3′), a control primer (5′-CTTTGCTATCATTCCAGGAA-3′), and a common reverse primer (5′-TA ACCTCATTCAGGACTTCC-3′) were used. For the position −137 forward

primers, two sequence-specific primers (5′-CCCCAACTTTTACGGAA GAAAAG-3′ or 5′-CCCCAACTTTTACGGAAGAAAAC-3′), a control primer (5′-CCAATAGGACTGATTATTCCGCA-3′), and a common reverse primer (5′ AGGAGGGCAAAATGCACTGG-3′) were used. Control primers were used to amplify fragments covering the polymorphic sites as an internal positive amplification control. Reactions were carried out in a MJ Research PTC-200 thermal cycler. Products were visualized by 2% agarose gel electrophoresis stained by ethidium bromide. To confirm our results, PCR products of A and C homozygotes in locus −607 and C and G homozygotes in locus −137 were sequenced with the same control primers used for amplification on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Stafford, TX). χ^2 test or Fisher's exact probability test were used to estimate the differences in the distribution of alleles, genotypes, and haplotypes between the studied groups (SAS/STAT version 6.12; SAS, Cary, NC and Statistica 5.5; StatSoft, Tulsa, OK). *P* values were corrected for the number of different haplotypes tested (*Pc*). Statistical significance was defined as $P < 0.05$.

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