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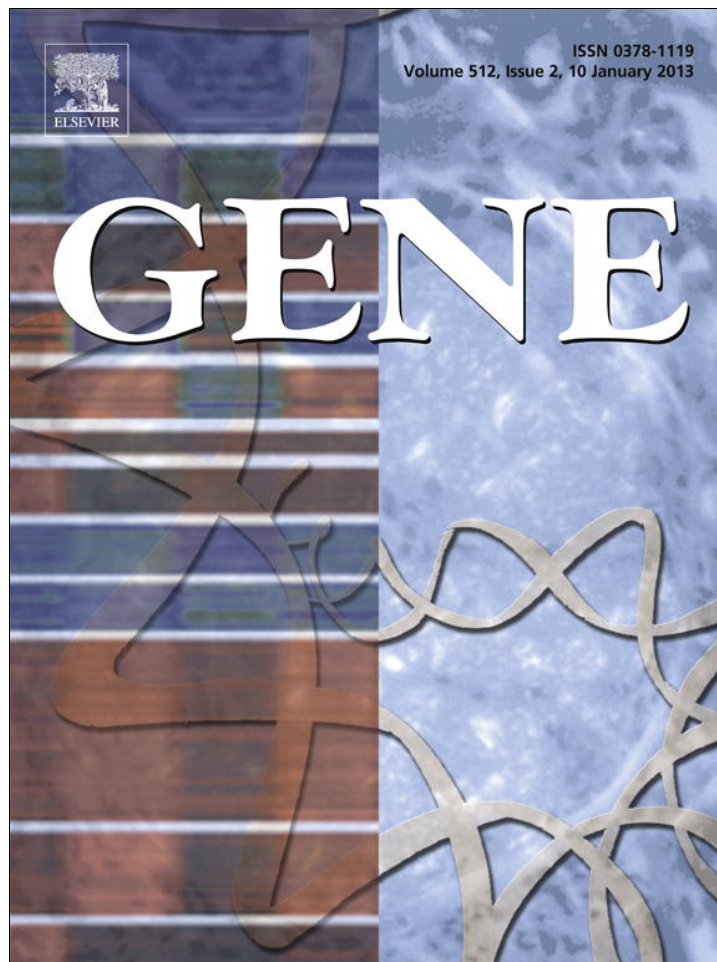

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Short Communication

Polymorphisms in the *IL-18* and *IL-12B* genes and their association with the clinical outcome in Croatian patients with Type 1 diabetes ☆

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ABSTRACT

Genetic variants of *IL-18* and *IL-12B* may be important in immunoregulatory abnormalities, observed in the patients with Type 1 diabetes mellitus (T1DM), that contribute to individual differences in response to a treatment. Therefore, we examined the significance of *IL-18*-137G/C, *IL-18*-607C/A, and *IL-12B* A/C polymorphisms in Croatians (187 patients, 236 controls), not only as factors that contribute to susceptibility to T1DM, but also as determinants of the clinical presentation of disease.

The polymorphism screening has been performed using PCR sequence-specific primers (*IL-18*) or PCR-RFLP (*IL-12B*) approach. Results were evaluated by GraphPad Prism and Sigma Stat 3.5, Arlequin software and calculator for Hardy–Weinberg equilibrium.

The genotype, allele and haplotype distribution were not statistically different between the patients and control subjects. The clinical parameter analysis revealed that patients with minor alleles at each locus, *IL-18*-137C/-607A, were significantly younger at T1DM onset than carriers of major alleles, *IL-18*-137G/-607C (20 vs 23.5 years). Moreover, the concomitant presence of minor alleles not only of *IL-18* but also of *IL-12B*, is associated with the risk of disease progression even at younger age. These patients developed diabetes at 16 years of age, what is significantly earlier ($p=0.044$) compared to 25.5 years of age in patients with common alleles *IL-18*-137G/-607C/*IL-12B* A. Furthermore, combined genotype analysis of *IL-18* and *IL-12B* has pointed out that patients with CC/AA/AA genotype have the worst glucose control based on HbA1c (8.7%, range 6.8–13.1%).

In conclusion, susceptibility to T1DM in Croatians is not strongly associated with *IL-18*-137/-607 and *IL-12B* polymorphisms. These SNPs are associated with the higher risk of earlier disease development and might be implicated in the effectiveness of glycemic control.

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1. Introduction

Type 1 diabetes (T1DM) or insulin dependent diabetes mellitus is a Th1 lymphocyte-mediated disease, and both genetic and environmental factors play a role in its pathogenesis.

Development of T1DM begins as peripheral Th2 type insulitis and progresses to destructive Th1 type insulitis which is driven by the innate immune system. Among numerous cytokines, interleukin-18 (IL-18) is a unique cytokine that enhances both Th1 and Th2 derived immune responses (Nakanishi et al., 2001). IL-18 contributes to the pathogenesis of T1DM acting as a proinflammatory cytokine and, in synergy with interleukin-12 (IL-12), promotes development of Th1 lymphocyte response by induction of gamma-interferon (IFN-γ) production, modulates activity of NK cells, increases TNF-alpha (TNF-α) and IL-1 production by macrophages, upregulates the expression of adhesion molecules, and induces nitric oxide production in the area of chronic inflammation (McInnes et al., 2000; Nakahira et al., 2002). Rise in IL-18 precedes development of the acute phase of autoimmune diseases like diabetes (Rothe et al., 1997), Crohn's disease (Monteleone et al., 1999) and multiple sclerosis (Balashov et al., 1999). Increased level of the cytokine correlates with the severity of autoimmune pathologies in experimental models of autoimmunity

Abbreviations: EDTA, ethylenediaminetetraacetic acid; CI, confidence intervals; HbA1c, glycated hemoglobin; IFN-γ, interferon-gamma; IL-1, interleukin-1; IL-12, interleukin-12; IL-18, interleukin-18; HWE, Hardy–Weinberg equilibrium; OGTT, oral glucose tolerance test; OR, odds ratio; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; T1DM, type 1 diabetes mellitus; TNF-α, tumor necrosis factor-alpha; WHO, World Health Organization.

☆ Conflict of interest: The authors declare that they have no conflict of interest.

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and also in clinical situations (Esfandiari et al., 2001). Moreover, IL-18 deficient mice are significantly more resistant to the induction of diabetes and do not develop the typical mononuclear cell infiltration of the islets (Lukic et al., 2003).

Two functional SNPs within *IL-18* promoter affecting transcription factor binding affinity have been identified. Minor alleles at the positions -137C and -607A increase transcriptional activity of the promoter upon binding its transcription factor (H4TF-1 nuclear factor and cAMP-responsive element-binding protein, respectively) (Giedraitis et al., 2001).

It has been shown that IL-18 acts in synergy with IL-12 in enhancing IFN-γ mRNA transcription and that IL-12 upregulates IL-18 receptor expression on target cells, T lymphocytes and NK cells (Morahan et al., 2001; Takeda et al., 1998; Yoshimoto et al., 1998). IL-12 drives the differentiation of lymphocytes towards autoreactive T cell responses, thus increasing susceptibility to autoimmunity. Moreover, IL-12p40 polypeptide subunit (coded by *IL-12B* gene) is expressed by activated macrophages and B cells, and its expression may be upregulated by IFN-γ (Huang et al., 2000). Several polymorphisms have been identified within *IL-12B* gene. SNP rs321227 (A/C) showed strong association with T1DM susceptibility in some, but not all studies (Morahan et al., 2001; Nerup and Pociot, 2001). Given the importance of the *IL-18* and the *IL-12B* in the immune system, genetic variants of these genes may be important in immunoregulatory abnormalities that occur during autoimmune diabetes. These variants might therefore influence individual response of T1DM patients to treatment recommended by the World Health Organization (WHO).

In this work we examined the significance of *IL-18*-137G/C (rs187238), *IL-18*-607C/A (rs1946518) and *IL-12B* A/C (rs321227) polymorphisms, not only in susceptibility to T1DM in Croatian population, but also in determination of clinical parameters (the age at onset and glycated hemoglobin). Since the two analyzed genes cooperate, we also examined their potential synergistic effect.

2. Material and methods

2.1. Subjects

A total of 423 subjects were enrolled in this study (Table 1). Unrelated patients with T1DM (N = 187) were recruited from the University Clinic Vuk Vrhovac, Zagreb, in the period between 2010 and 2011. The University Clinic is the WHO collaborating center for diabetes and diagnosis was based on fasting plasma glucose levels, 2-hour plasma glucose during an oral glucose tolerance test (OGTT), insulin and C-peptide profile, and the presence of GAD65 and IC auto-antibodies. All patients required insulin for glycemic control. Glycated hemoglobin (HbA1c) was measured at 3-month intervals by ion-exchange high-performance liquid chromatography (Pharmacia, Uppsala, Sweden) in a central laboratory at the University Clinic as a part of every patient's regular medical control. Each patient was assessed by the mean value of four HbA1c measurements for the duration of 1 year, before entering the study, since there were not significant variations between measurements.

The control group (N = 236) consisted of unrelated volunteers recruited from Croatian Institute for Transfusion Medicine, Zagreb. Control subjects were carefully assessed to ensure the absence of family history of T1DM and any diagnostic sign of autoimmune disease. Fasting plasma glucose level of control subjects was determined, as in patients, in central laboratory at the University Clinic.

Ethical approval by the relevant research ethics committees was obtained for all collected samples. All patients and control subjects were informed of the purpose of the study and gave their informed consent before anonymously participating in the study.

Table 1
Clinical characteristics of T1DM patients and controls.

Characteristics	Patients	Controls
N	187	236
Sex (male/female)	75/112	186/50
Current age (years)[a]	36 (29–45)	51 (44–56)
Age at onset of diabetes (years)[a]	21 (14–28)	–
Duration of diabetes (years)[a]	15 (9–26)	–
Fasting blood glucose (mmoldm^{-3})[b]	8.8 ± 3.1	5.4 ± 0.4
HbA1c (%)[b]	7.3 ± 1.4	–
Insulin dose (U/kg/day)[b]	0.7 ± 0.2	–

T1DM—Type 1 diabetes mellitus.
[a] Median (25–75 percentiles).
[b] Mean ± standard deviation.

2.2. DNA extraction and genotyping

DNA was isolated from 3 mL of peripheral blood by salting-out procedure (Miller et al.,1988), whose concentration was spectrophotometrically measured (Biospec, Shimadzu). The appropriate fragments were amplified by polymerase chain reactions (PCR). The PCR mixture contained 100 ng DNA, 10 pmol of each primer, 50 μM of each dNTP, 1× PCR buffer with $MgCl_2$, and 1 U Hot Start Taq DNA polymerase (Eppendorf, Hamburg, Germany). PCR was started with initial denaturation at 95 °C for 15 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C (*IL-18*-137) or 60 °C (*IL-18*-607) or 62 °C (*IL-12B*) for 30 s and extension at 72 °C for 30 s. The reaction was completed by final extension at 72 °C for 30 s.

IL-18 polymorphisms were detected by using PCR sequence-specific primers. For the position -137 (rs187238) one of the two forward sequence-specific primers (5′ CCC CAA CTT TTA CGG AAG AAA AG 3′ or 5′ CCC CAA CTT TTA CGG AAG AAA AC 3′), a control primer (5′ CCA ATA GGA CTG ATT ATT CCG CA 3′), and a common reverse primer (5′ AGG AGG GCA AAA TGC ACT GG 3′) were used.

For the position -607 (rs1946518) one of the two forward sequence-specific primers (5′ GTT GCA GAA AGT GTA AAA ATT ATT AC 3′ or 5′ GTT GCA GAA AGT GTA AAA ATT ATT AA 3′), a control primer (5′ CTT TGC TAT CAT TCC AGG AA 3′) and a common reverse primer (5′ TAA CCT CAT TCA GGA CTT CC 3′) were used.

Control primers were used to amplify fragments covering the polymorphic sites as an internal positive amplification control. PCR products were separated by electrophoresis on 2.5% agarose gel containing ethidium bromide and visualized under UVC light.

The SNP in *IL-12B* gene (rs 3212227) was detected by forward 5′ TTT GGA GGA AAA GTG GAA GA 3′ and reverse 5′ AAC ATT CCA TAC ATC CTG GC 3′ primers. The resulting PCR products (3 μL) were digested with 5 units of restriction enzyme *TaqI*α (Fermentas GmbH, St. Leon-Rot, Germany) overnight at 65 °C. Genotype of each sample was determined by electrophoresis in vertically 12% poly (acrylamide/bis-acrylamide) gel (16 cm long) containing 1× Tris-borate–ethylenediaminetetraacetic acid (EDTA; TBE) buffer. DNA fragments were stained with SYBR Gold dye (Molecular Probes, Leiden, The Netherlands) and visualized under ultraviolet light. The presence of *TaqI*α (TCGA) site indicates the transversion of A to C creating two fragments.

15% randomly selected samples were re-tested for these SNPs. Obtained results confirmed the previous findings.

2.3. Statistical analysis

Statistical analysis was performed using GraphPad Prism and Sigma Stat 3.5 (Jandell Scientific Corp. San Raphael, California, USA). Genotype frequencies were calculated for patient and control subjects by direct counting. The differences between groups were evaluated using Pearson χ^2 test. The level of statistical significance was set at p value less than 0.05 (two-tailed test).

Web-based calculator made by Michael H. Court, (http://www.tufts.edu/~mcourt01/useful_links.htm) was used to determine whether

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observed genotype frequencies were consistent with Hardy–Weinberg equilibrium (HWE). p values >0.05 were considered consistent with HWE.

Haplotype frequencies were determined using Arlequin software (http://lgb.unige.ch/arlequin/). Odds ratios (OR) and 95% confidence intervals (CI) were calculated using a Web-based OR calculator for unmatched case–control studies (http://www.hutchon.net/ConfidOR.htm).

The Kolmogorov–Smirnov test was used to evaluate normality of clinical variables. The *t* test was applied to compare means of normally distributed variables, while the Mann–Whitney test was used for the means comparison of variables that are not normally distributed.

3. Results

The distribution of genotype and allele frequencies at *IL-18*-137 (rs187238), *IL-18*-607 (rs1946518) and *IL-12B* (rs321227) loci did not significantly differ between T1DM patients and controls (Table 2). All analyzed SNPs fulfilled the HWE criteria in both patient and control groups (Table 2).

The analysis of haplotype distribution of SNPs in the *IL-18* gene promoter region showed no significant association of any haplotype with susceptibility to diabetes (Table 3).

To analyze the association of SNPs in *IL-18* or/and *IL-12B* genes with the patient's clinical presentation (age at onset, HbA1c) the whole patients group was divided in genotype-based subgroups. Patients with the minor allele -137C or -607A of *IL-18* gene developed diabetes at earlier age at onset than patients with major allele (G or C) at the same positions, but the difference did not reach significant level (Table 4). However, the minor alleles at each of the two *IL-18* loci (-137C/-607A) were associated with the disease occurrence at significantly younger age compared to carriers of major alleles (-137G/-607C) (Table 4).

The significantly different age at diagnosis between patients with A or C allele at polymorphic site in the *IL-12B* gene was not found, although we observed a trend of earlier onset of disease in carriers of minor (C) allele (19 years vs 21 years, p = 0.137).

Moreover, the concomitant presence of polymorphic (minor) alleles, *IL-18*-137C, *IL-18*-607A and *IL-12B* C, is associated with the earliest onset of T1DM among our patients. Their median age at the time of diagnosis (16 years) was significantly lower compared to the age of patients (25.5 years) that carry common alleles, *IL-18*-137 G, *IL-18*-607C and *IL-12B* A (Table 4). Their risk of onset of diabetes before 21 years of age (it is median age at T1DM onset of all patients included in the study) is OR = 2.72 (95% CI = 0.99–7.42).

Furthermore, we observed some differences in effectiveness of long-term glucose control, based on HbA1c level, among T1DM patients with different *IL-18*-137/-607 or/and *IL-12B* genotypes. The patients with two minor alleles at both IL-18 loci (137CC/-607AA) had higher HbA1c level than patients homozygous for major alleles (-137GG/-607CC), but without significant difference (Table 5).

Furthermore, patients with the dominant AA genotype of the *IL-12B* gene had significantly higher HbA1c level than those with AC genotype (Table 5). HbA1c level of patients genotyped as CC was similar to those found in carriers of AA genotype (7.4 ± 1.2 vs 7.4 ± 1.4, p = 0.769).

Moreover, the concomitant presence of high risk-genotypes of both genes in the patients, induced even higher HbA1c levels, worsening the glucose control (Table 5). Namely, the analysis of combined genotypes of *IL-18* and *IL-12B* genes pointed out that patients with CC/AA/AA genotype have significantly higher HbA1c level (8.7%) than patients with GG/CC/AC genotype (7.0%, p = 0.029). At the same time, these two patient subgroups did not take significantly different doses of insulin (units/kg/day) for their glycemic control (0.71 ± 0.2 vs 0.54 ± 0.2, p = 0.146).

Table 2
Genotype and allele frequencies of *IL-18*-137G/C, *IL-18*-607 C/A and *IL-12B* A/C polymorphisms in patients with T1DM and healthy controls.

Locus	Genotypes	Patients (n = 187)		Controls (n = 236)		p value	HWE p value	
	Alleles	N	(%)	N	(%)		Patients	Controls
IL-18-137	GG	92	(49.2)	104	(44.1)	0.557	0.868	0.6
	GC	79	(42.2)	108	(45.8)			
	CC	16	(8.6)	24	(10.1)			
	G	263	(70.3)	316	(66.9)	0.33[a]		
	C	111	(29.7)	156	(33.1)			
IL-18-607	CC	58	(31.0)	67	(28.4)	0.766	0.256	0.225
	CA	99	(52.9)	126	(53.4)			
	AA	30	(16.1)	43	(18.2)			
	C	215	(57.5)	260	(55.1)	0.529[a]		
	A	159	(42.5)	212	(44.9)			
IL-12B	AA	123	(65.8)	152	(64.4)	0.907	0.053	0.130
	AC	52	(27.8)	70	(29.7)			
	CC	12	(6.4)	14	(5.9)			
	A	298	(79.7)	374	(79.2)	0.943[a]		
	C	76	(20.3)	98	(20.8)			

T1DM—Type 1 diabetes mellitus.
HWE—Hardy–Weinberg equilibrium.
[a] Yates corrected.

Table 3
Distribution of the *IL-18*-137/-607 haplotypes in T1DM patients and controls.

Haplotypes -137/-607	Patients		Controls		p value	OR at 95% CI
	N	(%)	N	(%)		
G/C	211	(56.4)	247	(52.4)	0.265	1.2 (0.9–1.6)
C/A	107	(28.6)	143	(30.3)	0.647	0.9 (0.7–1.2)
G/A	52	(13.9)	69	(14.6)	0.845	0.9 (0.6–1.4)
G/A	52	(13.9)	69	(14.6)	0.845	0.9 (0.6–1.4)
C/C	4	(1.1)	13	(2.7)	0.137	0.4 (0.1–1.2)

T1DM—Type 1 diabetes mellitus.
OR—odds ratio.
CI—confidence interval.

4. Discussion

In the present study we demonstrated that *IL-18*-137G/C (rs187238), *IL-18*-607 C/A (rs1946518) and *IL-12B* A/C (rs321227) polymorphisms in Croatian patients, are not associated with the susceptibility for the development of type 1 diabetes mellitus.

It was known that testing for Hardy–Weinberg equilibrium (HWE) is strongly advocated as an important quality control step in population genetic studies (Gomes et al., 1999; Hosking et al., 2004), in order to

Table 4
Comparison of age at onset of T1DM patients according to *IL-18* or/and *IL12B* gene polymorphisms.

Gene	Genotypes	Age at onset[a]	p value
IL-18-137	GG vs GC,CC	21.5 (1–29.75) vs 21 (12–27)	0.061
IL-18-607	CC vs CA,AA	24 (16.75–30) vs 21 (12–27)	0.054
IL-18-137/-607	GG/CC vs GC,CC/CA,AA	23.5 (16.25–30) vs 20 (11.75–27)	0.031*
IL-18-137/-607/*IL-12B*	GG/CC/AA vs GC,CC/CA,AA/AC,CC	25.5 (17.75–30.25) vs 16 (11.5–23)	0.044*

T1DM—Type 1 diabetes mellitus.
[a] Median age at onset (year) of T1D patients (25–75 percentiles).
* Significantly different.

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Table 5
Association of HbA1c value with *IL-18* or/and *IL-12B* gene polymorphisms.

Gene	Genotypes	HbA1c	p value
IL-18-137/-607	GG/CC	7.2 ± 1.3	0.06
	vs	vs	
	CC/AA	8.2 ± 1.9	
IL12B	AA	7.3 ± 1.4	0.034*
	vs	vs	
	AC	6.9 ± 1.3	
IL-18-137/-607/*IL-12B*	GG/CC/AC	7.0 ± 1.1	0.029*
	vs	vs	
	CC/AA/AA	8.7 ± 1.9	

HbA1c (%)—mean ± standard deviation.
* Significantly different.

reduce the chance of false-positive associations. Both patient and control groups in our study fulfilled the HWE criteria at three polymorphic loci.

So far, the contradictory results have been obtained by studying the SNPs of *IL-18* gene promoter -137G/C and -607C/A in different populations with autoimmune diabetes. To our knowledge, only a few case–control studies reported the significant association of these SNPs with T1DM. In Polish population, the significant association of genotypes with T1DM was found at both loci (Kretowski et al., 2002). However, the disease risk due to genotype at -137 differs depending on the combination with *IL-18*-607 genotype, suggesting their interaction. The combined genotype association with T1DM in their study included GC or CC at -137, and genotype AA or CA at -607 loci. However, we could not confirm any -137/-607 genotype combination as a risk factor for T1DM in Croatian population.

In the Chinese (Dong et al., 2007) or Japanese (Ide et al., 2004) populations, significantly different distribution of the -607 genotypes in T1DM patients compared to controls appears due to decreased frequency of patients carrying the AA genotype. We also detected a lower frequency of the AA genotype at -607 among Croatian diabetic subjects (16.1%) relative to control (18.2%), but without significant difference.

In contrast, many studies, including this one, have demonstrated no association of *IL-18*-137 and -607 polymorphisms with autoimmune diabetes (Martin et al., 2005; Mojtahedi et al., 2006; Novota et al., 2005; Szeszko et al., 2006). Except for one case–control study of T1DM patients from Dalmatian region of Southern Croatia, there are no other reports about an association between the *IL-18* gene polymorphisms and autoimmune diabetes in the general population (Boraska et al., 2006). In this study, authors investigated allelic variation only at -137 promoter position, and a positive correlation with the development of T1DM was not found (Boraska et al., 2006). Furthermore, recently published meta analysis, that pooled six individual association studies with T1DM patients (in total 2388 cases and 2738 controls), suggests that *IL-18* gene promoter -607C/A polymorphism does not contribute substantially to T1DM susceptibility (Pan et al., 2011). Therefore, the findings of these genetic studies do not support pathological studies about interleukin-18's role in the development of autoimmune disease (Amerio et al., 2002; Bresnihan et al., 2002; Wong et al., 2000). Different multi-genetic backgrounds of diabetes type 1 and environmental exposures of the studied populations could lead to discrepant results. Moreover, the influence of the allele might be masked by the presence of other as-yet unidentified causal genes involved in the development of autoimmune diabetes.

Lack of association of A/C *IL-12B* gene polymorphism with T1DM in Croatians is in agreement with the data previously obtained in the Italian, Northern Ireland and Norwegian populations (Johansson et al., 2001; McCormack et al., 2002; Nistico et al., 2002). In contrast, some other studies, that investigated Caucasian American families (Davodi-Semiromi et al., 2002), Western Australian (Windsor et al., 2004) and Japanese subjects (Yang et al., 2006), reported a linkage between that polymorphism and susceptibility to the disease. These inconsistent results might be related to genetic heterogeneity between populations, heterogeneity in the enrollment of patients, or even interactions of *IL-18* and *IL-12B* polymorphisms and other polymorphisms in the genetic pool.

It is known that coordinate action of interleukins IL-18 and IL-12, as well as TNF-α, has been required for the progression of disease stages, from insulitis to clinical diabetes (Andre-Schutz et al., 1999). It has been suggested that these interleukins could be diabetogenic effectors. Since the *IL-18* and *IL-12B* SNPs can influence not only the susceptibility to autoimmune diabetes, but also the clinical presentation of disease, we investigated the association of *IL-18*-137/-607 and/or *IL-12B* polymorphisms with age at T1DM onset. A trend of a younger age at onset of disease has been observed in the patients who carry one or two copies of *IL-18*-137C or -607A allele compared to the homozygous carriers of common allele; -137G or -607C. Similar observation at -137 position was observed among Turkish patients with type 1 diabetes (Altinova et al., 2010). In this study, homozygous patients for *IL-18*-137C allele, were diagnosed at significantly younger age than the patients homozygous for G allele (10.2 *vs* 17.4 years, $p = 0.02$). Furthermore, we demonstrated that the concomitant presence of minor -137C and -607A alleles even further decreased the age of disease onset, what was not observed in UK population (Martin et al., 2005).

In our study, whereas *IL-12B* A/C SNP did not show an association with the age at disease onset, it exhibited it in combination with *IL-18* SNPs. Namely, diabetes was diagnosed significantly earlier (at 16 years of age) in patients with at least three minor alleles (*IL-18*-137C/-607A/*IL-12B* C), than in those without these alleles (at 25 years of age).

It seems that the difference of age at onset of disease could be attributed to alleles at a single locus, suggesting that both genes could contribute to the variability of age. It further concurs with the fact that genetic factors might regulate the intensity of beta cell destruction and therefore influence the age of diabetes onset (Sabbah et al., 2000). However, the small number of Croatian patients in each subgroup (38 vs 17), should be acknowledged. Therefore, we should conduct a comprehensive study in the future.

Several recent studies, that investigated the relation of the IL-18 serum level (Altinova et al., 2008; Katakami et al., 2007; Mahamoud et al., 2004) or genetic variation within *IL-18* gene (Smart et al., 2011) with the patients' clinical parameters, have been suggested a possible role of the interleukin IL-18 in glucose and energy homeostasis, as well as the effectiveness of the glycemic control. The important biochemical marker of long-term glucose control is glycated hemoglobin (HbA_{1c}). Altinova et al. (2010) detected a genetic correlation of *IL-18* or *IL-12B* SNPs with HbA1c level among Turkish T1DM patients. Patients with high expressing *IL-18*-607CC genotype showed higher levels of HbA1c than patients with AC genotype (10.0 ± 0.4 vs 8.5 ± 0.3, $p = 0.004$). Earlier, the same authors also reported the association between levels of circulating IL-18 and HbA1c (Altinova et al., 2008). T1D patients with poor glycemic control (HbA1c > 7.0%) had significantly increased ($p = 0.02$) concentrations of circulating IL-18. Thus, these two studies have shown the correlation of HbA1c and IL-18 at both genetic and protein expression levels.

In contrast, the effectiveness of glycemic control (indicated by HbA1c level) in Croatian patients, was evaluated according to the presence of the major or minor alleles at both *IL-18* positions. Higher HbA1c level (8.2%) was found in patients that carry low expressing genotypes (*IL-18*-137CC/-607AA) compared to homozygous patients for both common alleles (HbA1c = 7.2%; *IL-18*-137GG/-607CC).

We also investigated the HbA1c level in relation to *IL-12B* genotypes. In our study AC genotype was connected with lower HbA1c level than AA genotype (6.9 vs 7.4, $p = 0.034$). In contrast, results obtained in Turkish T1DM population (Altinova et al., 2010)

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described higher levels of HbA1c in patients with AC than in patients with AA genotype (9.7 vs 8.5, $p = 0.01$).

Our results disagree with those obtained among Turkish T1DM patients probably due to lack of sufficient data about HbA1c level for their patients. Namely, HbA1c is a variable dependent on the method of detection, treatment regimen and life style of the patient. HbA1c of our patients was measured at 3-month intervals by ion-exchange high-performance liquid chromatography, as a part of every patient's regular medical control. As there were no significant variations between the measurements, each patient was assessed by the mean value of four HbA1c measurements for the duration of 1 year, before entering the study.

Since IL-12 synergizes with IL-18, we examined the effect of the genetic variations within both genes on HbA1c level. The carriers of *IL-18*-137CC/-607AA/*IL-12*AA genotype exhibited the worst glycemic control (HbA1c mean was 8.7%; min–max 6.8%–13.1%). In contrast, a subgroup of patients who have genotype associated with lower HbA1c at all three loci, showed significantly lower HbA1c level (mean 7.0%, min–max 5.5–9.5%). However, at the same time, the significant difference between the average daily insulin treatment requirements was not observed. These results could possibly suggest that it is more difficult to regulate glucose level if an individual possesses *IL-18*-137CC/-607AA/*IL-12*AA genotype. However, it should be pointed out that a small number of subjects per group have been studied. Therefore, a prospective clinical study following a larger number of patients with relevant clinical data, such as insulin dose, insulin resistance, physical activities, and adequate or non-adequate diet, is needed to establish the importance of *IL-18* and *IL-12B* genotype in diabetes care.

In conclusion, we found no association of *IL-18*-137/-607 and *IL-12B* genotype, allele or haplotype with T1DM development in Croatians. However, the results suggest an association of genotypes with age at onset of T1DM and HbA1c level.

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