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Research paper

Multilocus associations of inflammatory genes with the risk of type 1 diabetes

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ABSTRACT

Background: Genome-wide association studies have captured a large proportion of genetic variation related to type 1 diabetes mellitus (T1D). However, most of these studies are performed in populations of European ancestry and therefore the disease risk estimations can be inaccurate when extrapolated to other world populations.

Methods: We conducted a case-control study in 1866 individuals from the three major populations of the Republic of Bashkortostan (Russians, Tatars, and Bashkirs) in Russian Federation, using single-locus and multilocus approach to identify genetic predictors of T1D.

Results: We found that *LTA* rs909253 and *TNF* rs1800629 polymorphisms were associated with T1D in the group of Tatars. Meta-analysis of the association study results in the three ethnic groups has confirmed the association between the T1D risk and *LTA* rs909253 genetic variant. *LTA* rs909253 and *TNF* rs1800629 loci were also featured in combinations most significantly associated with T1D.

Conclusion: Our findings suggest that *LTA* rs909253 and *TNF* rs1800629 polymorphisms are associated with the risk of T1D both independently and in combination with polymorphic markers in other inflammatory genes, and the analysis of multi-allelic combinations provides valuable insight in the study of polygenic traits.

1. Introduction

Type 1 diabetes mellitus (T1D) is a chronic disorder characterized by hyperglycemia with subsequent development of vascular and neuropathic complications, caused by cellular-mediated autoimmune destruction of the beta cells of the pancreas. It affects approximately 20 million people worldwide, and is detected in children and young adults. The incidence of T1D varies greatly across global populations, ranging from a low of 0.1/100,000 per year in China and Venezuela to a high of 36.5/100,000 in Finland and 36.8/100,000 per year in Sardinia (Maahs et al., 2010). The observed discrepancies in T1D incidence in world populations suggest that risk allele frequencies and/or effect sizes may also vary. In 2016, the incidence of T1D in Russian Federation (RF) was 6.15/100,000 per year, in the Republic of Bashkortostan – 7.86/100,000 per year (Dedov et al., 2017).

T1D has a significant genetic component with concordance rate for monozygotic twins estimated between 0.45 and 0.53 (Kumar et al., 1993; Kyvik et al., 1995). Numerous studies have shown that HLA-DRB1 and DQB1 loci are major genetic determinants of T1D (reviewed in (Noble, 2015)). However, a considerable proportion of T1D patients do not carry high-risk HLA genotypes, and their frequencies are shown to decline over time (Steck et al., 2011). Large-scale genome-wide association studies (GWAS) have identified multiple non-HLA single nucleotide polymorphisms (SNP) associated with T1D (Wellcome Trust Case Control C, 2007; Hakonarson et al., 2007; Sharma et al., 2018). Although the functional role of most of the identified loci has not been properly established yet, a vast number of them belong to the pathways involved in inflammation, immunity, apoptosis, *etc.* Notably, according

Abbreviations: FDR, false discovery rate; GWAS, genome-wide association study; LD, linkage disequilibrium; PCR, polymerase chain reaction; RF, Russian Federation; SNV, single nucleotide variant; T1D, type 1 diabetes

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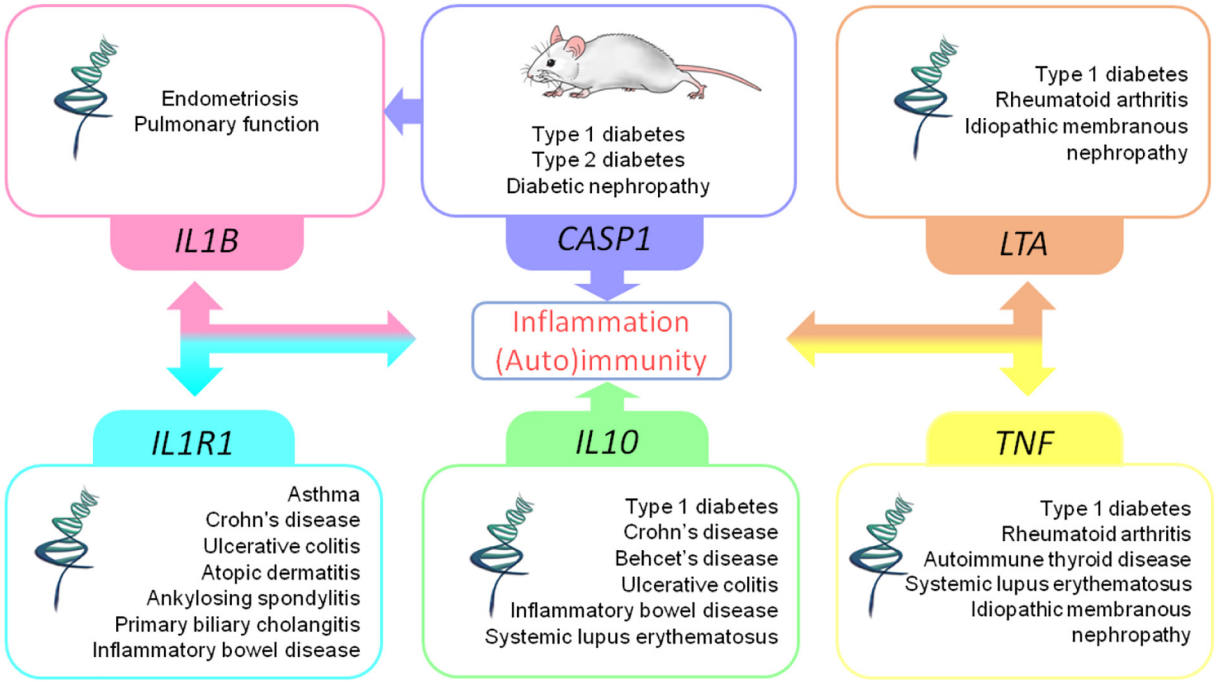

Fig. 1. Genes associated with type 1 diabetes and other autoimmune and inflammatory traits, according to the results of GWAS (*IL1B, IL1R1, IL10, TNF, LTA*) and animal studies (*CASP1*).

to the results of GWAS and animal studies, there is often an overlap among T1D and other autoimmune and inflammatory traits loci (Fig. 1).

The key players in the process of inflammation and immune response (tumor necrosis factor alpha, interleukin 1 beta, and interleukin 10) are implicated in numerous autoimmune and inflammatory diseases including T1D, systemic lupus erythematosus, rheumatoid arthritis, Crohn's disease, ulcerative colitis, and inflammatory bowel disease, which points to the existence of common genetic mechanisms in the development of these conditions (Hakonarson et al., 2007; Okada et al., 2014; Lee et al., 2012; Jostins et al., 2012; Franke et al., 2010; Franke et al., 2008). GWAS signals located in these genes can be related to the impairment of immune system and inflammation regulation either due to their functional role or *via* linkage with a true causative variant (Xavier and Rioux, 2008). Genotyping of additional variants in linkage disequilibrium (LD) with GWAS loci can increase the power to detect variants that directly influence the development of T1D (Wu et al., 2013). Migration flow due to various historical events affects LD patterns in different populations; therefore, genetic markers of the disease identified in European population need to be validated in other world populations (The International HapMap C, 2005). *Trans*-ethnic studies have demonstrated that genetic determinants of complex traits are diverse across the groups with different ancestry, which may partly explain the discrepancies in disease prevalence seen among various ethnic groups even in the same geographic region (Musunuru et al., 2012; Kim et al., 2018). The population of the Republic of Bashkortostan, located in the Volga-Ural region of Russian Federation (RF), is comprised of three distinct ethnic groups (Bashkirs, Russians, and Tatars) characterized by a unique genetic structure including both Asian and Caucasian components, which makes it a compelling object for genetic study (Yunusbayev et al., 2015). In this research, we aimed to evaluate the role of polymorphic variants in inflammatory genes in susceptibility to T1D, focusing on *IL1B*, *TNFA*, *IL6, IL12B*, *IL10* and related genes, as well as interactions between them, in the ethnic groups of Bashkirs, Russians, and Tatars from the Republic of Bashkortostan, RF.

2. Materials and methods

2.1. Study group

The study protocol was approved by the ethics committee of the Institute of Biochemistry and Genetics of Ufa Federal Research Centre of Russian Academy of Sciences (IBG UFRC RAS). All participants provided written informed consent. The study was conducted in accordance with the Helsinki declaration (2000). Study group consisted of 340 patients with T1D (167 women, mean age 28.66 ± 12.32 yrs., and 173 men, mean age 32.66 ± 12.21 yrs) and 1526 healthy individuals (428 women, mean age 36.79 ± 10.6 yrs.; 1098 men, mean age 37.91 ± 14.74 yrs) without clinical symptoms and family history of diabetes. Patients with T1D admitted at the Department of Endocrinology of the City Hospital No. 21 (Ufa, Republic of Bashkortostan, RF) were invited to participate in the study. Criteria for exclusion were: unwillingness to consent, the presence of concurrent chronic illnesses. Clinical characteristics of the group of patients are given in Table 1. Healthy controls were recruited at the Republic Centre of Blood Transfusion (Ufa, Republic of Bashkortostan, RF). Questionnaires assessing information about the ethnicity and the place of birth of participants' ancestors in three generations were administered to each individual enrolled in the study. All persons included in study permanently lived in the Republic of Bashkortostan and belonged to the Russian (n = 760), Tatar (n = 707), or Bashkir (n = 399) ethnic group. Persons of mixed ancestry were excluded from genotyping.

Data are shown as mean values ± SD. BMI - body mass index, WHR - waist-hip ratio, HbA1C - glycated haemoglobin, HDL - high density lipoproteins, VLDL - very low density lipoproteins, LDL - low density lipoproteins, AC - atherogenic coefficient, Insulin AB - anti-insulin antibodies, ICA - islet cell antibodies, GADA - glutamic acid decarboxylase antibodies.

2.2. Genotyping

DNA was isolated from 6 ml of whole venous blood using standard phenol-chloroform extraction. Genotyping was performed using PCR

Table 1
Demographic and clinical characteristics of the participants with type 1 diabetes.

Characteristic	Russians N = 135	Tatars N = 129	Bashkirs N = 76
Age	30,92 ± 14.15	31.08 ± 14.0	28.51 ± 11.75
Sex (female, n, %)	65 (48%)	66 (51%)	38 (50%)
BMI	23.43 ± 3.6	26.05 ± 24.2	23.22 ± 4.08
WHR	0.85 ± 0.09	0.82 ± 0.06	0.82 ± 0.1
Fasting blood glucose (mmol/l)	9.49 ± 4	10.02 ± 3.6	9.83 ± 4.34
HbA1C (%)	8.6 ± 2.55	8.58 ± 2.8	8.7 ± 2.6
Duration of T1D (years)	11.34 ± 9.32	11.3 ± 11.04	9.78 ± 9.28
Age at onset	19.16 ± 9.91	18.73 ± 9.93	19.83 ± 9.08
Insulin (Unit/kg)	0.78 ± 0.24	0.76 ± 0.3	0.76 ± 0.23
Total cholesterol	4.48 ± 1.06	4.6 ± 1.16	4.41 ± 0.98
Triglycerides	1.2 ± 0.58	1.43 ± 0.93	1,15 ± 0.85
HDL	1.36 ± 0.92	1.37 ± 0.54	1.84 ± 1.75
VLDL	0.55 ± 0.27	0.66 ± 0.47	0.52 ± 0.39
LDL	2.58 ± 1.19	2.55 ± 1.14	1.91 ± 1.69
AC	2.8 ± 1.18	2.88 ± 1.53	2.39 ± 1.38
C-peptide	0.61 ± 0.2	0.76 ± 0.74	0.77 ± 0.48
AB insulin	5.27 ± 3.77	3.34 ± 2.4	2.55 ± 1.83
ICA	0.35 ± 0.33	0.28 ± 0.28	0.06 ± 0.13
GADA	1.62 ± 0.82	1.65 ± 0.99	2.75 ± 1.02
Insulin	11.35 ± 8.21	13.2 ± 10.6	10.2 ± 8.1

followed by restriction endonuclease digestion. Primer sequences were designed using PrimerSelect 5.05 software (DNAStar Inc., Madison, WI, USA) (Table 2). Gene sequences for primers designing were obtained from NCBI (National Center for Biotechnology Information) database (http://www.ncbi.nlm.nih.gov/SNP). The concentrations of primers and probe were optimised leading to the following PCR conditions: 0.2 mM of both primers, 20 ng of DNA template, 30 mM Tris-HCl (pH 8.6/25 °C), 16.6 mM $(NH_4)_2SO_4$, 2.5$MgCl_2$ 0.2 mM of each dNTPs (Thermo Fischer Scientific, Lithuania), and 0,5 U of Taq-polymerase enzyme. The amplification was performed in a T100™ thermal cycler (BioRad, USA) programmed for initial denaturation step (95 °C for 1 min) followed by 28 cycles of amplification (denaturation at 95 °C for 20 s, primer annealing at specific temperature for 30 s, elongation at 72 °C for 30 s) and a final extension (72 °C for 4 min). Restriction analysis was performed by incubating 5 µl of PCR product with 2.5 U of restriction endonuclease in a 10 µl reaction volume for 16 h (enzymes and digestion temperatures are listed in Table 2). PCR and restriction products were separated by the electrophoresis on 2% agarose gel and identified using Mega-Bioprint 1100 gel documentation system (Vilber Lourmat, Collégien, France). As quality control, 5% of the studied samples were randomly selected for re-genotyping; all subsequent results were identical to initially obtained genotyping data.

2.3. Statistical analysis

Study data were stored and managed using IBM SPSS Statistics for Windows, version 22.0. Power calculations were performed using QUANTO software version 1.2.4. Sample size required to detect an association with odds ratio (OR) of 1.5 was calculated taking into account the minimally acceptable 80% power level, minor allele frequency, and T1D prevalence in the studied population. In 2016, the prevalence of T1D in the Republic of Bashkortostan was 160.74 per 100,000 individuals (Dedov et al., 2017). For each SNP, Hardy-Weinberg equilibrium and linkage disequilibrium was tested using Arlequin 3.0 software (Excoffier et al., 2005) (the detailed algorithm description is available elsewhere (Excoffier and Slatkin, 1998)) in the Tatar, Russian, and Bashkir ethnic groups (Table 3). Association between the studied polymorphic loci and T1D was analyzed in each group separately using logistic regression analysis under additive genetic model adjusted for sex implemented in PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) (Purcell et al., 2007). Additive model

Table 2
Primer sequences and PCR conditions for amplification of the studied SNPs.

No.	Locus	SNP	Alternative names	Chr:Position	Function	Primer sequences	Annealing temperature	Restriction enzyme	Digestion temperature	Alleles, fragment length, bp
1	*IL10*	rs1800872	–627C > A	1:206946407	Promoter	5`-cct agg tca cag tga cgt gg-3` 5`-ggt gag cac tac ctg act agc-3`	62°	*RsaI*	37°	*A (236 + 176) *C (412)
2	*IL1R1*	rs2287047	3653 T > G	2:102774054	Intron 1	5'- aat act taa ctt acc agc ctc cag – 3' 5'- act ctt tat ccc ttt ttg ttc c-3'	62°	*BsrI*	65°	*C (210 + 77) *T (286)
3	*IL1B*	rs16944	–511 T > C	2:113594867	Promoter	F 5'-gcc tga acc ctg cat acc gt-3' R 5'-gcc aat agc ccg ccc tgt ct-3'	65°	*AvaI*	37°	*C (89 + 64) *T (153)
4	*IL12B*	rs3212227	1159A > C	5:158742950	3`-UTR	5`-ttt gga gga aaa gtg gaa ga-3` 5`-aac att cca tac atc ctg gc-3`	59°	*TaqI*	65°	*C (185 + 138) *A (323)
5	*LTA*	rs909253	252A > G	6:31540313	Intron 1	5'-ccg tgc ttc gtg gtt tgg act a-3' 5'-aga ggg gtg gat gct tgg gtt c-3'	63°	*NcoI*	37°	*A (331) *G (234 + 97)
6	*TNF*	rs1800629	–308G > A	6:31543031	Promoter	F 5'-agg caa tag gtt ttg agg gcc at-3' R 5'-tcc tcc ctg ctc cga ttc cg-3'	61°	*NcoI*	37°	*G (87 + 20) *A (107)
7	*IL6*	rs1800796	-636G > C (–572G > C)	7:22766246	Promoter	5`-gag acg cct tga agt aac tg-3` 5`-tgc agc tta ggt cgt cat tg-3`	58°	*BsrBI*	37°	*G (376 + 60) *C (434)
8	*CASP1*	rs481736	–877C > T	11:104906710	Promoter	5'- gag cca ccc acc cat cct g-3' 5'- aaa taa tct gca aat aat cat cct-3'	63°	*TaqI*	65°	*C (85 + 253) *T (388)

Chr:Position - chromosome number and position (bp) according to GRCh37.p13.

Table 3
Results of the analysis of association between the studied SNPs and the risk of T1D in the three ethnic groups.

No.	Locus SNP	EA/NEA	Ethnic groups	N cases/N controls	EAF cases/controls	HWE *P*-value	OR (CI 95%)	*P*-value	P_{FDR}
1	*IL10*	A/C	Tatars	128/530	27.34/28.4	0.087	0.96 (0.7–1.31)	0.802	0.912
	rs1800872		Russians	125/574	28.8/25.78	0.828	1.14 (0.83–1.57)	0.407	0.795
			Bashkirs	66/112	33.08/29.02	0.36	1.22 (0.76–1.96)	0.418	0.772
2	*IL1R1*	A/G	Tatars	124/246	41.13/39.63	0.79	0.98 (0.72–1.34)	0.912	0.912
	rs2287047		Russians	126/339	36.5/34.51	0.186	1.08 (0.8–1.44)	0.619	0.795
			Bashkirs	66/88	37.69/41.48	1	0.86 (0.54–1.36)	0.506	0.772
3	*IL1B*	T/C	Tatars	113/559	47.35/42.84	0.143	1.17 (0.87–1.57)	0.29	0.58
	rs16944		Russians	103/406	34.47/41.87	0.185	0.76 (0.49–1.18)	0.217	0.79
			Bashkirs	64/96	58.59/43.23	0.299	1.99 (1.18–3.34)	0.009	0.076
4	*IL12B*	C/A	Tatars	130/532	22.69/22.27	0.9	0.97 (0.68–1.38)	0.865	0.912
	rs3212227		Russians	122/595	20.08/19.41	0.512	1.09 (0.78–1.54)	0.608	0.795
			Bashkirs	67/135	20.45/18.15	0.383	1.16 (0.68–1.98)	0.579	0.772
5	*LTA*	G/A	Tatars	125/501	39.2/29.24	0.13	**1.79 (1.32–2.43)**	**$1.95*10^{-4}$**	**$7.82*10^{-4}$**
	rs909253		Russians	127/588	34.25/25.94	0.39	1.45 (1.08–1.97)	0.015	0.121
			Bashkirs	65/84	38.28/36.31	0.236	1.11 (0.66–1.89)	0.695	0.794
6	*TNF*	A/G	Tatars	124/567	19.76/11.02	0.524	**2.12 (1.43–3.14)**	**$1.83*10^{-4}$**	**$7.82*10^{-4}$**
	rs1800629		Russians	113/412	17.26/13.96	0.681	1.06 (0.59–1.88)	0.004	0.84
			Bashkirs	65/132	16.41/17.19	0.765	0.95 (0.53–1.71)	0.867	0.867
7	*IL6*	C/G	Tatars	134/545	14.18/14.77	0.866	1.14 (0.77–1.7)	0.514	0.822
	rs1800796		Russians	127/599	9.06/11.27	1	0.77 (0.47–1.26)	0.296	0.79
			Bashkirs	68/128	14.93/18.36	0.767	0.76 (0.42–1.36)	0.351	0.772
8	*CASP1*	T/C	Tatars	110/475	23.18/18.84	0.764	1.3 (0.89–1.9)	0.18	0.481
	rs481736		Russians	114/522	16.67/17.53	0.071	0.92 (0.62–1.37)	0.7	0.795
			Bashkirs	57/31	16.07/27.42	0.068	0.41 (0.15–1.06)	0.065	0.258

Multiple regression analysis was performed with sex as covariate under additive genetic model. Abbreviations: Chr – chromosome; Position (bp) according to GRCh37.p13; SNP – single nucleotide polymorphism; EA - effect (risk) allele; NEA – non-effect allele; EAF – effect allele frequency, %, HWE *P*-value – Hardy-Weinberg *P*-value; P_{FDR} - significance level corrected for multiple testing using FDR approach; OR – odds ratio, CI – confidence interval. The OR is aligned to the SNP risk allele. Bold text denotes the results that are statistically significant at $P < .05$ level.

assumes that carrying two copies of an allele has twice the effect on phenotype compared to the presence of only one copy. Meta-analysis of the study results was performed under fixed-effects and random-effects models. *P* values of < 0.05 were considered statistically significant. Association between allele and/or genotype combinations and the risk of T1D was tested using APSampler 3.6.0, the program and its description are available at http://apsampler.sourceforge.net/, common algorithm has been described elsewhere (Favorov et al., 2005). Correction for multiple testing was performed using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995).

3. Results

Using logistic regression analysis with sex as covariate, we detected two polymorphic loci associated with T1D in the group of Tatars: *TNF* rs1800629 (for the A allele: OR = 2.12, $P_{FDR} = 7.82*10^{-4}$) and *LTA* rs909253 (for the G allele: OR = 1.79, $P_{FDR} = 7.82*10^{-4}$) (Table 3). In the group of Russians, nominally significant associations were observed for *LTA* rs909253 (for the G allele: OR = 1.45, *P* = .015) and *TNF* rs1800629 (for the A allele: OR = 1.06, *P* = .04), but they did not reach the level of significance after the correction for multiple testing was applied (Table 3). No association with T1D risk was found for the studied loci in the group of Bashkirs (Table 3).

Meta-analysis of the association study results in the three ethnic groups has revealed the association with the disease risk of *LTA* rs909253*G allele (OR = 1.51, $P = 3.77*10^{-4}$ under random effects model, OR = 1.53, $P = 3.03*10^{-5}$ under fixed effects model) and *TNF* rs1800629*A allele (OR = 1.48, *P* = .007 under fixed effects model) (Table 4).

Using the APSampler algorithm, we obtained 833 and 252 genotype/allele combinations of the studied polymorphic variants associated with T1D in the groups of Tatars and Russians, respectively. Combinations that remained significantly associated with T1D after the FDR test was applied (with OR ≥ 2 for combinations associated with increased risk of T1D, and OR ≤ 0.5 for those associated with decreased risk of T1D), are presented in Table 5. A total of 7 genotype/allele combinations fulfilled the above mentioned criteria (five in the group of Tatars, two in the group of Russians). *TNF* rs1800629 or *LTA* rs909253 or both were present in all of the identified patterns. *CASP1* rs481736 was frequently featured as well, but only in the group of Tatars (Table 5). Four patterns were associated with the increased risk of T1D, and three conferred a protective effect against the disease. Interestingly, *TNF* rs1800629 appeared to be able to change the direction of the effect of other SNPs: *CASP1* rs481736*C allele was associated with an increased risk of T1D as part of the combinations containing *TNF* rs1800629*A allele, but together with *TNF* rs1800629*G/G genotype it was conferring reduced risk of the disease (OR = 0.47, CI:0.3–0.73, $P_{FDR} = 0.029$).

Using Arlequin 3.0 program, we performed the linkage disequilibrium test between *TNFA, LTA* and *HLA-DRB1, HLA-DQB1* loci (methods used for *HLA* genotyping have been previously described elsewhere (Avzaletdinova et al., 2012; Avzaletdinova et al., 2005)), but failed to detect any linkage (*TNFA-DRB1* *P* = .257 Russians, *P* = .153 Tatars, *P* = .644 Bashkirs; *TNFA-DQB1* *P* = .371 Russians, *P* = .396 Tatars, *P* = .354 Bashkirs; *LTA-DRB1* *P* = .573 Russians, *P* = .393 Tatars, *P* = .907 Bashkirs; *LTA-DQB1* *P* = .0.666 Russians, *P* = .424 Tatars, *P* = .119 Bashkirs).

4. Discussion

We studied associations of eight SNPs in inflammatory genes with T1D in the ethnic groups of Russians, Tatars, and Bashkirs from the Republic of Bashkortostan (Russian Federation), and found that *TNF* rs1800629 and *LTA* rs909253 were associated with T1D in Tatars. The results of the meta-analysis have shown that *LTA* rs909253 polymorphism conferred the risk of diabetes in the three ethnic groups. Furthermore, we found seven genotype/allelic combinations of the studied loci that were significantly associated with T1D in the groups of Tatars and Russians, including SNPs that did not show any association with the disease when analyzed individually.

LTA rs909253 polymorphism was associated with T1D in the Tatars ethnic group and in the meta-analysis population. *LTA* rs909253 was

Table 4
Meta-analysis of the association studies in the three ethnic groups.

No.	Locus	SNP	N cases/N controls	EA/ NEA	OR (R)	*P*-value (R)	OR (F)	*P*-value (F)
1	*IL10*	rs1800872	320/1216	A/C	1.08	0.481	1.08	0.481
3	*IL1R1*	rs2287047	317/673	A/G	1	0.986	1	0.986
2	*IL1B*	rs16944	280/1061	T/C	0.97	0.9	1.02	0.847
4	*IL12B*	rs3212227	319/1261	C/A	1.05	0.649	1.05	0.649
6	*LTA*	rs909253	318/1173	G/A	**1.51**	**$3.77*10^{-4}$**	**1.53**	**$3.03*10^{-5}$**
5	*TNF*	rs1800629	302/1111	A/G	1.34	0.294	**1.48**	**0.007**
7	*IL6*	rs1800796	330/1272	C/G	0.92	0.564	0.92	0.566
8	*CASP1*	rs481736	282/1028	T/C	0.92	0.739	1.02	0.877

Chr – chromosome; Position (bp) according to GRCh37.p13; SNP – single nucleotide polymorphism; EA - effect (risk) allele; NEA – non-effect allele; EAF – effect allele frequency, %, *P*-value (R) – random-effects meta-analysis *P*-value; *P*-value (F) – fixed-effects meta-analysis *P*-value; OR (R) – random-effects odds ratio; OR (F) – fixed-effects odds ratio. The OR is aligned to the SNP risk allele. Bold text denotes the results that are statistically significant at $P < .05$ level.

reportedly associated with T1D according to the results of family-based linkage studies (Boraska et al., 2009; Noble et al., 2006). GWAS studies identified an association between *LTA* rs909253 and rheumatoid arthritis, as well as association of its proxy SNP, rs1041981, with T1D and idiopathic membranous nephropathy (Okada et al., 2014; Stanescu et al., n.d.). In addition, rs909253 polymorphism also exhibited eQTL properties, altering *TNF* gene expression in peripheral blood cells (Westra et al., 2013). TNF-α is a pleiotropic inflammatory cytokine produced mainly by macrophages and activated monocytes. It has been established that TNF-α induces NF-kB-mediated *NOS2* expression which plays a key role in beta cell apoptosis in the early stages of T1D. *In vitro* studies indicate that *A allelic form of rs1800629 polymorphism in 5′ upstream promoter region of *TNF* gene results in two-fold increase of *TNF* transcription as compared to the rs1800629*G form (Kroeger et al., 1997). *TNF**A allele frequency was higher in non-responders to treatment with TNF-α blockers, indicating that the rs1800629*A variant predicts poor response to TNF-α inhibitors (O'rielly et al., 2009). Notably, *TNF**A allele frequency shows substantial variation across world populations, with higher frequencies observed in Caucasian populations and lower frequencies detected in Asian populations, which corresponds with T1D prevalence. In our study, we observed significant association between rs1800629*A allele and the increased risk of T1D in the group of Tatars. *TNF* also appeared in three combinations associated with diabetes in Tatars, and in one combination associated with T1D in Russians. *TNF* rs1800629 is associated on genome-wide level with systemic lupus erythematosus and co-located with a T1D-associated SNP (rs2857595) (Bentham et al., 2015; Tomer et al., 2015). Considering that *LTA* gene is clustered with other members of the tumor necrosis factor (TNF) superfamily within the central MHC (Major Histocompatibility Complex) class III region on chromosome 6, it is possible that the associations detected in the current study reflect a linkage disequilibrium (LD) with some major risk HLA alleles. Previous studies have identified *HLA-DRB1**04/*17 genotype and *HLA-DRB1**04, *HLA-DRB1**17, *HLA-DQB1**0301, *HLA-DQB1**0602–08 alleles as common T1D markers, and *HLA-DRB1**15 allele as a protective marker in all three ethnic groups of the Republic of Bashkortostan (Avzaletdinova et al., 2012). Additionally, lower T1D risk was associated with *HLA-DRB1**11 allele in Russians, and *HLA-DRB1**01 in Tatars. *HLA-DRB1**04/*17 genotype conferred increased risk of T1D in Bashkirs and Russians, while *HLA-DQB1**0302 was an independent risk marker of T1D in Tatars (Avzaletdinova et al., 2012). However, an LD analysis of *TNF* rs1800629, *LTA* rs909253, and *HLA-DRB1-HLA-DQB1* did not reveal any significant linkage. This suggests that the associations observed in our study are independent or reflect a LD with some other loci.

CASP1 genetic variant was one of the most widely represented across the identified patterns. This gene encodes caspase-1 (formerly known as IL-converting enzyme), which is required for cleavage of interleukin-1beta (IL-1B) from its precursor form. *CASP1* rs481736 has demonstrated an ambiguous effect in our study: in the presence of *TNF* rs1800629*G/G genotype it exhibited protective effect against T1D, but combined with *TNF* rs1800629*A allele it conferred an increased risk of the disease. The observed associations could reflect the influence of *TNF* rs1800629, but also could be a result of rs481736 action on gene expression, as it has been shown to affect the expression not only of *CASP1*, but also *CASP5*, *CARD16*, and *COP1* genes, thus potentially impacting the processes of inflammation, apoptosis, and protein degradation (Westra et al., 2013; Fehrmann et al., 2011; Zeller et al., 2010).

IL1B rs16944*T allele together with *CASP1**T and *LTA**A alelles was associated with an increased risk of diabetes (Table 5). IL-1B is an inflammatory cytokine coordinating the recruitment of inflammatory cells to pancreatic islets and mediating direct cytotoxic effects on beta-cells. *IL1B* rs16944 polymorphism (C to T substitution at −511 position in the promoter of *IL1B* gene) has been previously reported to increase the expression of *IL1B* – individually, together with *IL1RN* VNTR

Table 5
Allele and genotype combinations most significantly associated with T1D.

No.	*IL10* rs1800872	*IL1R1* rs2287047	*IL12B* rs3212227	*IL1B* rs16944	*LTA* rs909253	*TNF* rs1800629	*CASP1* rs481736	Control (%)	Patients (%)	*P*-value	P_{FDR}	OR	CI_{OR}
Tatars													
1		G			G			35.45	52.94	$1.37*10^{-3}$	0.03	2.05	1.3–3.22
2				T	G		T	10.64	23.08	$1.87*10^{-3}$	0.037	2.52	1.42–4.46
3						A	C	19.87	36.19	$4.08*10^{-4}$	0.03	2.29	1.44–3.62
4	C					A	C	17.94	35.39	$1.64*10^{-4}$	0.03	2.49	1.56–4
5						G/G	C	76.28	60	$6.95*10^{-4}$	0.029	0.47	0.3–0.73
Russians													
1			A		A			90.05	76.67	$1.27*10^{-4}$	0.032	0.36	0.22–0.6
2					A	G		93.38	81.98	$5.14*10^{-4}$	0.043	0.32	0.17–0.6

P-value - significance level estimated using Fisher's exact test, P_{FDR} - significance level corrected for multiple testing using FDR approach, OR – odds ratio, CI_{OR} – 95% confidence interval.

polymorphism *2 allele, and as a part of the haplotype consisting of *IL1B* -3893*G, −1464*G, −511*C and − 31*T alleles (Landvik et al., 2009). Interestingly, it has been noted that IL-1B promotes beta cell proliferation at low concentrations while causing functional impairment and cell death at higher concentrations, which suggests that it can have various effect depending on transcriptional activity of the gene (Maedler et al., 2006). *IL1B* rs16944*T has been shown to affect gene expression in lymphoblastoid cell lines and in testes (Carithers et al., 2015; Nica et al., 2011). *IL1R1*G allele paired with *LTA* rs909253*G allele was modestly associated with an increased risk of T1D. The rs2287047 polymorphism has been previously shown to be located within a predicted transcription factor binding site with the conserved allele matching the consensus sequence, and it has been linked with other complex diseases such as osteoarthritis (Näkki et al., 2010). It reportedly alters *IL1R1* gene expression in esophageal mucosa (Carithers et al., 2015). *IL1R1* rs2287047*T allele correlated with *IL1B* rs13019803*T allele, which, in turn, has been reportedly associated with decreased ST2 levels in Framingham Heart Study, increased mortality in the CHARGE consortium, and increased risk of coronary artery disease in the CARDIoGRAM consortium (Ho et al., 2013). ST2/IL-33 axis has been implicated in several immunological and inflammatory diseases; ST2 deletion reportedly enhances the development of T cell-mediated autoimmune disorders, including T1D (Ho et al., 2013). *IL10* rs1800872*C allele was a part of a pattern associated with an increased risk of T1D. IL-10 is a potent anti-inflammatory cytokine, down-regulating the expression of pro-inflammatory mediators, such as TNF-α and IL-1. Extensive study of *IL10* gene promoter revealed three polymorphic loci (rs1800896A > G, rs1800871C > T, and rs1800872C > A), affecting transcriptional activity of *IL10*. It has been demonstrated that the A-T-A haplotype was associated with lower *IL10* expression level than either the G-C-C or the A-C-C haplotype (Crawley et al., 1999). It has also been shown that, due to the strong linkage disequilibrium, the analysis of the rs1800872 polymorphism could determine the occurrence of the common haplotype A-T-A, which was associated with decreased synthesis of IL-10 *in vitro* and *in vivo* (Eder et al., 2007). *IL10* rs1800872 and a proxy SNP (rs1518111) were also associated with Behcet's disease according to the GWAS results (Mizuki et al., 2010; Remmers et al., 2010). *IL10* rs1800872 is located in a region that mediates negative regulatory function (Lazarus et al., 1997). Upon encountering apoptotic signals, PARP-1 (poly ADP-ribose polymerase 1) acts as a transcriptional factor by directly binding an octamer motif that contains rs1800872 polymorphism, thus repressing *IL10* transcription. The rs1800872*C allele containing sequence leads to weaker binding of PARP-1 (weaker repression), which results in higher IL-10 production in carriers of rs1800872*C allele (and, contrarily, decreased IL-10 synthesis in individuals carrying rs1800872*A allele) (Chung et al., 2007). It is unclear whether the association between the allelic pattern containing *IL10* rs1800872*C reflects its functional role or is due to the influence of the other genes in the combination (*TNF* and *CASP1*).

We did not detect an association between the studied polymorphisms and T1D in Bashkirs. The observed differences in the genetic makeup of the disease in the three study group may reflect the differences in the genetic structure of the population. The ethnic structure of the study sample (40.73% Russians, 37.89% Tatars, 21.38% Bashkirs) corresponded with the proportions of these groups in the population structure of the Republic of Bashkortostan (39.27% Russians, 28.42% Tatars, 21.91% Bashkirs) (Vasilieva, 2006). Among them, the population of Russians is the most admixed due to the historical migration flows in the Volga-Ural region. Bashkirs, despite being an aboriginal population of the Republic of Bashkortostan, are characterized by geographical detachment: the majority of Bashkirs (57.6%) live in rural areas on the territories east of the Ural Mountains, while 82.7% of Russians and 67% of Tatars live in urban areas located to the west of the Ural Mountains (http://bashstat.gks.ru/). These factors may have influenced both the genetic structure of the population and the T1D susceptibility in different ethnic groups.

5. Study limitations

The main limitation of our study is a small sample size that can affect statistical significance. To address this limitation, we performed power analysis to ensure that our sample size was sufficient to detect associations with OR ≥ 1.5 with minimally acceptable 80% power level; however, more modest associations (OR 1.1–1.2) may have been missed. Associations that we report for *LTA* and *TNF* polymorphisms remained statistically significant after correction for multiple testing and adjustment for sex. The strengths of the study include populations' homogeneity and well-defined clinical phenotype. Importantly, we identified both single locus associations and genotype- and allelic combinations associated with T1D risk. Further research is needed to establish whether the observed associations are due to the biological role of the studied polymorphic variants or they reflect the influence of nearby located functional variants.

6. Conclusion

T1D is a polygenic autoimmune disorder, and cytokines are thought to be major players in its development. Our study has identified a number of polymorphic variants in cytokine genes associated with T1D, both individually and in combination. It is worth noting that the analysis of genotype and allelic combinations has revealed some associations that remained obscure after the single-marker analysis was performed. It suggests that the analysis of multi-allelic combinations may help in identifying complex trait loci. However, the results obtained will require further validation in an independent study sample.

Declaration of interests

The authors have no conflict of interest to disclose

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