Evidence for a Type 1 Diabetes Susceptibility Locus (*IDDM10*) on Chromosome 10p11-q11 in a Russian Population

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Abstract

Around 20 susceptibility loci for type 1 diabetes mellitus (T1DM) have been mapped. One of these loci, *IDDM10*, was found on chromosome 10p11-q11. Here, we investigated whether the *IDDM10* locus contributes in the susceptibility to T1DM in a Russian family dataset. One hundred and fourteen simplex Russian families, each containing two siblings (one affected with T1DM diagnosed and one nondiabetic sibling), and 97 multiplex families, containing 106 affected full sibling pairs, were studied. Genomic DNA from the venous blood of the patients was genotyped by PCR using 12 microsatellites (*D10S193*, *D10S548*, *D10S565*, *D10S586*, *D10S588*, *D10S675*, *D10S1243*, *D10S1426*, *D10S1733*, *D10S1772*, *D10S1780* and *D10S1783*) located on chromosome 10p11-q11. Using the multipoint linkage analysis, the region of suggestive linkage, with a multipoint logarithm of odds (LOD) ratio (MLS) value of more than 2.2, was found between markers *D10S1733* and *D10S1780*, an area of 9.0 cM on the genetic map. The maximum linkage peak (MLS = 2.85 and nonparametric logarithm = 2.68) was observed between markers *D11S565* and *D11S1243*. Using the transmission disequilibrium test, an association of these markers, *D10S565* (*P* overall = 0.0082) and *D10S1243* (*P* overall = 0.017), with T1DM was shown. These results suggest the evidence for the *IDDM10* susceptibility locus on chromosome 10p11-q11.

Introduction

Type 1 diabetes mellitus (T1DM) is an organ-specific disorder that arises from autoimmune destruction of the insulin-producing β cells of the pancreas. The cause of T1DM is an interaction between genetic and environmental factors. Genome searches have established that T1DM is a polygenic disease. To date, genome-wide scans have revealed about 20 genomic regions linked to T1DM [1]. Of them, the human leucocyte antigen (*HLA*) region (locus *IDDM1*), located on chromosome 6p21.3, shows the major effect. *HLA* contribution to total family clustering of the disease is about 44% [1]. In comparison with the *HLA* region, the effects of most of other non-*HLA* diabetes-predisposing loci are much weaker.

A first genome-wide screen for susceptibility loci to T1DM revealed a 24-cM region of linkage to the disease in on chromosome 10p11-q11 in the UK nuclear families [2]. The susceptibility locus was designated *IDDM10* and further confirmed in the extended UK family dataset, with a peak multipoint logarithm of odds (LOD) score (MLS) of 4.7 [3–5]. In fact, this was the strongest non-*HLA* linkage observed in the latter study. A peak marker of linkage, *D10S193*, showed an association with T1DM in UK Caucasian diabetic patients [3]. In addition, a transmission from parents to diabetic children, of one of the alleles of *D10S588*, a marker, located 4.8 cM apart from *D10S193*, has been shown to be significantly increased in UK and US Caucasian families but not in families from Norway, Italy and Sardinia [3]. In the consensus analysis of US and UK diabetic multiplex families, evidence of linkage was also observed at chromosome 10p11, near the *D10S565* marker, located 1.6 cM telomeric to *D10S193*, with a LOD score of 2.8 [5]. On the other hand, using many of the same British families as Mein *et al.* [4] as well as numerical American families, Concannon *et al.* [6] found little evidence for linkage to *IDDM10*

(MLS of 0.4). *IDDM10* locus has not been replicated in other Caucasian populations: two recent whole-genome scan failed to find a suggestive evidence for linkage to T1DM on the chromosomal region 10p11-q11 in Scandinavians [7] and isolated Dutch population [8].

In this connection, it would be interesting to examine whether the *IDDM10* locus contributes to the genetic susceptibility to T1DM in Russian patients. Here, we evaluated a set of 12 polymorphic microsatellite markers, including *D10S193*, *D10S565* and *D10S588*, on chromosome 10q11.1-10p11.2 for linkage and association with T1DM in a Russian population.

Materials and methods

Patients. We studied 114 simplex Russian families, each containing two siblings (one affected with T1DM diagnosed before the age of 17 years and one nondiabetic sibling). Sixteen families were collected from the Samara Diabetic Centre, the other being recruited from the Endocrinology Research Centre in Moscow. Ninety-seven multiplex families, containing 116 affected full sibling pairs (mean age at onset of diabetes 13.2 ± 2.3 years), were recruited from the Endocrinological Research Centre. The research protocol was approved by the Ethics Committee of the Endocrinology Research Centre and performed according to the principles of the Declaration of Helsinki. Informed consent was obtained from all subjects before participation in this study.

Diabetes was diagnosed according to the criteria defined by the National Diabetes Data Group [9]. T1DM was classified based on the presence of ketosis, low body mass index and the need for insulin treatment. In all subjects, diagnosis of the disease was confirmed by the presence of at least one of the three major islet autoantibodies: islet cell antibodies, GAD65 antibodies and/or antityrosine phosphate-like molecule (ICA512) antibodies [10, 11].

DNA typing. Genomic DNA was extracted from whole-blood samples collected in disodium EDTA (3 mg/ml) according to the established protocol [12]. Microsatellite markers (*D10S193*, *D10S548*, *D10S565*, *D10S586*, *D10S588*, *D10S675*, *D10S1243*, *D10S1426*, *D10S1733*, *D10S1772*, *D10S1780* and *D10S1783*) were taken from the public databases and analysed by PCR. Primer sequences were as described in the GENOME Database (http://www.gdb.org). For each microsatellite marker, one primer was fluorescently labelled with either 6-HEX or FAM (Eurogentec, Scraing, Belgium). The PCR cocktail contained 10 mM Tris–HCl pH 9.0, 50 mM KCl, 1.0–1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.2 mM each dNTP, 0.5 U AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA, USA), 5 μM each primers and 100 ng of genomic DNA in a total volume of 10 μl. PCR was carried out on a GeneAmp® PCR System 2700 (Applied Biosystems) at 95 °C for 3 min followed by 30 cycles of denaturation at 95 °C for 15 s, annealing at 55–60 °C for 15 s and extension at 72 °C for 30 s, with final extension at 72 °C for 10 min. Fluorescence-based genotyping was performed with an ABI PRISM 310 DNA Sequencer (Applied Biosystems) and GENESCAN analysis software version 3.1.2. PCR-amplified microsatellite alleles were numbered according to the number of repeat units.

HLA-DRB1, *HLA-DQA*1 and *HLA-DQB1* alleles were determined by the use of a locus-specific amplification procedure, with subsequent hybridization of PCR products with a corresponding allele-specific oligonucleotide probe as previously described [13, 14].

Three single-nucleotide polymorphisms (SNP) located within corresponding candidate genes (*GAD2*, *CREM* and *TCF8*) were also genotyped in simplex families using PCR restriction fragment length polymorphism (PCR-RFLP) approach. These SNP were taken from the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/). PCR-RFLP assays for detection of each SNP are summarized in Table 1.

Statistical analysis. Multipoint linkage analysis was performed in affected sibling pairs using GENEHUNTER 2.1 software [15]. MLS and nonparametric LOD (NPL) values were computed using estimate LOD command of the software and allele frequencies derived from parents of affected sibs. Marshfield sex-average map distances were used in the linkage analysis [16]. According to criteria proposed by Lander and Kruglyak [17], for mapping genes involved in complex traits, a LOD score of 2.2 was considered to indicate suggestive linkage, whereas a LOD score of 3.6 was assessed as evidence for significant linkage.

Using the GENEHUNTER software, the transmission disequilibrium test (TDT) was performed in simplex families to identify alleles preferentially transmitted from heterozygous parents to diabetic offspring [18]. *P*-value (P_c) of less than 0.05 after correction for multiple alleles (62) was considered significant. For each microsatellite marker, an overall TDT *P*-value was also calculated and corrected by multiplying it by a number of markers (8) studied in the TDT analysis. For each SNP, *P*-value was multiplied by a total number of alleles tested (6) to obtain corrected *P*-value (P_c).

The degree of pairwise linkage disequilibrium between markers was calculated using the 2LD software [19], expressed as D', which represents the proportion of the maximum possible allele association given the allele frequencies and the direction of association. $D' = 1$ corresponds to complete disequilibrium.

Results

Linkage analysis

We studied 12 polymorphic microsatellites that span a 20.3-cM centromeric region of chromosome 10q11.1-10p11.2. Markers *D10S193*, *D10S565* and *D10S588*

Table 1 Description of molecular assays to detect single-nucleotide polymorphism (SNP) within three candidate genes studied for association with susceptibility to type 1 diabetes in a Russian sample

SNP	dbSNP ID	Gene	Location within the gene (position from the transcription start, bp)	PCR primers, 5′→3′	Annealing temperature (°C) ([Mg^{2+}], mM)	Restriction enzyme to digest PCR product	Digestion buffer (digestion temperature, °C)	Duration of digestion (h)	Definition of alleles (length of digestion products, bp)
Ala/Gly	2839678	GAD2	Codon 326	F: CAGACGTGTCTGTTAGGGAG R: CTCACGAGGAAAGGAACAAAG	60 (1.0)	*Eco*471	Fermentas B (37)	3	Ala allele: undigestable (130) Gly allele: (108 + 22)
C/T	1531550	CREM	Promoter region (−11)	F: GTAGGCCAGTCATATTAGTG R: CCTGATCCAGCCACAGAAAGG	55 (1.5)	*Kpn*I	Fermentas *Kpn*I buffer (37)	2	C allele: undigestable (118) T allele (20 + 98)
A/G	2839664	TCF8	Intron 4 (182905)	F: GCCAGTGGTCATGATGAAAATG R: CTATAGTAGGAGCAGGTTCCCATTC	60 (1.0)	*Xba*I	Fermentas Y (37)	2	A allele (27 + 83) G allele: undigestable (110)

that showed significant linkage and association with T1DM in previous studies [3, 20] have been also estimated in our analysis.

Multipoint linkage analysis in the Russian multiplex family dataset showed a suggestive evidence of linkage to T1DM, with a MLS of 2.75 (Fig. 1) and NPL of 2.68 ($P = 0.00368$) (Fig. 2). The maximum linkage peak was observed between markers *D10S565* and *D10S1243*. The region of suggestive linkage, with a MLS value of more than 2.2 [17], was situated between markers *D10S1733* and *D10S1780*, an area of about 9.0 cM on the genetic map [16] (Fig. 1).

Transmission disequilibrium test

Eight microsatellite markers located within or close to the region of suggestive linkage (Fig. 1) were further evaluated for association with the disease using TDT analysis in 114 Russian simplex families (Table 2). Among the microsatellites tested, a positive allele-wise TDT result was obtained for two markers, *D10S565* (P_c overall = 0.0082) and *D10S1243* (P_c overall = 0.017), located within the region of maximum linkage with T1DM in the Russian family dataset (Figs 1 and 2). For *D10S565* and *D10S1243*, this significant result was produced largely due to preferential transmission of alleles 26 ($P_c = 0.014$) and 16 ($P_c = 0.025$), respectively, from parents to affected children. The random transmission of alleles was observed

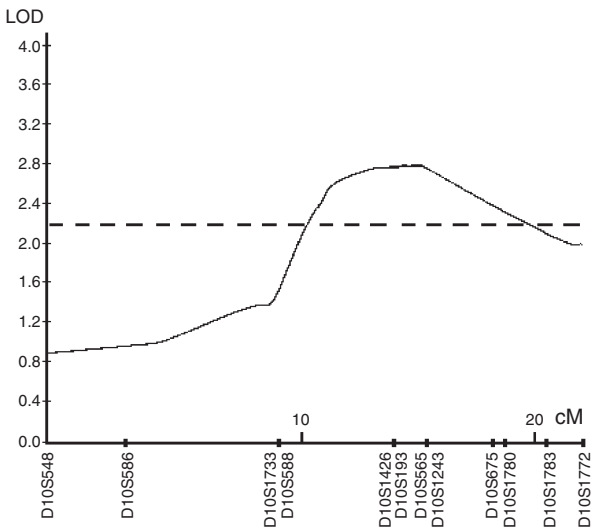

Figure 1 Multipoint logarithm of odds (LOD) ratio for type 1 diabetes on chromosome 10p11-q11 obtained using the «estimate» command of GENEHUNTER 2.1, assuming dominance variance may exist, and using allele frequencies from parents of affected sibs in the Russian dataset, consisting of 106 identical-by-descent sibling pairs affected with diabetes. Dotted line indicates the suggestive level of linkage, with a LOD score that is equal or more than 2.2 [18]. A scale of genetic distances between markers is presented in centimorgans (cM) according to the Marshfield sex-average map [17].

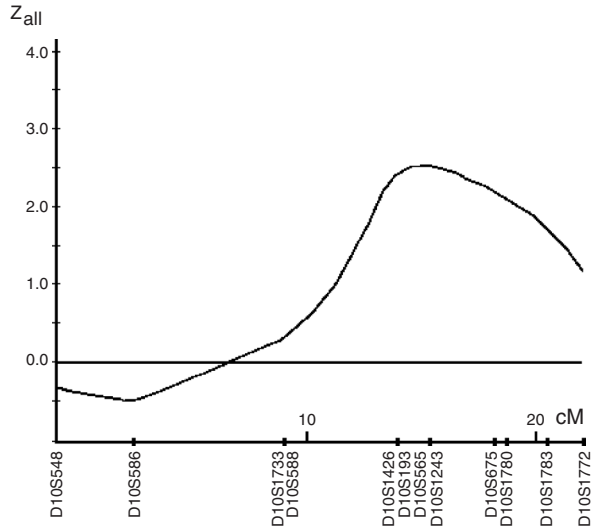

Figure 2 Multipoint nonparametric logarithm (NPL) profile for type 1 diabetes on chromosome 10p11-q11 obtained using the «estimate» command of GENEHUNTER 2.1, assuming dominance variance may exist, and using allele frequencies from parents of affected sibs in the Russian dataset, consisting of 106 identical-by-descent sibling pairs affected with diabetes. A scale of genetic distances between markers is shown in centimorgans (cM) according to the Marshfield sex-average map [17].

in unaffected offspring that hence excludes segregation distortion (Table 2).

To investigate a possible interaction between *IDDM10* and major *IDDM1* susceptibility markers, we performed TDT analysis for these eight microsatellites in the subset of 42 simplex families that have probands, carrying *HLA-DR4* haplotype (*DRB1*04-DQB1*0302*), the commonest *HLA* class II risk haplotype for T1DM in the Russian family dataset (data not shown). We found that an excess of alleles 16 (*D6S1243*) and 26 (*D6S565*) in diabetic progeny was more significant in the *HLA-DR4*-positive subset compared to those in the whole simplex family dataset (Table 2). This did not occur due to the segregation distortion because the allele transmission to unaffected sibs maintained a random pattern. This observation suggests that *IDDM1* and *IDDM10* susceptibility markers could synergically interact resulting in increasing genetic risk for T1DM in human carriers of risk haplotypes sharing predisposing alleles of both susceptibility loci.

D10S565 and *D10S1243* are separated by a physical distance of 510 kbp and show significant pairwise linkage disequilibrium ($D' = 0.81$, $P < 0.001$). Among haplotypes, a combination between alleles 16 (*D10S1243*) and 26 (*D10S565*) was found to be the commonest. In simplex families, this haplotype showed a significant preferential transmission from parents to diabetic progeny (37 transmissions versus 15 nontransmissions, $P = 0.00105$).

Three candidate genes for susceptibility to T1DM encoding islet glutamic acid decarboxylase (*GAD2*), cAMP-responsive element modulator (*CREM*) and transcription factor 8 (*TCF8*) lie at the region of suggestive linkage. Using the TDT analysis, three common SNP markers, each of them is located within a corresponding candidate gene, were studied for association with T1DM in the Russian simplex family dataset. No significant association with the disorder have been found for the Gly326-Glu *GAD2* and C(−11)T *CREM* molecular variants (Table 3). For adenine-to-guanine substitution, located in intron 4 of the *TCF8* gene, significant preference in transmission of the G allele from parents to affected offspring was initially found (uncorrected $P = 0.023$). The significance has been failed after correction for multiple alleles (Table 3). However, preference in transmission of the G allele remained significant even after correction when the simplex family dataset has been stratified for the *HLA-DR4* haplotype (Table 3). This finding suggests that the A182905G molecular variant of *TCF8* could confer susceptibility to T1DM in individuals carrying the *HLA-DR4* risk haplotype.

Discussion

Using multipoint linkage analysis, we showed suggestive evidence of linkage of the chromosomal region 10p11-q11 to T1DM, with a maximum linkage peak between markers *D10S565* and *D11S1243*. In addition, this pair of markers and two other microsatellites showed an association with the susceptibility to T1DM in a Russian population.

One of the markers, *D10S565*, has been previously studied in other ethnic groups. Consensus analysis in the combined US+UK dataset of 767 multiplex families found a maximum peak of linkage in the vicinity to marker *D10S565* [20]. In our study, this marker showed sufficient association and linkage to T1DM.

Therefore, our results suggest a positive evidence for the *IDDM10* susceptibility locus on chromosome 10p11-q11 that has been previously found and strongly replicated in the UK Caucasians [3–5, 20]. However, this conclusion is preliminary because of a relatively small sample size and should be re-evaluated using a larger dataset.

The region of suggestive linkage found in this study spans around 8.0 Mb on chromosome 10. Three putative candidate genes for susceptibility to T1DM lie within this region. There are *GAD2*, *CREM* and *TCF8* genes. The *GAD2* gene encoding islet glutamate decarboxylase (GAD65), a major autoantigen in T1DM, was mapped to chromosome 10p11.23, a region that lies within *IDDM10* locus [21, 22]. The *GAD2* gene has been considered as a strong positional candidate for contribution to the disease susceptibility. However, linkage and association studies failed to found a significant relationship between the gene and susceptibility to T1DM [2, 23, 24]. Recently,

Table 2 Transmission disequilibrium test of chromosome 10p11-q11 microsatellite markers in 114 Russian discordant sibling pairs with type 1 diabetes and in the subset of 42 sibling pairs, with a proband, carrying the predisposing *HLA-DR4* haplotype

	All sibling pairs ($n=114$)										Sibling pairs with the *HLA-DR4* haplotype ($n=42$)									
	Probands					Non-affected sibs					Probands					Non-affected sibs				
Allele	T*	NT†	χ^2 ($df=1$)	P	P_c	T*	NT†	χ^2 ($df=1$)	P	P_c	T*	NT†	χ^2 ($df=1$)	P	P_c	T*	NT†	χ^2 ($df=1$)	P	P_c
D10S1733																				
4	32	36	0.28	0.60		35	33	0.12	0.73		11	10	0.10	0.75		12	9	0.86	0.35	
5	2	0	2.01	0.16		2	0	2.01	0.16		0	0	0	1.00		0	0	0	1.00	
7	11	6	1.54	0.21		7	10	1.06	0.30		5	4	0.22	0.64		4	5	0.22	0.64	
8	8	16	2.84	0.09		11	13	0.33	0.57		4	9	2.63	0.10		8	5	1.39	0.24	
35	14	8	1.73	0.19		9	13	1.45	0.23		10	7	1.06	0.30		8	9	0.12	0.73	
36	85	74	1.25	0.26		76	83	0.62	0.43		26	22	0.67	0.41		20	28	2.67	0.10	
37	41	49	0.91	0.34		51	39	3.20	0.07		15	17	0.55	0.46		18	14	1.00	0.32	
38	9	13	0.77	0.38		11	11	0	1.00		4	4	0	1.0		4	4	0	1.00	
Overall ($df=7$)		10.21	0.18				5.39	0.61				2.60	0.86				3.12	0.79		
D10S588																				
10	11	4	3.41	0.06		6	9	1.20	0.27		4	3	0.29	0.59		4	3	0.29	0.59	
11	31	42	1.73	0.19		37	36	0.03	0.86		10	15	2.00	0.16		12	13	0.08	0.78	
12	33	45	0.84	0.36		41	37	0.41	0.52		10	11	0.10	0.75		12	9	0.86	0.41	
13	106	90	1.12	0.29		97	99	0.05	0.82		47	42	0.56	0.45		43	46	0.21	0.65	
Overall ($df=7$)		5.82	0.12				0.84	0.84				1.47	0.69				0.71	0.87		
D10S1426																				
11	7	5	0.34	0.56		6	6	0	1.0		4	4	0	1.00	3	5		1.00	0.32	
12	28	18	2.47	0.12		19	27	2.78	0.10		12	7	2.63	0.10	9	10		0.11	0.74	
13	66	82	2.83	0.09		78	70	0.86	0.35		30	36	1.09	0.30	31	35		0.49	0.48	
14	45	30	3.74	0.053		34	41	1.31	0.25		15	13	0.29	0.59	16	12		1.15	0.28	
15	24	28	0.36	0.55		30	22	2.46	0.12		7	6	0.15	0.70	8	5		1.39	0.24	
16	27	32	0.85	0.36		30	29	0.04	0.85		7	8	0.13	0.72	8	7		0.13	0.72	
17	0	2	2.00	0.16		0	2	2.01	0.16		0	1	1.00	0.32	0	1		1.00	0.32	
Overall ($df=7$)		9.97	0.13				5.72	0.46				3.15	0.79				3.13	0.79		
D10S193																				
18	3	6	2.00	0.16		5	4	0.22	0.64		1	3	2.00	0.16		1	3	2.00	0.16	
19	1	0	1.00	0.32		0	1	1.00	0.32		1	0	1.00	0.32		0	1	1.00	0.32	
20	14	24	2.91	0.09		21	17	0.84	0.36		8	12	1.60	0.21		13	7	3.60	0.06	
21	12	19	1.71	0.19		15	16	0.07	0.80		4	7	1.63	0.20		7	4	1.63	0.20	
22	57	44	2.25	0.13		50	51	0.02	0.89		22	19	0.44	0.51		17	24	2.39	0.12	
23	11	9	0.21	0.65		10	10	0	1.00		4	4	0	1.00		5	3	1.00	0.32	
24	48	60	1.83	0.18		61	47	3.63	0.06		17	20	0.49	0.22		21	16	1.35	0.25	
25	33	25	1.29	0.26		23	35	4.97	0.023	>0.05	15	10	2.00	0.16		11	14	0.31	0.58	
26	14	10	0.71	0.40		11	13	0.33	0.56		5	4	0.22	0.64		3	6	2.00	0.16	
27	5	1	2.34	0.13		2	4	1.33	0.25		2	0	2.01	0.16		1	1	0	1.00	
Overall ($df=7$)		13.86	0.13				6.71	0.67				7.19	0.62				8.14	0.52		

Table 2 Continued

	All sibling pairs ($n=114$)										Sibling pairs with the *HLA-DR4* haplotype ($n=42$)									
	Probands					Non-affected sibs					Probands					Non-affected sibs				
Allele	T*	NT†	χ^2 ($df=1$)	P	P_c	T*	NT†	χ^2 ($df=1$)	P	P_c	T*	NT†	χ^2 ($df=1$)	P	P_c	T*	NT†	χ^2 ($df=1$)	P	P_c
D10S5652																				
1	20	43	10.01	1.6×10^{-3}	>0.05	34	29	0.79	0.37		9	19	7.14	7.5×10^{-3}	>0.05	16	12	1.14	0.29	
22	12	17	0.93	0.33		15	14	0.007	0.79		6	9	1.20	0.27		8	7	0.13	0.72	
23	8	9	0.12	0.73		8	9	0.12	0.73		3	3	0	1.00		4	2	1.33	0.25	
25	10	5	1.73	0.19		6	9	1.20	0.27		4	3	0.29	0.59		3	4	0.29	0.59	
26	132	96	13.68	2.2×10^{-4}	0.014	113	115	0.004	0.85		49	25	15.57	7.0×10^{-5}	4.3×10^{-3}	31	43	3.27	0.07	
27	13	25	4.20	0.040	>0.05	19	19	0	1.0		4	16	14.40	1.4×10^{-4}	8.7×10^{-3}	13	7	3.60	0.06	
Overall ($df=5$)		20.46	1.0×10^{-3}	8.2×10^{-3}								19.30	1.7×10^{-3}	1.4×10^{-2}			6.79	0.34		
D10S675																				
14	52	65	2.11	0.15		63	54	1.39	0.24		20	24	0.72	0.39		21	23	0.18	0.67	
15	19	24	0.66	0.42		26	17	3.77	0.052		5	10	3.33	0.07		9	6	1.20	0.27	
16	64	50	2.49	0.11		53	61	1.12	0.29		25	16	3.95	0.047	>0.05	20	21	0.05	0.82	
17	45	41	0.24	0.62		39	47	1.49	0.22		21	20	0.05	0.82		21	20	0.05	0.82	
18	5	5	0	1.00		4	6	0.80	0.37		1	2	0.34	0.56		1	2	0.34	0.56	
Overall ($df=7$)		3.93	0.42				4.28	0.37				4.36	0.36				1.07	0.90		
D10S1243																				
8	0	3	3.02	0.08		2	1	0.34	0.56		0	2	2.01	0.16		1	1	0	1.00	
10	5	9	1.18	0.28		8	6	0.57	0.45		2	4	1.33	0.25		3	3	0	1.00	
12	25	29	0.34	0.56		32	22	3.70	0.054		9	11	0.21	0.65		13	7	3.60	0.06	
14	22	29	1.09	0.30		25	26	0.46	0.50		9	11	0.21	0.65		10	10	0	1.00	
15	4	0	4.04	0.044	>0.05	3	1	2.00	0.16		1	0	1.00	0.32		0	1	1.0	0.32	
16	48	21	12.53	4.0×10^{-4}	0.025	30	39	2.35	0.13		23	8	14.50	1.4×10^{-4}	8.7×10^{-3}	13	18	1.61	0.20	
17	3	13	6.49	0.011	>0.05	10	6	2.00	0.16		0	5	10.0	2.2×10^{-3}	>0.05	4	1	3.60	0.06	
18	26	37	2.24	0.14		32	31	0.03	0.86		8	9	0.12	0.73		6	11	2.94	0.09	
19	2	0	2.01	0.16		1	1	0	1.0		0	0	0	1.00		0	0	0	1.00	
20	20	19	0.03	0.86		17	22	1.29	0.26		4	5	0.22	0.64		6	3	2.00	0.16	
21	15	20	0.77	0.38		21	14	2.80	0.09		5	9	1.18	0.28		6	8	0.57	0.45	
22	35	23	2.86	0.09		25	33	2.21	0.14		10	8	0.44	0.51		11	7	1.78	0.18	
23	13	15	0.15	0.70		14	14	0	1.00		4	4	0	1.00		3	5	1.00	0.32	
24	9	11	0.21	0.65		10	10	0	1.00		3	2	0.4	0.53		3	2	0.40	0.53	
30	13	11	0.18	0.67		10	14	0.71	0.40		3	3	0	1.00		2	4	1.33	0.25	
Overall ($df=7$)		33.87	2.2×10^{-3}	0.017			9.49	0.80				35.18	1.4×10^{-3}	0.011			8.15	0.88		
D10S1780																				
21	17	23	1.01	0.31		21	19	0.2	0.65		6	7	0.15	0.70		8	5	1.39	0.24	
22	13	19	1.23	0.27		18	14	1.00	0.32		8	5	1.39	0.24		6	7	0.15	0.70	
23	1	5	2.71	0.10		4	2	1.33	0.25		0	2	2.01	0.16		1	1	0	1.00	
25	6	13	2.72	0.10		11	8	0.95	0.33		4	3	0.29	0.59		2	5	2.57	0.11	
26	129	112	3.55	0.06		117	124	0.41	0.52		49	50	0.02	0.89		48	51	0.18	0.67	
27	14	9	1.16	0.28		9	14	2.17	0.14		4	4	0	1.00		6	3	2.00	0.16	
28	2	1	0.34	0.56		2	1	0.34	0.56		0	0	0	1.00		0	0	0	1.00	
Overall ($df=7$)			9.89	0.13				3.36	0.76			2.92	0.71					3.14	0.68	

*T – transmitted.
†NT – nontransmitted.

Table 3 Transmission disequilibrium test of Ala326Gly *GAD2*, C(−11)T *CREM* and A182905G *TCF8* single-nucleoide polymorphism (SNP) in 114 Russian discordant sibling pairs with type 1 diabetes and in the subset of 42 sibling pairs, with a proband, carrying the predisposive *HLA-DR4* haplotype

			All sibling pairs ($n = 114$)								Sibling pairs with the *HLA-DR4* haplotype ($n = 42$)							
			Probands				Non-affected sibs				Probands				Non-affected sibs			
Gene	SNP	Allele	T*	NT†	χ^2 ($df = 1$)	P	P_c	NT†	T*	χ^2 ($df = 1$)	P	P_c	NT†	T*	χ^2 ($df = 1$)	P	P_c	NT†	T*	χ^2 ($df = 1$)	P	P_c
GAD2	Ala326Gly	Gly	44	56	2.88	0.09		53	47	0.72	0.40		24	30	1.33	0.25		28	26	2.00	0.16	
CREM	C (-11)T	T	58	47	2.31	0.13		48	57	1.54	0.21		33	25	2.21	0.14		27	31	0.55	0.46	
TCF8	A182905G	G	51	36	5.17	0.023	>0.05	40	47	1.13	0.29	–	29	17	6.26	0.012	0.037	20	26	1.57	0.21	–

*T – transmitted.
†NT – nontransmitted.

no major role of the *GAD2* gene in T1DM has been shown in large UK and Finnish family datasets, using a high-density map of SNP located within the gene [25]. We also showed no association of the Gly326Glu dimorphism of the *GAD2* gene with T1DM in a Russian sample. These data suggest that genetic variation within this gene is unlikely to contribute significantly to the pathogenesis of the disease.

cAMP-responsive element modulator, product of the *CREM* gene, regulates production of interleukin-2 (IL-2), a cytokine that directs the growth and proliferation of T cells [26]. CREM protein is increased in T cells of patients with systemic lupus erythematosus, an autoimmune disease with the production of antibodies to components of the cell nucleus in association with a diverse array of clinical manifestations, and this protein has been considered responsible for the decreased production of IL-2 [27]. Moreover, the involvement of CREM protein in impaired insulin gene transcription in the pancreas of diabetic rats was found [28]. These data suggest in favour of *CREM* gene to be evaluated as a likely candidate for susceptibility to T1DM. However, our study failed to detect any association of the C(−11)T dimorphism of the *CREM* gene with T1DM. It is possible that other polymorphic marker could be involved in the susceptibility to the disease. Further investigations are required to conclude whether polymorphic variants of the *CREM* gene are involved in the development of T1DM.

Transcription factor 8 negatively regulates the expression of CD4 and IL-2 by T cells [29, 30], and their gene therefore might be a likely candidate for the susceptibility to T1DM. The *TCF8* gene is located between markers *D10S565* and *D10S1243*, which showed association and maximal strength of linkage to T1DM in the Russian family dataset. We found that the A/G nucleotide variation in intron 4 of the *TCF8* gene is associated with the disease in the subset of Russian simplex families, containing affected sibs with the common predisposing *HLA-DR4* haplotype (Table 3). This suggests that the *TCF8* gene could represent the *IDDM10* susceptibility gene in a Russian population. However, *TCF8* seems to have a modest effect in conferring T1DM susceptibility and rather modulates disease development in *HLA-DR4*-positive subjects. In the future, it is necessary to re-estimate a role of the intron 4 polymorphic site as well as to examine relevance of other genetic variations within the *TCF8* gene to the susceptibility to T1DM in a larger sample size. Finding a common disease-associated *TCF8* haplotype will help to clarify, exactly, whether the *TCF* gene is a true contributor to the T1DM susceptibility within *IDDM10* locus.

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