biochemical autoantibody (p=0.003, Kaplan-Meier log rank test), although the genotype was unrelated to disease development among those with more advanced autoimmunity. Subsequent analysis of ZnT8 antibodies revealed that this antibody was significantly associated with development of diabetes (p=0.047, Fisher´s Exact test) but the DR3/DR4 genotype association with progression to clinical diabetes tended to remain after exclusion of these cases.

Conclusion: These results suggest that autoimmunity spreading associated with the high-risk HLA class II genotype is taking place in subjects positive for only one biochemical autoantibody although such spreading cannot be detected by current autoantibody tests.

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HLA-haplotypes, autoantibodies to beta cells: their role in the prediction of type 1 diabetes

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Background and aims: Risk of Type 1 Diabetes (T1D) development in mainly defined by susceptible haplotypes of HLA DR and DQ genes carriage and specific autoantibodies (Ab) presence. Prediction of early preclinical stage of T1DM.

Materials and methods: Predisposing and protective haplotypes (HLA-DRB1, genes DQ) together with immunological markers (ICA, GADA, IAA) have been studied in 143 discordant families, in normal siblings (N=171; mean age - 11,9±5,8 years). Simultaneously HLA-genotyping was performed in 599 patients with Type 1 Diabetes (mean age - 7,5±6,2 years). Control group included 200 individuals.

Results: During the trial the haplotypes susceptible to T1D [DRB1*4-DQA1*301-DQB1*302 (OR=4,7); DRB1*17-DQA1*501-DQB1*201 (OR=2,7); DRB1*4-DQA1*301-DQB1*304 (OR=4,0); DRB1*1-DQA1*101-DQB1*501 (OR=1,9); DRB1*16-DQA1*102-DQB1*502/4 (OR=2,4)] and protective [DRB1*15-DQA1*102-DQB1*602/8 (OR=0,08); DRB1*11-DQA1*501-DQB1*301 (OR=0,14); DRB1*13-DQA1*103-DQB1* 602/8 (OR=0,16)] were defined in 599 T1D patients, belonging to Russian population. Analyzing of HLA haplotypes prevalence in patients' families and in control group it was revealed that susceptible haplotypes incidence was lower in normal siblings [DRB1*4-DQA1*301-DQB1*302, DRB1*17-DQA1*501-DQB1*201] - 45,6% and 31%, comparing with patients - 61,5% and 51,7%, respectively ($\chi2$=7,93, p<0,001; $\chi2$ =13,93, p<0,0001); though it was higher comparing with control group - 8,5% and 10%, respectively ($\chi2$ =66,7, p<0,0001; $\chi2$ =25,7, p<0,0001). It should be noted that protective haplotypes [DRB1*15-DQA1*102-DQB1*602/8/DRB1*13-DQA1*103-DQB1*602/8] incidence was higher in normal siblings comparing with patients - 22,8% and 4,9%, respectively ($\chi2$ =19,98, p<0,001) and didn't differ from the incidence in control group -22% ($\chi2$=0,03, p=0,85). It is possible to suggest that protective haplotypes play their role in disease susceptibility lowering disease incidence among normal siblings. Three risk groups were selected in siblings of patients with T1D, considering that heterozygosis according to haplotypes [DQA1*0501-DQB1*0201 (DQ2) and DQA1*0301-DQB1*0302 (DQ8)] specifies the highest risk of T1D development. The high genetic risk group (DQ2/DQ8) included 13,5%, moderate risk group (DQ2/X, DQ8/X - 59,7% and low risk group (X/X) - 36,8%, where X is any haplotype, besides DQ2;DQ8. Incidence rate of two and three Ab types in high risk group was higher than in moderate and low risk groups (26,1%, 14,1%, 11,1% respectively; p>0,05; 8,7%, 2,4%, 0% respectively, p<0,05). In this case GAD was defined significantly more often in the group with DQ2/DQ8 genotype, comparing with groups of moderate and low genetic risk (65%; 37,9%; 18%, respectively; p<0,05). Ab to insulin were revealed in 19%; 12,9%; and 3,7% cases, respectively (p<0,05). T1D manifestation was observed in 11 individuals during 11 years of surveillance (6,4%). The disease was detected in 8,7% cases from high risk group, in 8,0% - from moderate risk group and in 3,3% - from low risk group. From those in worm disease developed high risk haplotypes (DQ2; DQ8) were revealed in 82% cases and only 18,2% from examined individuals didn't have susceptible haplotypes.

Conclusion: The data obtained confirmed once more once more, that DQ2, DQ8 haplotypes presence in normal siblings forecast high risk of T1D development, but even these haplotypes absence doesn't completely protect from disease development.

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Role of the repair enzyme PIMT in type 1 diabetes: studies in patients, dogs with diabetes and PIMT knock-out mice

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Background and aims: Post-translational protein modifications may create new antigenic epitopes and elicit autoimmunity. The enzyme PIMT, encoded by PCMT1, repairs isomerised Asn and Asp residues (IsoAsp). We showed that a compound up-regulating PCMT1, delays diabetes onset in the BB rat. The aim of this study was to assess the role of PIMT/PCMT1 in diabetes by studying several models.

Materials and methods: Patients (with GAD65 and/or IA2 antibodies) with a short duration (<5years) of T1D were selected. As control groups, patients with T2D (<5 years since diagnosis and no complications) and healthy subjects matched for age and gender were selected. Samples from diabetic and non-diabetic dogs were collected. The expression of PCMT1 mRNA was assessed by qPCR. Transcription levels were normalized to β-actin by comparative Ct. Red blood cells were filtered and lysed. The cytosol was separated and depleted of haemoglobin and Western Blot for PIMT was performed. Heterozygous PIMT+/- mice were crossed and their offspring, genotyped. PIMT knock-out mice were compared with their littermates at 5-6 weeks of age. Oral glucose (2g/Kg) and insulin tolerance tests (0.3 U/Kg) were performed. Differences were analysed using Mann-Whitney's U test.

Results: Blood samples are available from 82 participants, though only 52 (50% women) have been analysed. Median [range] PCMT1 expression tended to be reduced in the 19 T1D (3.03 [0.49-16]; p=0.083) as compared to the 28 controls (6.57 [0.81-32.67]), whereas no difference was found in the small group (N=5) with T2D (6.73[0.42-15.56]). When only the 15 matched control-T1D pairs were analysed, differences were non-significant (p=0.24). PCMT1 expression was similar in the 8 diabetic (0.95 [0.31-3.89]) and the 9 non-diabetic (0.91 [0.88-1.09]) dogs (p>0.2). Western Blot analysis has so far been performed in 15 human subjects (5 per group), where it showed higher PIMT expression in controls than in T1D and T2D patients. The first litter of mice analysed included one knock-out, 5 heterozygous and 3 wildtype mice. Glucose averages 15 min. after the oral glucose load, were 351 mg/dl in the knock-out, 300 mg/dl in the heterozygous and 262 mg/dl in the wildtype subjects (p>0.2). The glucose nadir after insulin administration was 42, 39 and 40% of baseline, respectively (p>0.2).

Conclusion: PCMT1 mRNA expression was normal, both in patients with T1D and in dogs with diabetes. However, preliminary results point towards possible differences in protein expression, suggesting that it might be post-transcriptionally regulated. Furthermore, although sample sizes are still small, the Pcmt gene seems to have a dose effect on glucose tolerance in mice. Ongoing experiments and sample collection should add to these results.

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