STRESZCZENIE W JĘZYKU ANGIELSKIM

Analysis of selected rs2476601 A/G-PTPN22, rs20541 A/G-IL13, rs29941 A/G-KCTD15 gene polymorphisms in the pathogenesis of type 1 diabetes in children.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by pancreatic β-cell damage leading to insulin deficiency. The pathogenesis of autoimmune type 1 diabetes is a complex process with still unclear, multifactorial etiology. Genetic predisposition, environmental and immunological factors play an important role in it. Current epidemiological studies indicate an increase in new cases of T1DM in Poland by 7% per year, up to currently 17.3 new cases under 14 years of age per 100,000 per year. The largest increase was recorded in children aged 5-9 years, with no differences due to gender. Due to the highest increase in the incidence of T1DM in the group of younger children, we can assume that genetic factors and epigenetic factors influencing gene expression play an important role in the pathomechanism of the development of type 1 diabetes.

Type 1 diabetes has a polygenic basis. About half of the risk of developing T1DM is determined by human leukocyte antigens (HLA). Due to the diverse role of HLA genes in T cell selection, antigen presentation and immune response, there is a strong relationship between HLA and the risk of T1DM occurrence and progression. HLA DR and DQ allele combinations determine genetic predisposition. To date, more than 60 chromosomal regions associated with increased susceptibility to T1DM have been identified. Susceptibility genes for the development of autoimmune diseases and immunomodulatory genes were identified.

OBJECTIVE OF THE WORK

The main aim of the work was:

Determination of relationships between the occurrence of selected polymorphisms: rs2476601 of the PTPN22 gene, rs20541 of the IL-13 gene and rs29941 of the KCTD15 gene with the occurrence of type 1 diabetes in the study group compared to the control group.

Additional goals were:

- Determination of the relationships between the occurrence of polymorphisms rs2476601 of the PTPN22 gene, rs20541 of the IL-13 gene, rs29941 of the KCTD15 gene in the group of patients with T1DM and the age of onset of type 1 diabetes.

- Determination of the relationships between the occurrence of rs2476601 polymorphisms of the PTPN22 gene, rs20541 of the IL-13 gene, rs29941 of the KCTD15 gene in the group of patients with T1DM and the presence of antidiabetic antibodies ICA, GAD, IA2, ZnT8, IAA.

- Determination of relationships between the occurrence of polymorphisms: rs2476601 of the PTPN22 gene, rs20541 of the IL13 gene, rs29941 of the KCTD15 gene, and anthropometric parameters.

- Assessment of the value of glycated hemoglobin (HbA1c) at the time of diagnosis of type 1 diabetes depending on the occurrence of polymorphisms rs2476601 of the PTPN22 gene, rs20541 of the IL13 gene, rs29941 of the KCTD15 gene.

MATERIAL AND METHODS

The study included 183 patients: 82 girls (44.8%)/101 boys (54.6%) diagnosed with type 1 diabetes based on the guidelines of the Polish Diabetology Society. The mean age in the study group was 10.35±3.9 years. The control group included 160 healthy volunteers: 75 girls (46.9%)/ 85 boys (53.1%), unrelated to T1DM patients. The participants in the study were aged 16.3 ± 3 years, with informed consent, without concomitant autoimmune diseases. The gender distribution in both groups was comparable.

Blood samples for EDTA were collected in all children to determine the polymorphisms of the genes PTPN22 (rs2476601), IL-13 (rs20541), KCTD15 (rs29941). Isolation of genomic DNA from peripheral blood leukocytes was carried out by the so-called salting-out method. salting out", followed by the actual PCR reaction (Life Technologies, USA). Molecular probes of the TaqMan type (Life Technologies, USA) were used to determine polymorphisms. In addition, the analysis took into account anthropometric data: sex, height, body weight, BMI and BMI standard deviation index (BMI SDS) were calculated, as well as the age of the patients in the study group at the time of onset of T1DM. In addition, the value of glycated hemoglobin (HbA1c) at the time of diagnosis of T1DM and the number of insulin units per kilogram of body weight per day and the presence of antidiabetic antibodies were recorded from the medical records.

The obtained results were subjected to statistical analysis. Contingency analysis was performed to determine the strength of the association between the frequencies of specific alleles/genotypes and the incidence of type 1 diabetes. Fisher's exact test was used for this purpose. Additionally, the value of the odds-ratio and the related exact confidence level of 95% were determined. The exact confidence interval for the odds ratio and the associated probability value p were determined using the median-unbiased odds ratio estimator. In order to detect potential relationships between alleles/genotypes and numerical traits, the t-test and the non-parametric Wilcoxon test were used. The choice of the test was conditioned by the fulfillment of the following assumptions: (a) distribution of the feature close to the normal distribution, verified by the Shapito-Wilk test; (b) of variance homogeneity, verified by Leven's test. If a significant result was obtained by the t-test or the Wilcoxon test, a post-hoc analysis was performed consisting in pairwise testing. In cases of multiple testing (e.g. post-hoc), the p-value correction – FDR – was applied. In addition, as a supplement to the contingency analysis, proportion tests were performed. The R statistical environment was used for the calculations, and the significance level was set at alpha = 0.05.

RESULTS

Based on the conducted analysis, the following conclusions were made regarding polymorphism:

rs29941 KCTD15

The occurrence of the allele A rs29941 of the KCTD15 gene was found to be 2.39 times more frequent in patients with type 1 diabetes compared to the control group than in the presence of the allele G rs29941. It was observed that with increasing number of G allele in SNP rs29941 KCTD15, both for genotype G/G vs. A/A and A/G vs. A/A, the risk of T1DM decreases. In the group of girls with the rs29941 KCTD15 G/G genotype and in the group of boys with the G/G rs29941 KCTD15 genotype, a lower incidence of type 1 diabetes was found. No statistical

significant has been found in distribution of A/A, A/G and G/G genotypes of KCTD 15 by sex in the group of patients with type 1 diabetes. Girls with genotype A/A rs 29941 of the KCTD gene were characterized by a lower average age at the time of diagnosis of T1DM (8.04 years) compared to genotype A/G (10.72 years). There were no differences between the average BMI and HbA1c values at the time of the diagnosis of T1DM and the occurrence of individual rs29941 alleles of the KCTD15 gene. In girls with allele A rs29941, a significantly higher percentage of positive ICA antibodies (>50%) was found. In the group of boys with T1DM having the rs29941 KCTD15 polymorphism, it was shown that the G allele is more common in the group of patients with positive ICA antibodies than in the group with negative antibodies. It was shown that in the case of the rs29941 KCTD15 polymorphism in boys, the chance of ICA antibody appearance rises with the increase in the number of G alleles.

rs20541 IL-13

Patients with the rs20541 polymorphism of the IL-13 gene with the G allele had higher mean daily insulin doses (insulin units/body weight (kg)/day) than patients with the A allele, respectively 0.84 U/body weight(kg)/day vs. 0.66 U/body weight(kg)/day. Boys with the G/G rs20541 genotype were on average 3 years younger at the time of diagnosis of T1DM compared to those with the A/A rs20541 genotype, 10.47 years vs. 13.24 years, respectively. In the presence of the allele G rs20541, a significantly higher percentage (>50%) of positive ICA and ZnT8 antibodies was found in the group of women than in the presence of the A allele.

rs2476601 PTPN22

It was found that with the appearance of the allele A rs2476601 of the PTPN22 gene, the risk of developing type 1 diabetes is 1.93 times the risk of developing diabetes in the presence of the allele G. In girls, with the occurrence of the allele A rs2476601, the risk of developing the disease is 2.23 of the risk of developing the disease with the presence of the allele G. rs2476601 of the PTPN22 gene in the group of girls showed a statistically significant difference in the average age of onset in girls with the A/A genotype of 6.5 years on average vs. the G/G genotype of 10.3 years on average. Comparing the average BMI at the time of T1DM diagnosis, it was shown that the AA genotype rs 2476601 PTPN 22 was characterized by a higher average BMI in the group of boys than the GG genotype, 25.6 kg/m2 vs. 19.41 kg/m2, respectively. A significantly higher percentage of positive ICA antibodies (>50%) was found in the group of girls with T1DM with the allele G. In the group of boys, it was shown that with the appearance of the A allele rs 24766012 PTPN 22, the probability of ICA antibodies increases.

CONCLUSIONS

The analysis of selected gene polymorphisms: rs2476601 PTPN22, rs29941 KCTD15, rs20541 IL13 may indicate their participation in the pathogenesis of type 1 diabetes.

rs 2476601 polymorphism of the PTPN 22 gene is involved in the pathogenesis of type 1 diabetes in children. The A allele of rs 2476601 shows a predisposing effect on T1DM, the predisposition is particularly expressed in females. On the other hand, the G rs 2476601 allele of the PTPN 22 gene has a protective effect.

The rs29941 KCTD 15 polymorphism is involved in the pathogenesis of type 1 diabetes. The G allele has a protective effect against the development of type 1 diabetes. The relationship is marked both in the distribution of alleles and genotypes, with no differences depending on sex.

In the group of children with T1DM, the occurrence of the A allele of the rs20541 polymorphism of the Interleukin-13 gene was significantly higher than in the group of healthy people.

In the group of girls with T1DM with genotype A/A rs 2476601, a significantly lower mean age of onset is observed compared to genotype G/G, 6.5 vs. 10.3 years. In the group of boys with T1DM with genotype G/G rs 20541 IL-13, the average age of onset of T1DM is significantly lower by 3 years compared to genotype A/A rs 20541, respectively 10.47 vs. 13.24 years old. Girls with the rs 29941 polymorphism with the A/A genotype developed T1DM more than 2 years earlier compared to the A/G genotype.

Boys with the rs2476601 polymorphism are more likely to develop ICA with the appearance of the A allele, similarly for girls with the rs2476601 polymorphism with the G allele and rs20541 with the G allele. rs20541 with the G allele correlates with a higher prevalence of ZnT8 antibodies than in the group with the A allele.

Correlations of rs2476601, rs20541 and 29941 polymorphisms with the BMI standard deviation showed that the A/A rs2476601 PTPN22 genotype was characterized by a significantly higher average BMI in the group of boys compared to the GG genotype. It was found that with an increase in the number of G alleles, the chance of obesity decreases compared to the A allele.