Investigation of associations between genetic polymorphisms and type 1 diabetes mellitus

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PhD Thesis

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Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease where the insulin-producing β cells of the islets of Langerhans are destroyed by the immune system, leading to a lifelong demand for exogenous insulin. Approximately 0.4 % of Caucasians develop T1DM. Both genetic and environmental factors are important for disease development, as seen from the 50 % concordance rate between monozygotic twins compared to 0% to 5-6% in dizygotic twins. Although rare mono or oligogenic forms of T1DM exist, the inheritance pattern of common forms of the disease is polygenic. HLA region genes collectively contribute the major susceptibility (almost 50 %). The remaining susceptibility genes have much lower overall contribution to diabetes risk. Identification and functional analysis of additional loci will provide pathophysiological insights necessary for the development of preventive interventions and individual risk prediction.

Studies over the last 20 years have examined the possible involvement of cytokines in the pathogenesis of T1DM. A variety of cytokines have been found to be expressed in the insulitis lesion of autoimmune diabetes-prone non-obese diabetic (NOD) mice and Biobreeding (BB) rats, as well as in the pancreata of humans with T1DM.

Coeliac disease (CD) is characterised by severe inflammation of the small intestine, which is triggered by gliadin. Prevalence of celiac disease in type 1 diabetes mellitus children is higher than that in nondiabetic children. This phenomenon is probably because of several common immunpathogenic factors. The environment of the ongoing diabetic autoimmunity may be a stimulant to the development of CD, a disease that possesses autoimmune features.

The genes of the inflammatory proteins which contribute to the development of T1DM, coeliac disease or both may contain certain polymorphisms. These polymorphisms or combinations of polymorphisms might play an essential role in the pathogenenesis of T1DM, CD or both by influencing the quality or the quantity of the protein coded by the gene.

TNF-α and IL-1β are pro-inflammatory cytokines produced primarily by activated macrophages that infiltrate the islets during the pathogenesis of diabetes. IL-6 may contribute not only to inflammatory processes that occur in autoimmune diabetes, but also to cellular neogenesis, which may indicate a role in tissue repair. The expression of heat shock protein (HSP) is probably one of the most conservative mechanisms of cellular protection from stress and HSPs have been indicated to play a cytoprotective role against a variety of toxic mediators. Protecting ß-cells against the toxic effect of free radicals HSP can reduce the cytotoxic effect of TNFα.

TNFα $^{-308}$A carrier cells sectret higher amount of TNFα than cells with $^{-308}$GG genotype. The presence of $^{-238}$A allele was associated with autoimmune and inflammatory diseases, however the role of TNFα G^{-238}A polymorphism in the excretion of TNFα is controversial. The carrier state of the IL-1β ^{3954}T allel is associated with enhanced cytokine production. The IL-6 $^{-174}$G allel carrier state is associated with higher amount of IL-6 both in vitro and in vivo. HSPA1B ^{1267}A allele is associated with enhanced protein production.

CD14 and Toll-like receptor 4 (TLR4) are part of the lipopolysaccharide (LPS) receptor complex of the innate immune system. Functional SNPs of CD14 and TLR4 might alter the innate mucosal immune response to bacterial antigens, thereby eliciting a stronger reaction to infection. The consequent inflammation in the jejunal mucosa might disrupt the intestinal

permeability barrier, leading to an increased gluten load in the lamina propria and to the development of CD.

TLR-4 ^{896}G allel carrier state is associated with less amount of inflammatory cytokines.

The homozygous CD14 $^{-260}$TT genotype causes an increased CD14 expression compared to the C/T and C/C genotypes

Aims

1. Investigation of associations and joint associations between polymorphisms of cytokines and inflammatory proteins and risk of T1DM or age-at-onset of T1DM:

- TNFα promoter region $G^{-308}A$ polymorphism
- IL-1β exon 5 $C^{3954}T$ polymorphism
- IL-6 promoter region $G^{-174}C$ polymorphism
- HSPA1B (HSP72) $A^{1267}G$ polymorphism

2. Investigation of associations between polymorphisms of cytokines and inflammatory proteins or HLA haplotypes and coeliac disease in T1DM patients:

- TNFα promoter region $G^{-308}A$ - and $G^{-238}A$ polymorphisms
- CD14 promoter region $C^{-260}T$ polymorphism
- TLR-4 $A^{896}G$ polymorphism
- HLA DQ haplotypes

Patients and methods

Studied population

Children, treated with T1DM or coeliac disease in the first Department of Paediatrics, Semmelweis University was involved in the study. The control groups represented healthy hungarian blood donors and term infants (fifth postnatal day metabolic screening). The distribution of HLA-DQ genotypes of the patients was compared to that of 2080 consecutive Hungarian cadaveric organ donors typed at the Hungarian National Blood Transfusion Service, Budapest, Hungary.

Genotyping

Total genomic DNA was extracted from whole blood using the method of Miller et al. Polymorphisms of TNFα, IL-1β, IL-6, HSPA1B, TLR4, CD14 were determined by polymerase chain reaction (PCR) – restriction fragment length polymorphism (RFLP) method. PCR-based HLA-DQ typing was performed using a low-resolution kit.

Statistical analysis

Hardy-Weinberg equilibrium was calculated to evaluate the relationship between gene frequencies and genotype frequencies. Chi square test and Fisher test were used to compare categorical data. Associations between SNPs and continuous variables were analysed with t-test, one-way ANOVA, Mann-Whitney or Kruskal-Wallis and Dunn's test. We used multiple regression to the analysis of the association between SNPs and age-at-onset of T1DM. For calculations SPS 11.5 and S.A.S. 8.2 were used.

Results

1. TNFα promoter region $G^{-308}A$ polymorphism was not associated with age-at-onset of T1DM. The prevalence of TNFα $^{-308}$A allele was higher among children with T1DM, than the healthy reference value.
2. IL-1β exon 5 $C^{3954}T$ polymorphism was not associated with age-at-onset of T1DM. The prevalence of IL-1β ^{3954}T allele was higher among children with T1DM, than the healthy reference value.
3. IL-6 $^{-174}$G allele carrier state was associated with older age-at-onset of T1DM but only in the presence of high IL-1β (^{3954}T carrier state) and TNFα ($^{-308}$A carrier state) producer genotypes.
4. The prevalence of HSPA1B ^{1267}G allele was associated with T1DM. The presence of TNFα $^{-308}$A allele together with HSPA1B ^{1267}G carrier state was associated with increased risk of T1DM compared to individuals with no TNFα $^{-308}$A and HSPA1B ^{1267}G allele.
5. A TNFα $G^{-308}A$ polymorphism was not associated with increased risk of coeliac disease in T1DM patients. TNFα $G^{-238}A$ polymorphism was associated with increased risk of coeliac disease in T1DM patients.
6. TLR-4 $A^{896}G$ polymorphism was not associated with increased risk of T1DM or CD. The prevalence of CD14 $^{-260}$TT genotype was lower only in T1DM group not in T1DM+CD group.
7. In T1DM the frequency of the homozygous HLA-DQ8 genotype was significantly higher than in CD while the frequency of homozygous or heterozygous HLA-DQ2 genotypes without DQ8 did not differ from controls. In patients with CD both homozygous and heterozygous HLA-DQ2 (DQ8-) genotypes were significantly more frequent compared to the control and the T1DM group, while no elevation in the frequency of the HLA-DQ8 genotypes (DQ2-) were observed. In T1DM and in CD+T1DM

the occurrence of HLA-DQ2/8 heterozygosity was significantly higher compared to both children with CD only and population controls.

In our study we found an association between the joint presence of high TNFα ($^{-308}$AA and AG) and low HSP72 (^{1267}AG and GG) producer genotypes in one hand and the risk of T1DM on the other. Higher production of TNFα may contribute to the development/maintenance of destructive insulitis and lower level of HSP 72 makes ß-cells less resistant to the autoimmune process, and hereby might contribute to the development of T1DM. We found an association between IL-6 $^{-174}$G allele carrier state and older age-at-onset of T1DM, but only in the presence of high IL-1β (^{3954}T allele carrier state) and TNFα producer genotypes. The higher IL-6 production associated with the $^{-174}$G allele in Langerhans islets, might have a protective effect against the autoimmune process and might delay the destruction of the β-cells. We found a significantly higher rate of carriers of TNFα $^{-238}$A allele in the histology-proven CD group than in the non-CD group. The significance of this finding is still unclear. The functional importance of G^{-238}A polymorphism has not been clarified yet. We found that in children with T1DM the frequency of the high CD14 producer $^{-260}$TT genotype was decreased, but in children affected by both CD and T1DM the occurrence of the CD14 TT homozygous mutant genotype was not decreased. CD14 is an important factor of inflammation in coeliac disease, but may have some protective effect in the pathogenesis of T1DM.
Both CD and T1DM are associated with the presence of DQ2 and DQ8. Our results confirm that for CD the major susceptibility factor is DQ2, while for type 1 diabetes DQ8 is a stronger susceptibility factor than DQ2 also in the Hungarian population. We also confirm that DQ2/DQ8 heterozygosity is the strongest risk factor for the development for T1DM

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