THE EFFECT OF GENETIC FACTORS ON THE CLINICAL PHENOTYPE IN
DIFFERENT FORMS OF DIABETES

Ph.D Thesis

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1. **INTRODUCTION**

Since the heterogeneity of diabetes mellitus has been accepted the disease is divided into two main clinical entities: type 1 diabetes (T1D), which develops as a result of immune-mediated destruction of pancreatic β-cells in patients with genetic susceptibility and type 2 diabetes (T2D), which develops as an interaction of β-cell dysfunction based on mainly genetic susceptibility and insulin resistance caused by principally environmental factors. A special subgroup of T1D, the latent autoimmune diabetes in adults (LADA) is considered as a slowly progressive form of the immunity-mediated type 1 diabetes.

Recently, a lot of research has made new important discoveries regarding the pathogenesis of diabetes, for example β-cell specific immune cells have also been demonstrated in T2D patients and insulin resistance has also been recognized in T1D. Another surprising result that LADA shares genetic features with type 2 diabetes.

Based on these observations a new theory emphasising the continuity of diabetes-spectrum has been introduced.

2. **AIMS OF THE STUDY**

One way leading to understand the pathophysiological processes is to detect the genetic background of diseases accurately. In the case-control studies I assessed the contribution of genetic factors to the risk of different clinical types of diabetes in the Hungarian and European populations. Additionally, I examined the geographical variability of risk alleles and the heterogeneity of gene effects among diabetes groups and the populations with European ancestry. The primary aims of my clinical studies were to detect the phenotype-genotype interactions and to
analyse the possible associations between the diabetes risk allele carrier status and the disease progression.

1. study: Genetic studies in patients with latent autoimmune diabetes in adults

The WHO classifies latent autoimmune diabetes in adults as a slowly progressive subgroup of type 1 diabetes. However, some characteristics of LADA resemble rather type 2 diabetes and the recent studies suggest that the type 2 diabetes-associated gene variant of transcription factor 7-like 2 (TCF7L2) has been proposed to be associated with latent autoimmune diabetes in adults. The genetic results related to populations of European ancestry are controversial.

Questions:

1. Do the type 2 diabetes-associated gene variants, the TCF7L2 gene rs7903146, the CDKN2A/2B (cyclin-dependent kinase inhibitor 2A and 2B) gene rs10811661 and the PPARG (peroxisome proliferator-activated receptor gamma) gene rs1801282 polymorphisms increase the risk of latent autoimmune diabetes in adults in the Hungarian population?

2. Can the disease association of TCF7L2 gene rs7903146 polymorphism be confirmed in Europeans?

3. Are there any interactions between the studied diabetes risk alleles and the clinical data?

2. study: Genetic studies in patients with type 2 diabetes

Type 2 diabetes has a polygenic inheritance; till now the genome-wide association scans have proved the connection of the 40 genetic loci with the disease. These
genes show low penetrance and mild diabetogenic effect individually and their population variance changes between wide ranges.

Questions:

1. Are the strongest type 2 diabetes-susceptibility gene variants, the TCF7L2 gene rs7903146, the CDKN2A/2B gene rs10811661 and the PPARG gene rs1801282 polymorphisms associated with type 2 diabetes in the Hungarian population?
2. Are there any interactions between the carrier status of the studied diabetes risk alleles and the clinical data?
3. What are the geographic variability of TCF7L2 gene variants and the heterogeneity of gene effect among populations and between patients with LADA and type 2 diabetes?

3. study: Genetic studies in patients with type 1 diabetes

Type 1 diabetes has a polygenic inheritance; till now the genome-wide association scans have proved the connection of the 41 genetic loci with the disease. The HLA-DR-DQ locus plays a major role, and is responsible for 40-50% of familial clustering. Another 15% is defined by insulin gene and PTPN22 (protein tyrosine phosphatase non-receptor 22) gene, which are the most potential minor susceptibility factors for type 1 diabetes.

Questions:

1. Do the insulin gene rs689 and the PTPN22 gene rs2476601 polymorphisms contribute to the risk of type 1 diabetes in the Hungarian population?
2. Are there any interactions between the carrier status of the studied diabetes risk alleles and the clinical data?
4. study: Analysis of the genetic background of an autoimmune triad

Autoimmune diseases are initiated by interaction between genetic and environmental factors and caused by the loss of immunologic tolerance to self-antigens. They cluster within families and individuals, but the aggregation in a triad is quite rare.

I report a case of a young girl affected by three organ-specific autoimmune disorders, among them type 1 diabetes developed first, followed by Hashimoto’s thyroiditis and juvenile rheumatoid arthritis. A detailed genetic study included genotyping of HLA-DR-DQ, CTLA4 and PTPN22 (cytotoxic T-lymphocyte associated antigen 4) gene regions was performed. These genes have been associated with autoimmunity in general and their variants confirm increased risk to all three diseases.

Question:
1. What is the importance of interaction between major and minor genetic factors in the determination of the individual clinical phenotype?

3. Patients and Methods

Diagnostic criteria: the diagnosis of the different diabetes forms was based on the criteria of the World Health Organisation (WHO) accepted in 1998.

Type 1 diabetes: All T1D patients were under the age of 15 years at diagnosis, they had classic diabetic symptoms (polyuria, polydipsia and weight reduction). The patients had lower fasting serum C-peptide levels (<0.35 nmol/l or <1.07 ng/ml) and diabetes-associated autoantibody-positivity [islet cell antibody (ICA),
GAD antibody (GADA), insulin autoantibody (IAA) and insulinoma-associated protein 2 antibody (IA-2A)]. They required prompt insulin therapy at diagnosis.

**Latent autoimmune diabetes in adults:** LADA diagnosis was established if the age at onset was above 35 years, at least one of the circulating islet autoantibodies (ICA, GADA, IAA, IA-2A) was detected, and insulin treatment was not indicated in the first 6 month after the diagnosis.

**Type 2 diabetes:** Type 2 diabetic individuals were above 35 years, antibody-negative (ICA, GADA, IAA, IA-2A) and insulin-independent at the time of diagnosis.

**Controls:** In the Hungarian part of the **case-control studies Nº 1-3.** the healthy controls with normal fasting glucose levels and without a family history of diabetes were recruited among blood donors from Budapest. The collection of blood samples was occurred related to public blood donations organized by the *Regional Blood Transfusion Centre of Buda* (1113 Budapest, Karolina út 19-21.). Using a questionnaire survey I excluded those donors from the study who are not of European descent, or who has relatives with any type of diabetes, an autoimmune disease or genetic syndrome.

In the **meta-analyses Nº 1-2.** controls were recruited among people with healthy metabolism from the studied country. The **study Nº 4.** did not required controls.

All participants of the studies were European ancestry.

Informed consent was obtained from participants. The studies were approved by the National Ethics Committees and were conducted according to the principles of the Declaration of Helsinki.
Table 1: The participants of studies

<table>
<thead>
<tr>
<th>Number of the study</th>
<th>Genes</th>
<th>Type of the study</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nº 1.</td>
<td><em>TCF7L2</em>, <em>CDKN2A/2B</em>, <em>PPARG</em></td>
<td>case-control</td>
<td>211 LADA, 545 T1D</td>
<td>1497</td>
</tr>
<tr>
<td></td>
<td></td>
<td>meta-analysis</td>
<td>999 LADA, 1880 T1D</td>
<td>5358, 5163</td>
</tr>
<tr>
<td>Nº 2.</td>
<td><em>TCF7L2</em>, <em>CDKN2A/2B</em>, <em>PPARG</em></td>
<td>case-control</td>
<td>1297 T2DM</td>
<td>1497</td>
</tr>
<tr>
<td></td>
<td></td>
<td>meta-analysis</td>
<td>4529 T2DM</td>
<td>5163</td>
</tr>
<tr>
<td>Nº 3.</td>
<td><em>PTPN22</em>, <em>INS</em></td>
<td>case-control</td>
<td>572 T1DM</td>
<td>236</td>
</tr>
<tr>
<td>Nº 4.</td>
<td><em>HLA DRB1-DQA1-DQB1</em>, <em>PTPN22</em>, <em>CTLA4</em></td>
<td>case-report</td>
<td>1 subject</td>
<td>-</td>
</tr>
</tbody>
</table>

**Clinical data:**

**Fasting C-peptide concentrations** were analysed with radioimmunoassay, normal value was 0.35 to 1.15 nmol/l (1.05-3.45 ng/ml). The assay had no detection limit.

**Glycated haemoglobin A₁c levels** (HbA₁c) were measured with column assay; normal range was determined as 4 to 6%.

**Body mass index** (BMI) was calculated as weight/height². BMI data were divided into two subgroups according to the International Classification and Child Growth Standards of the WHO:
non-overweight: BMI <25 kg/m² for adults or BMI less than 1 SD above the mean for children,
overweight: BMI ≥25 kg/m² for adults or BMI 1 SD or more above the mean for children.

**Genetic studies:**
Total DNA was isolated from peripheral blood lymphocytes using salting out method. Quant-iT™ PicoGreen® dsDNA Assay Kit was using to DNA quantitation. Genotyping of the polymorphisms of PTPN22 (rs2476601), INS (rs689), CTLA4 (rs3087243), TCF7L2 (rs7903146), CDKN2A/2B (rs10811661) and PPARG (rs1801282) gene was carried out using an allelic discrimination assay (TaqMan®, Applied Biosystems, Foster City, CA, USA). The HLA DR-DQ genotype was defined by a low-resolution full house typing with a lanthanide labelled oligonucleotide hybridization method (DELFIA®, PerkinElmer Inc., Turku, Finland).

**Statistical analyses**
Before starting the case-control studies I estimated the required number of cases to ensure the adequate statistical power for the examination of gene polymorphism.

Each analysis was performed using SPSS for Windows (version 17.0; SPSS, Chicago, IL, USA). Disease association was assessed by odds ratio (OR) and 95% confidential interval (95% CI) using logistic regression model adjusted for age, sex and BMI. The genotype distributions were compared with Hardy-Weinberg equilibrium.
The databases searched included MEDLINE and the Cochrane Library. Meta-analysis was performed using **MIX software**. Between-study heterogeneity assessing by Cochran’s Q-test was not statistically significant. Thus, fixed-effect method of Mantel Haenszel was performed to pool the allelic OR. The Breslow–Day test was used to assess allelic heterogeneity. Population attributable risk was calculated as \( \text{PAR} = \frac{(X-1)}{X} \), assuming the multiplicative model where \( X = (1-f)^2 + 2f(1-f)\gamma + f^2\gamma^2 \); \( \gamma \) is the allelic OR, \( f \) is the frequency of the risk allele.

The normality and homogeneity of variables were checked by Shapiro-Wilk’s W and Levene’s tests. The results were given by descriptive method as the sample size (n) for discrete variable (e.g. gender) and as means\( \pm \)SD (standard deviation) for the continuous variables (e.g. age, BMI, C-peptide level). Group comparisons were carried out by one-way analysis of covariance (ANCOVA with age and gender as covariates), followed by Tukey’s test as post-hoc analysis. Categorical variables were compared using Pearson’s \( \chi^2 \) test.

All reported \( p \)-values were two-tailed and differences were considered to be statistically significant at \( p<0.05 \).

4. **RESULTS**

In all studies no deviation from the Hardy–Weinberg equilibrium was found in patients and control groups (all \( p \)-values are > 0.005).

1. **study**: Genetic studies in patients with latent autoimmune diabetes in adults

In the Hungarian dataset, the \( TCF7L2 \) gene rs7903146 polymorphism showed significant disease associations with LADA (OR 1.34, 95% CI 1.07, 1.68;
\( p=0.0127 \), but not with type 1 diabetes (OR 0.98, 95% CI 0.73, 1.31; \( p=0.5219 \)). The PAR of the \textit{T-allele} for LADA was 15.21% in the Hungarian population.

In the Hungarian dataset, the \textit{CDKN2A/B} gene (\textit{T-allelic} OR 1.07, 95% CI 0.82-1.40; \( p=0.6221 \)), and the \textit{PPARG} gene (\textit{C-allelic} OR 1.03, 95% CI 0.74-1.42; \( p=0.8628 \)) were not associated with LADA.

The meta-analysis of published studies and our results comprised a total of 999 LADA patients and 5,358 control individuals of European ancestry, and yielded a modest, but significant effect for the \textit{TCF7L2} rs7903146 \textit{T-allele} on LADA risk (OR 1.28, 95% CI 1.15, 1.42; \( p<0.0001 \); Fig. 1). Between-study heterogeneity was not significant (\( p=0.9288 \)). The PAR of the \textit{T-allele} for LADA was assessed 12.99% in the European populations.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Population Size</th>
<th>LADA/Controls</th>
<th>MAF</th>
<th>OR (95% CI)</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervin et al.</td>
<td>Swedish/Finnish</td>
<td>357/1639</td>
<td>27.31/22.57</td>
<td>1.28 (1.07-1.54)</td>
<td>0.0069</td>
<td></td>
</tr>
<tr>
<td>Szerpiewska et al.</td>
<td>Polish</td>
<td>68/195</td>
<td>31.62/24.87</td>
<td>1.39 (0.91-2.14)</td>
<td>0.1263</td>
<td></td>
</tr>
<tr>
<td>Pettersen et al.</td>
<td>Norwegian</td>
<td>113/1482</td>
<td>27.88/25.24</td>
<td>1.14 (0.84-1.54)</td>
<td>0.3801</td>
<td></td>
</tr>
<tr>
<td>Zampetti et al.</td>
<td>Italian</td>
<td>250/545</td>
<td>39.60/34.22</td>
<td>1.26 (1.01-1.56)</td>
<td>0.0380</td>
<td></td>
</tr>
<tr>
<td>Lukacs et al.</td>
<td>Hungarian</td>
<td>211/1497</td>
<td>31.75/25.89</td>
<td>1.34 (1.07-1.68)</td>
<td>0.0127</td>
<td></td>
</tr>
<tr>
<td>Meta-analysis</td>
<td></td>
<td>999/5358</td>
<td>31.68/25.50</td>
<td>1.28 (1.15-1.42)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1:** Association of the \textit{TCF7L2} rs7903146 polymorphism with LADA in European populations (MAF: minor allele frequency)
The metaanalysis proved that the TCF7L2 gene rs7903146 polymorphism was not associated with type 1 diabetes in individuals of European ancestry (OR 1.09, 95% CI 0.98-1.22; \( p=0.0788 \)) (Fig. is not shown).

Then we further analysed the possible interaction between the TCF7L2 gene rs7903146 polymorphism and clinical data in the Hungarian population. The associations were age- and sex-independent. T-allele carriers among LADA patients tended to have lower fasting serum C-peptide levels than patients with CC genotype, but the difference was not significant (mean±SD, CT/TT genotype: 0.24±0.18 nmol/l vs. CC genotype: 0.29±0.23 nmol/l, \( p=0.0841 \)).

T-allele carriers had significantly lower mean BMI values than patients with the CC genotype in the LADA (mean±SD: CT/TT genotype: 4.29±2.74 vs. CC genotype: 26.26±2.82 kg/m², \( p=0.0021 \)). A similar interaction was not seen in controls and type 1 diabetes.

We further analysed the effect of this TCF7L2 variant on diabetes risk in non-overweight vs. overweight BMI categories. T allele carrying was associated with LADA only in the non-overweight group (OR 1.61, 95% CI 1.21, 2.12; \( p=0.0011 \)); polymorphism-related susceptibility to diabetes was increased by 2.84-fold in non-overweight compared with overweight LADA patients (\( p=0.0013 \)).

2. study: Genetic studies in patients with type 2 diabetes

The analysis of data proved that the TCF7L2 rs7903146 polymorphism (T-allelic OR 1.49, 95% CI 1.13-1.73; \( p<0.0001 \)), the CDKN2A/2B rs10811661 polymorphism (T-allelic OR 1.20, 95% CI 1.04-1.39; \( p=0.0111 \)) and the PPARG rs1801282 polymorphism (C-allelic OR 1.20, 95% CI 1.01-1.43; \( p=0.0345 \)) were
associated with type 2 diabetes in the Hungarian population. The PARs of genes for T2D were 16.96%, 26.46% and 28.24%, respectively.

We further analysed the possible interaction between gene variants and clinical data. The associations were age- and sex-independent in cases of all there gene polymorphisms.

However, in the Hungarian dataset the TCF7L2 rs7903146 polymorphism was associated both with the fasting serum C-peptide level measured at the time of diagnosis and BMI values in patients with type 2 diabetes. T-allele carriers among type 2 diabetic patients had significant lower fasting serum C-peptide levels (mean±SD, CT/TT genotype: 0.81±0.23 vs. CC genotype: 0.87±0.24 nmol/l, p=0.0352) and BMI values (mean±SD, CT/TT genotype: 29.10±5.06 kg/m² vs. CC genotype: 30.04±4.77 kg/m², p=0.0013) than individuals with CC genotype. T-allele carriers showed an increased risk compared with controls, both in the non-overweight (OR 1.93, 95% CI 1.49, 2.50; p<0.0001) and in the overweight (OR 1.36, 95% CI 1.14, 1.63; p=0.0038) categories; polymorphism-related susceptibility was higher in non-overweight than in overweight individuals (p<0.0001).

In our study neither the CDKN2A/2B gene rs10811661 nor the PPARG gene rs1801282 polymorphisms showed any interactions with the fasting serum C-peptide level or BMI values.

The meta-analysis in regard to same European population comparison a total of 4,529 T2D patients and 5,163 control individuals proved that the TCF7L2 gene contributes to the risk of type 2 diabetes strongly significantly (OR 1.42, 95% CI 1.29-1.56; p<0.0001) (Fig. 2.). The Cochran-Q test showed the homogeneity of studies (p=0.8391).

The PAR of the T-allele for T2D was assessed 18.45% in the European populations.
Comparison of the effect size of the TCF7L2 gene variant rs7903146 C-to-T polymorphism among 999 LADA and 4,529 type 2 diabetic patients revealed homogeneity ($p=0.2423$).

The disease-associated T allele frequencies showed a north–south geographic gradient in background populations and patient groups, with the lowest presence in North (control/LADA/T2D in Finland and Sweden: 23/27/29%), with medium level in the Central European region (in Poland: 25/32/-% and in Hungary: 26/32/33%) and increasing southward (in Italy: 34/40/44%) (Fig. 3.).

**Fig. 2: Association of the TCF7L2 rs7903146 polymorphism with T2D in European populations (MAF: minor allele frequency)**

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>T2D/Control Number</th>
<th>MAF (MAF</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervin et al.</td>
<td>Swedish/Finnish</td>
<td>1621/1639</td>
<td>28.62/22.57</td>
<td>1.37 (1.16-1.61)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pettersen et al.</td>
<td>Norwegian</td>
<td>991/1482</td>
<td>33.91/25.24</td>
<td>1.51 (1.24-1.85)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Zampetti et al.</td>
<td>Italian</td>
<td>620/545</td>
<td>43.71/34.22</td>
<td>1.49 (1.13-1.96)</td>
<td>0.0041</td>
</tr>
<tr>
<td>Lukacs et al.</td>
<td>Hungarian</td>
<td>1297/1497</td>
<td>32.46/25.89</td>
<td>1.49 (1.13-1.73)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Meta-analysis</td>
<td></td>
<td>4529/5163</td>
<td>32.94/25.50</td>
<td>1.45 (1.30-1.60)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Figure 3.: The *T-allele* frequency of the rs7903146 polymorphism in *TCF7L2* gene and the standardized incidence of autoimmune diabetes in the European populations

3. study: Genetic studies in patients with type 1 diabetes

The *PTPN22 T-allele* showed a close relationship with type 1 diabetes in the Hungarian population (OR 2.01, 95% CI 1.44, 2.81; *p*=3.18x10⁻⁵). The distribution
of genotypes proved the minor T-allele dominant risk effect (in case of CT genotype OR 1.86, 95% CI 1.27, 2.71; \( p=1.26 \times 10^{-3} \)).

The association between PTPN22 gene C1858T polymorphism and type 1 diabetes was age- and sex-independent. However, at diagnosis T allele carriers had significant lower fasting serum C-peptide levels than patients with homozygote CC genotype (mean±SD, CT/TT genotype: 0.19±0.16 nmol/l vs. CC genotype: 0.29±0.22 nmol/l, \( p=0.026 \)).

The A-allele of insulin gene -23HphI polymorphism (a surrogate for VNTR class I allele) predisposed to type 1 diabetes (OR 2.33, 95% CI 1.59, 3.42; \( p=1.03 \times 10^{-5} \)), while the T-allele (a surrogate for VNTR class III allele) had a dominant protective effect in the Hungarian population (in case of AT genotype: OR 0.43, 95% CI 0.26, 0.70; \( p=7.42 \times 10^{-4} \)).

The association between insulin gene -23HphI polymorphism and type 1 diabetes was age- and sex-independent.

4. study: Type 1 diabetes associated with Hashimoto’s thyroiditis and juvenile rheumatoid arthritis: a case report with clinical and genetic investigations

The presently 17-year-old girl was born at term (with a birth weight of 2.9 kg and with a birth length of 47 cm) from the mother’s first uneventful pregnancy. There was no family history of autoimmune diseases.

At the age of two she was admitted to hospital in an unconscious state, the laboratory parameters confirmed severe diabetic ketoacidosis. Type 1A diabetes was diagnosed and insulin treatment was started. At the age of 11, autoimmune hypothyroidism and at the age of 15, seronegative polyarticular type of juvenile rheumatoid arthritis was diagnosed. With thyroid hormone replacement and methotrexate administration she became asymptomatic.
The Table 1 contains the laboratory data related to the diagnosis of the three autoimmune diseases.

Table 1: The laboratory data and radiological features at diagnosis of the three autoimmune diseases

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patient’s values</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type 1A diabetes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose</td>
<td>37.6 mmol/l</td>
<td>3.5-5.3 mmol/l</td>
</tr>
<tr>
<td>Fasting serum C-peptide</td>
<td>0.32 ng/ml</td>
<td>1.1-3.6 ng/ml</td>
</tr>
<tr>
<td>HbA1c</td>
<td>14.2 %</td>
<td>3.9-5.7 %</td>
</tr>
<tr>
<td>GADA</td>
<td>160 IU/ml</td>
<td>&lt;5 IU/ml</td>
</tr>
<tr>
<td><strong>Hashimoto’s thyroiditis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSH</td>
<td>100 µU/l</td>
<td>0.3-3.0 µU/l</td>
</tr>
<tr>
<td>FT3</td>
<td>2.06 pmol/l</td>
<td>1.23-3.08 pmol/l</td>
</tr>
<tr>
<td>FT4</td>
<td>2.12 pmol/l</td>
<td>7.72-23.17 pmol/l</td>
</tr>
<tr>
<td>anti-TPO</td>
<td>&gt;4000 U/ml</td>
<td>&lt;100 U/ml</td>
</tr>
<tr>
<td>TG</td>
<td>4744 U/ml</td>
<td>&lt;100 U/ml</td>
</tr>
<tr>
<td>TRAb</td>
<td>31.6 U/ml</td>
<td>&lt;14 U/ml</td>
</tr>
<tr>
<td>Thyroid ultrasonography</td>
<td>positive</td>
<td>-</td>
</tr>
<tr>
<td><strong>Juvenile rheumatoid arthritis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>We</td>
<td>36 mm/h</td>
<td>&lt;12 mm/h</td>
</tr>
<tr>
<td>CRP</td>
<td>6.6 mg/ml</td>
<td>&lt;5.0 mg/ml</td>
</tr>
<tr>
<td>RF</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>Technetium scintigraphy</td>
<td>positive</td>
<td>-</td>
</tr>
</tbody>
</table>

I analysed the common genetic risk factors of human autoimmunity, the *HLA class II genes*, the C1858T polymorphism of the protein tyrosine phosphatase non-receptor 22 gene (*PTPN22*) and the CT60 polymorphism of the cytotoxic T-lymphocyte-associated protein 4 (*CTLA4*) gene. Unfortunately, the parents did not consent to blood sampling and genetic investigation in other family members.

The patient had *HLA DRB1*0401-*DQA1*03-*DQB1*0301/(DR14)-*DQB1*0503; the *PTPN22* genotype was CT; the *CTLA4* genotype was also heterozygote, AG.
5. CONCLUSIONS

Latent autoimmune diabetes in adults - related findings:

1. The TCF7L2 variant rs7903146 C-to-T polymorphism contributes to susceptibility to LADA in people with European ancestry. The effect of TCF7L2 gene is population-independent among Europeans and its magnitude is comparable between LADA and type 2 diabetes. LADA is genetically an admixture of type 1 and type 2 diabetes suggesting that autoimmune and non-autoimmune processes play a role in the pathogenesis of the disease.

2. A north–south geographic gradient was seen in the frequency of the disease-associated T allele, both in LADA and control populations. It suggests that the non-autoimmune mechanisms have an increasing role in the pathogenesis of LADA towards the south.

3. The TCF7L2 gene rs7903146 polymorphism is associated with latent autoimmune diabetes also in the Hungarian population. In Hungarians, the disease-associated T allele increased susceptibility to LADA by 1.34-fold, which corresponds to a 15% PAR. The findings of Hungarian datasets suggest a phenotype-genotype interaction in LADA, with the TCF7L2 gene effect on diabetes risk possibly being modulated by BMI, such that the lower the BMI, the higher the gene effect.

4. Patients with LADA carrying TCF7L2 gene rs7903146 T allele variant tended to have less residual β-cell function at the onset of the disease. One putative effect of TCF7L2 gene may be a further decrease of insulin secretion via non-autoimmunity-mediated beta cell dysfunction.
Type 1 diabetes - related findings:

1. The *PTPN22* gene C1858T polymorphism shows a close relationship with type 1 diabetes in the Hungarian population; the T-allele carrying increases the risk on the disease by 2-fold. The association is age- and sex-independent. A phenotype-genotype interaction is found in case of *PTPN22* gene, the risk allele carriers have more extensive destruction of β-cells at the onset of type 1 diabetes.

2. The *INS* gene -23HphI polymorphism T-allele (a surrogate for VNTR class III allele) has a dominant protective effect in the Hungarian population, the AA genotype increases the susceptibility to type 1 diabetes by 3-fold. The association is age- and sex-independent.

3. The overlapping genetic loci of autoimmune diseases suggest that the genetic pathways in their pathogenesis are shared. However, the *HLA class II* genes are the primary determinants of human autoimmunity; nowadays more and more data prove that the importance of HLA-linked genetic susceptibility decreases and/or the environmental pressure increases and the interaction between major and minor genetic factors determines the individual clinical phenotype.

Type 2 diabetes - related findings:

1. The *TCF7L2* gene rs7903146, the *CDKN2A/B* gene rs10811661 and the *PPARG* gene rs1801282 polymorphisms are associated with type 2 diabetes in the Hungarian population. The associations are age- and sex-independent; the gene effects consistent with data published so far.
2. The findings of Hungarian datasets suggest that the effect of *TCF7L2* gene variant on diabetes risk may be modulated by BMI status, the disease-associated T allele increases the susceptibility to T2D in both bodyweight-categories, but the risk is twice higher in non-overweight than in overweight individuals.

3. The *TCF7L2* gene rs7903146 T allele carriers among T2D patients have significant lower residual β-cell function at the onset of the disease than patients with CC genotype. The diabetogen effect of *TCF7L2* gene may caused by direct decreasing of insulin secretion of β-cells.
PUBLICATIONS

On the subject of the Ph.D. thesis:

1. **Lukacs K**, Hosszufalusi N, Dinya E, Bakacs M, Madacsy L, Panczel P.: *The type 2 diabetes-associated variant in TCF7L2 is associated with latent autoimmune diabetes in adult Europeans and the gene effect is modified by obesity: a meta-analysis and an individual study*

2. Katalin H Nagy*/Krisztina Lukács*, Péter Sipos, Róbert Hermann, László Madácsy, Gyula Soltész: *Type 1 diabetes associated with Hashimoto’s thyroiditis and juvenile rheumatoid arthritis: a case report with clinical and genetic investigations* (*These authors contributed equally to the work.*)
   Pediatric Diabetes. 2010 Dec;11(8):579-82. **IF: 2,177**

3. **Lukács Krisztina**, Szatmári Ildikó, Jermendy Ágnes, Krikovszky Dóra, Körner Anna, Pánczel Pál, Madácsy László, Hermann Róbert: *A PTPN22 gén C1858T és az inzulin génrégió −23HphI polimorfizmusának összefüggése az 1-es típusú diabéteszsel magyar populációban*

4. **Lukács Krisztina**, Szatmári Ildikó, Hosszúfalusi Nóra, Pánczel Pál: *A TCF7L2 gén rs7903146 polimorfizmusának összefüggése a 2-es típusú diabéteszsel a magyar populációban*
   Diabetologia Hungarica 2010; 18(1): 29-37
On other subjects:


5. **Lukács Krisztina**, Pánczél Pál, Bakacs Márta, Madácsy László, Hosszúfalusi Nóra, Pánczél Pál: *A felnőttkori látens autoimmun diabétesz és a 2-es típusú diabétesz közös genetika hátterének vizsgálata* Diabetologia Hungarica. 2012; accepted for publication