Sex-specific association of PTPN22 1858T with type 1 diabetes but not with Hashimoto's thyroiditis or Addison's disease in the German population

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Abstract

Background: Endocrine autoimmune disorders share genetic susceptibility loci, causing a disordered T-cell activation and homeostasis (HLA class II genes, CTLA-4). Recent studies showed a genetic variation within the PTPN22 gene to be an additional risk factor.

Materials and Methods: Patients with type 1 diabetes (n = 220), Hashimoto’s thyroiditis (n = 94), Addison’s disease (n = 121) and healthy controls (n = 239) were genotyped for the gene polymorphism PTPN22 1858 C/T.

Results: Our study confirms a significant association between allelic variation of the PTPN22 1858 C/T polymorphism and type 1 diabetes mellitus (T1D). 1858T was observed more frequently in T1D patients (19.3% vs 11.3%, P = 0.0009; odds ratio for allele T = 1.88, 95% confidence interval [1.3 − 2.7]). Furthermore, we found a strong association in female patients with T1D (P = 0.0003), whereas there was no significant difference between male patients with type 1 diabetes and male controls. No significant difference was observed between the distribution of PTPN22 C/T in patients with Hashimoto’s thyroiditis or Addison’s disease and healthy controls.

Conclusion: The PTPN22 polymorphism 1858 C/T may be involved in the pathogenesis of type 1 diabetes mellitus by a sex-specific mechanism that contributes to susceptibility in females.

Introduction

Type 1 diabetes mellitus (T1D), Hashimoto’s thyroiditis and Addison’s disease are all caused by immune mediated self-destruction, where autoreactive cytotoxic T-lymphocytes appear to be the main effector cells (1−3).

So far, several proteins have been identified with genetic susceptibilities to autoimmune diseases that are involved in T-cell interaction. As the key determinant of the T-cell response to antigens, human leukocyte antigen (HLA) molecules have shown unequivocal evidence for a role in the susceptibility to type 1 diabetes, Hashimoto’s thyroiditis and Addison’s disease (4−7). Furthermore, single nucleotide polymorphisms (SNPs) within the cytotoxic T lymphocyte antigen-4 (CTLA-4) gene have also been shown to confer susceptibility to these diseases in several populations (8, 9).

Recently, evidence for another susceptibility locus has been reported: the protein tyrosine phosphatase N22 (PTPN22) gene (10). PTPN22 is localized on chromosome 1p13 encoding for the lymphoid-specific phosphatase (LYP), which is expressed in both immature and mature B- and T-cells (11). LYP is a powerful inhibitor of the T-cell antigen receptor signaling pathway. Thus, in conjunction with protein kinase Csk, LYP restricts the response to antigens by disrupting protein tyrosine phosphorylation events that control cell activation and differentiation. Subsequently, this negative control mechanism prevents spontaneous T-cell activations and reverts activated T-blasts to a resting phenotype (10).

The functional importance of LYP on susceptibility in autoimmune diseases was first implicated by Bottini et al. (10). They identified an SNP at nucleotide 1858 in codon 620 of PTPN22 that causes a substitution of arginine with tryptophan in the amino acid chain (10). In vitro experiments show that only LYP with Arg620 (allele 1858C) forms a complex with Csk whereas LYP with Trp620 (allele 1858T) binds less
efficiently (12). Accordingly, the PTPN22 1858T allele raises the potential for a ‘hyper-reactive’ pathogenetic T-cell response (13).

This assumption is supported by large case-control and family studies, showing an association of 1858T with susceptibility to autoimmune diseases such as type 1 diabetes (T1D), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Graves’ disease (GD) (10, 12–17).

Given the growing evidence that PTPN22 is associated with autoimmunity in general, we analyzed the PTPN22 1858 C/T polymorphism in German patients with type 1 diabetes, Addison’s disease (AD) and Hashimoto’s thyroiditis (HT).

Subjects and methods

Subjects

All patients were recruited from the endocrine outpatient clinics at the University Hospitals of Frankfurt am Main, Freiburg and Düsseldorf (Germany).

Altogether, 673 individuals (121 patients with AD, 94 patients with HT, 220 type 1 diabetic patients and 239 German healthy controls) were genotyped for the PTPN22 1858 C/T polymorphism.

Addison’s disease was diagnosed by primary adrenocortical insufficiency without evidence of tuberculosis or adrenoleukodystrophy. Adrenal autoantibodies were detected with indirect immunofluorescence on cryostat sections; in a subgroup of patients radioimmunoassay confirmed that these were directed against 21-hydroxylase. No neurological deficits could be detected.

The diagnosis of Hashimoto’s thyroiditis was established by positive thyroglobulin and/or thyroid peroxidase antibodies, reduced echogenicity on thyroid ultrasound, and normal or elevated thyrotropin levels.

Type 1 diabetes was diagnosed according to World Health Organization criteria. The median age of disease onset was 10 years (range 1–44 years).

Healthy controls (HC) were volunteer blood donors from the Red Cross Blood Transfusion Center in Frankfurt am Main (Germany), staff personnel or medical students from the University Hospital Frankfurt am Main (Germany) without a family history of type 1 diabetes mellitus, Hashimoto’s thyroiditis, Graves’ disease or Addison’s disease. Although the adrenal function of the controls was not formally assessed, they had normal thyroid function and were thyroid autoantibody negative.

All individuals were of Caucasian origin and were inhabitants from the surrounding area of Frankfurt am Main (Germany). The male:female ratio of patients with AD, HT, T1D and HC was 1:2.2, 1:4.5, 1:1.1 and 1:0.8 respectively. The average ages (in years) of patients with AD, HT, T1D and HC were 57.4 ± 16.0 (range 22–92), 51.6 ± 17.0 (range 23–95), 27.9 ± 12.9 (range 6–69) and 49.4 ± 16.6 (range 23–83) respectively.

The study protocol was approved by the Ethics Committee of the University Hospital, Frankfurt am Main, and written informed consent was obtained from all patients and controls.

Genotype analysis

DNA was extracted from whole blood according to standard protocols. The PTPN22 1858 C/T polymorphism (rs2476601) was analyzed by real-time PCR using the ABI 7300 (Applied Biosystems, Darmstadt, Germany). Primer and probe sequences were as follows: forward primer 5’-CAACTGCTCCAAGGATAGATG-3’; reverse primer 5’-CCAGCTTCCTCAACCACA-ATAAATG-3’; probe for C allele, 5’-FAM-TCAGGTGTCC-Table 1 Distribution of the PTPN22 1858 C/T polymorphism in German healthy controls and patients with autoimmune endocrinopathies.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 239)</th>
<th>Type 1 diabetes (n = 220)</th>
<th>Addison’s disease (n = 121)</th>
<th>Hashimoto’s thyroiditis (n = 94)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotypes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>187</td>
<td>142</td>
<td>96</td>
<td>67</td>
</tr>
<tr>
<td>CT</td>
<td>50</td>
<td>71</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td>TT</td>
<td>2</td>
<td>7</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><strong>P(HWE)</strong></td>
<td>0.8753</td>
<td>0.9008</td>
<td>0.7463</td>
<td>0.991</td>
</tr>
<tr>
<td><strong>Allele</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>424</td>
<td>355</td>
<td>214</td>
<td>159</td>
</tr>
<tr>
<td>T</td>
<td>54</td>
<td>85</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td><strong>OR [95% CI]</strong></td>
<td>1.88 [1.3–2.7]</td>
<td>1.03 [0.63–1.67]</td>
<td>1.43 [0.88–2.33]</td>
<td></td>
</tr>
</tbody>
</table>

1 Compared with controls; 2 P(HWE), probability of deviation of the observed from the expected genotype frequencies by chance; 3 odds ratio for the T allele compared with controls [CI, confidence interval].
GTACAGG-3'; probe for T allele 5'-VIC-TCAGGTGTCCA-
TACAGG-3'.

The interaction analyses used the CTLA4 49 A/G polymorphism and HLA DRB1, which have been reported previously (18, 19).

**Statistical analysis**

Patients and controls were compared using allele-wise and genotype-wise Chi-square testing. Differences in distribution of age of disease onset were analyzed by the nonparametric Wilcoxon-Mann-Whitney test. All calculations were performed using BiAS software (Epsilon, Weinheim, Germany) (20).

Due to multiple comparisons we applied the Bonferroni adjustment (21). We divided the chosen overall significance level ($\alpha = 0.05$) by the number of hypotheses to be tested ($n = 14$), considering this value to be the significance level ($\alpha^* = 0.0036$) for any single comparison.

Power calculation for each group was performed assuming an allele frequency of 11.3% (derived from controls used in this study) and a type I error rate of 5%. On the basis of these assumptions, we estimate that we have more than 80% power to detect an allelic odds ratio of 1.7 (T1D), 1.9 (Addison's disease) and 2 (Hashimoto's thyroiditis) for disease susceptibility in the case/control data set by using the program Power and Sample Size Calculations 2.1.30 (22).

The magnitude of associations was assessed using the odds ratio (OR) statistics. Confidence intervals (CI) were calculated for the OR by Woolf’s method (23).

**Results**

No deviations from Hardy-Weinberg equilibrium were observed for PTPN22 1858 C/T genotypes in controls or in any of the patient groups (Table 1).

**Type 1 diabetes mellitus**

Our results, given in Table 1, confirm that PTPN22 1858T is associated with T1D in the German population. Genotypes and allele frequencies show significant differences between patients with T1D and healthy controls ($P = 0.0027$ and $P = 0.0009$ respectively). 1858T was observed more often in T1D patients (19.3% vs 11.3%, $P = 0.0009$; OR for allele $T = 1.88$, 95% CI [1.3–2.7]). These data are consistent with previously published studies in other populations (13, 15).

A sex-stratified analysis reveals an interesting new observation (Table 2): comparison of female patients with female controls shows a significant difference in their genotype distribution ($P = 0.0003$; OR for allele $T = 3.12$, 95% CI [1.7–5.6]), whereas no significant difference was detected between patients and controls of the male sex. No sex-related differences were observed for the allele distribution within our controls.

Rigorous Bonferroni correction showed no statistically significant associations between T1D and other factors such as age at onset, HLA DRB1 or CTLA-4 variants (Table 3).

**Addison’s disease**

We found no evidence for an association of 1858T with Addison’s disease. Both genotypes and allele frequencies of patients and controls were similar (11.6% vs 11.3%, $P = 0.9879$; OR for allele $T = 1.03$, 95% CI [0.63–1.67]). No sex-related differences were observed.

**Hashimoto’s thyroiditis**

Patients with Hashimoto’s thyroiditis showed no significant difference in the distribution of the allele $T$ to controls (15.4% vs 11.3%, $P = 0.1863$; OR for allele $T = 1.43$, 95% CI [0.88–2.33]). Furthermore, no sex-related differences were detected.

**Table 2** Sex-related distribution of the PTPN22 1858 C/T polymorphism in German healthy controls and patients with type 1 diabetes mellitus.

<table>
<thead>
<tr>
<th></th>
<th>Controls ($n = 116$)</th>
<th>Type 1 diabetes ($n = 105$)</th>
<th>Controls ($n = 93$)</th>
<th>Type 1 diabetes ($n = 115$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>91</td>
<td>78.4</td>
<td>76</td>
<td>72.4</td>
</tr>
<tr>
<td>CT</td>
<td>23</td>
<td>19.8</td>
<td>28</td>
<td>26.7</td>
</tr>
<tr>
<td>TT</td>
<td>2</td>
<td>1.7</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>$P^1$</td>
<td>0.4432</td>
<td></td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>205</td>
<td>88.4</td>
<td>180</td>
<td>85.7</td>
</tr>
<tr>
<td>T</td>
<td>27</td>
<td>11.6</td>
<td>30</td>
<td>14.3</td>
</tr>
<tr>
<td>$P^1$</td>
<td>0.4918</td>
<td></td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>OR [95% CI]$^2$</td>
<td>1.27 [0.7–2.2]</td>
<td></td>
<td>3.12 [1.7–5.6]</td>
<td></td>
</tr>
</tbody>
</table>

$^1$Compared with controls; $^2$odds ratio for the T allele compared with controls [CI, confidence interval].
Table 3 Distribution of clinical and genetic parameters among patients with type 1 diabetes stratified by the presence of the PTPN22 T allele.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>PTPN22 T (+)</th>
<th>PTPN22 T (-)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*03 present</td>
<td>218</td>
<td>49 (34.5%)</td>
<td>79 (31.0%)</td>
<td>0.4712†</td>
</tr>
<tr>
<td>DRB1*04 present</td>
<td>218</td>
<td>54 (38.0%)</td>
<td>105 (41.1%)</td>
<td>0.5395†</td>
</tr>
<tr>
<td>CTLA4 49 G present</td>
<td>219</td>
<td>56 (72.7%)</td>
<td>98 (69.0%)</td>
<td>0.6749†</td>
</tr>
<tr>
<td>Age of onset in years (median, lower-upper quartile)</td>
<td>138</td>
<td>10 (6–15)</td>
<td>9 (5–13)</td>
<td>0.2318‡</td>
</tr>
</tbody>
</table>

\(n\), number of subjects available for analysis; the PTPN22 T(+) group contained T/T and C/T genotypes, whereas the PTPN22 T (−) group comprised C/C homozygotes.

† \(x^2\)-test; ‡ Wilcoxon-Mann–Whitney test.

Discussion

In the present study, we investigated the PTPN22 1858 C/T polymorphism in susceptibility to type 1 diabetes mellitus, Hashimoto’s thyroiditis and Addison’s disease, in an attempt to corroborate the general role of PTPN22 as a susceptibility locus for these diseases in the German population. The reported association between PTPN22 1858T and both Addison’s disease and Hashimoto’s thyroiditis in other studies could not be verified by our analysis. This may be explained by different PTPN22 allele frequencies in several populations. For each of the patient groups (AD or HT), there is only one available study confirming the association. Velaga et al. investigated PTPN22 1858 C/T genotypes of 104 patients with Addison’s disease and found a significant increase in 1858T (17). Criswell et al. analyzed 194 patients with Hashimoto’s thyroiditis selected from multiplex families with other autoimmune diseases such as rheumatoid arthritis or multiple sclerosis among others (15). Thereby, these diseases (T1D, RA, GD or SLE) (10, 12, 15, 16) contributed to an accumulation of a higher immunogenetic risk within these families. Due to the small number of analyzed patients with AD or HT, we cannot exclude the possibility that subtle effects of the PTPN22 gene polymorphism may alter the genetic susceptibility of these diseases in the German population.

In accordance with recent findings, our data show an association of 1858T with T1D in the German population. However, this susceptibility factor proved to be unique to the female sex, which is a novel observation. Smyth et al. evaluated the distribution of 1858 C/T genotypes on the basis of sex in 1600 T1D subjects and about 2000 families (14). Absolutely no effect of patient sex was observed in the large family-based cohort from Great Britain, Northern Ireland, USA and Romania (\(P = 0.951\)). However, in T1D subjects the obtained \(P\) value was of borderline significance for the patient sex (\(P = 0.029\)) (14). Given that allelic frequencies vary in each population, this discrepancy and also the inconsistency of the present study with the findings of Smyth et al. may be attributed to population stratification. Nevertheless, we cannot exclude the possibility that this effect in our study may be caused by the smaller sample size in comparison with that of Smyth et al. (14).

Sex-specific differences in the genetic susceptibility to type 1 diabetes have been reported for HLA DQ locus and early disease onset or its seasonality for males (24). Furthermore, a male/female bias has been observed for HLA DR3/non DR4 patients (25) that can only be explained by an X-chromosomal linked inheritance of an additional interacting susceptibility factor (26). Little is known, however, about the contribution of genetics to sex differences in autoimmune diseases. So far, steroidal hormones are considered to be the primary mediators of sex differences. This assumption has been borne out by the increased prevalence of autoimmune diseases in women, the sexual dimorphism of the immune response and in vitro modulatory effects of sex steroids on immune functions. Their genetic effects most probably operate at the level of the steroid receptors that may act as transcription factors for susceptibility genes such as MHC or even PTPN22. However, the role of sex hormones in regulating these genes is not known. Sex hormones may act as critical modulatory factors that can induce disease expression or not (27). In particular, fine-tuning of T-lymphocyte immunity is regulated by estrogens that augment the expression of FoxP3 in murine T-cells and can drive the proliferation of CD4+ and CD25+ regulatory T-lymphocytes (28). Given that females have higher absolute numbers of CD4+ T-lymphocytes and higher levels of Th1 cytokines with a subsequently increased immune response (27), PTPN22 1858T could drive immunity in females to the development of type 1 diabetes by a sex-specific mechanism that remains to be elucidated.

Acknowledgements

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