Evidence for susceptibility determinant(s) to psoriasis vulgaris in or near PTPN22 in German patients

U Hüffmeier, M Steffens, H Burkhardt, J Lascoz, F Schürmeier-Horst, M Ständer, R Kelsch, C Baumann, W Küster, R Mössner, K Reich, T F Wienker, H Traupe, A Reis

Introduction: Variant R620W of protein tyrosine phosphatase non-receptor type 22 (PTPN22) has consistently been reported as a susceptibility factor for several autoimmune diseases. We investigated its role in susceptibility to psoriasis, the relevance of possibly other disease-causing variants, and interdependency of the major risk factor for psoriasis at PSORS1.

Methods: R620W was tested in a case-control study initially with 375 German patients and then with an enlarged sample of an additional 418 patients. Analyses were extended to linkage disequilibrium (LD) based haplotypes. Potential interaction between risk haplotypes of PTPN22 and the PSORS1 associated risk allele was tested by regression analysis. PTPN22 coding sequence was determined in 20 patients carrying the risk haplotype. Association and regression analysis were also performed in the extended case-control study.

Results: R620W was not associated in either case-control study, while significant association (corrected for multiple testing) with one haplotype (C-4) of the LD block encompassing PTPN22 as well as another haplotype (B-3) within an adjacent telomeric LD block was detected. No evidence for interaction between risk haplotype C-4 and the PSORS1 associated risk allele was found. Sequencing excluded other coding variants within PTPN22 as a basis for association findings. Analysis of the extended study group confirmed association for haplotypes B-3 and C-4 and independence of risk haplotypes C-4 and PSORS1.

Discussion: We exclude a major role of *620W in German psoriasis patients but suggest that other susceptibility determinants within non-coding regions of PTPN22 or its proximity might exist acting independently of the major PSORS1 risk factor.

Abbreviations: hiSNP, haplotype tag SNP; LD, linkage disequilibrium; SNP, single nucleotide polymorphism
In order to reduce the risk of false positive association results, we corrected for multiple testing after Bonferroni; haplotypes of a frequency of more than 5% in the controls were considered, resulting in a correction for five tests in the case of haplotype blocks A and B and for four tests in the case of haplotype block C.

To estimate the influence of the PSORS1 risk allele, the risk haplotype HCR^WWCC within the coiled-coil alpha-helical rod protein 1 (CHCR1) gene was determined through two previously described htSNP alleles at positions 325 and 2327.\textsuperscript{16} Nineteen SNPs were genotyped and haplotypes were calculated as previously described.\textsuperscript{16}

**Regression analysis**

Logistic regression analysis was carried out with SAS (version 9.1) to analyse the role of HCR^WWCC and the PTNP22 risk haplotype as potential predictors for psoriasis vulgaris. The two groups, patients with psoriasis vulgaris and controls, were treated as categorical variables, controls being the reference category for the outcome, whereas the PTNP22 risk haplotype for psoriasis (C-4, C A G G, abbreviated rhPTPN22) and the HLA-Cw6 associated risk haplotype HCR^WWCC (abbreviated rhHCR) were treated as explanatory variables. We tested a reduced model with respect to both risk haplotypes including the independent variables rhHCR and rhPTPN22 and a saturated model including the additional interaction term, rhHCR^rhPTPN22.

**Sequencing of PTNP22**

The coding segments of PTNP22 were covered in 24 amplicons; 20 of the patients were sequenced with intron based primers (primer sequences are available upon request). Patients were selected for risk haplotype C-4: 14 probands were homozygous and six heterozygous for this risk haplotype. After performing PCR reactions in an MJ Research thermocycler (Biozym, Hess, Oldendorf, Germany), the PCR products were purified on a robotic system (Tecan Miniprep 75-2 with vacuum station; Tecan, Crailsheim, Germany) with Millipore Montage PCR Cleanup Filter Plates (Millipore, Schwalbach, Germany). Sequencing reactions were performed using the BigDye Terminator Cycle Sequencing Kit Version 3.1 (Applied Biosystems) according to the manufacturer's instructions and were purified on the same robotic system with Millipore Montage SEQ Sequencing Reaction Cleanup Kit. They were analysed in an ABI Prism Model 3730 Sequencer (Applied Biosystems) while sequencing analysis was performed with the software SeqMan II (DNA-Star, Madison, WI).

**Extension of the study**

An analysis of the second study group of patients and controls was performed for the two CHCR1 SNPs as well as for the htSNPs covering the haplotype blocks B and C (fig 1). In the combined data set we determined the CHCR1 risk haplotype and tested for association with haplotypes within LD blocks B and C. In addition we tested the hypothesis of possible interaction between those two risk factors in this combined data set by regression analysis as in the exploratory study.

**RESULTS**

**Lack of association with variant R620W**

In the screening set no association with PTNP22 1858C→T was observed (table 1). We also stratified the patients for the following criteria: carrier/non-carrier of PSORS1 associated risk allele, sex, and manifestation of the disease before age 16 versus manifestation between 16 and 40 years of age. After stratification no evidence for association with any allele of R620W in any subgroup was noticed (data not shown). The
allele and genotype frequencies of R620W were similar in the second case-control study. We observed no evidence for association after combining both data sets either before or after stratification for the above mentioned criteria.

**LD structure**
Genotypes of the 19 htSNPs in the exploratory case-control study composed of 375 psoriasis patients and 376 control subjects were used for LD analysis. We omitted rs6679677 due to its minor allele frequency of 0.1. Considering the data of the 751 individuals, we were able to confirm the LD structure based on HapMap data. The 335 kb large LD block comprising rs6679677 due to its minor allele frequency of 0.1.

**Associations at haplotype level**
Within the four LD blocks we observed five common haplotypes in block A (frequency of ≥5% in controls), six in block B, five in block C, and three in block D. These common haplotypes accounted for the majority of haplotypes (total coverage of 95.3–99.2%). Significant association with one haplotype in blocks A, B, and C (A-3, B-3, and C-4) was discovered while no association was observed with any haplotype in block D (table 2). After Bonferroni correction for multiple testing, association findings for B-3 and C-4 remained significant. Corresponding odds ratios and their confidence intervals (ORs (95% CI)) for the three haplotypes were in the range of 1.35 (1.07 to 1.74) to 1.42 (1.12 to 1.81).

**Interaction with PSORS1 associated risk haplotype**
In the exploratory study the regression analysis resulted in significant Wald $\chi^2$ values for both main effects. As expected, in terms of $p$ values the effect of rhHCR ($p<1.50 \times 10^{-14}$) was more than ten magnitudes stronger than the effect of rhPTPN22 ($p<0.0046$) (table 3). Since these tests were done on the same sample, $p$ values could be taken as a proxy for effect size, however in a non-linear way. We also observed a combined effect of the variables as both risk haplotypes act in the same direction (estimates of –0.60 for rhHCR and –0.22 for rhPTPN22). Regarding the interaction effect

**Table 1** Genotype frequencies of R620W (rs2476601) and results of $\chi^2$ statistics

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>No. of genotypes (%)</th>
<th>Statistical parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C/T-1/T</td>
<td>C/C</td>
</tr>
<tr>
<td>A</td>
<td>375 psoriasis vulgaris patients</td>
<td>782 (21.8)</td>
</tr>
<tr>
<td></td>
<td>376 controls</td>
<td>61 (18.3)</td>
</tr>
<tr>
<td>B</td>
<td>793 psoriasis vulgaris patients</td>
<td>158 (21.5)</td>
</tr>
<tr>
<td></td>
<td>937 controls</td>
<td>144 (18.9)</td>
</tr>
</tbody>
</table>

A: exploratory case-control study; B: combined case-control studies.
(rhHCR*rhPTPN22), which is mathematically defined as the coefficient of exposure products in the model, results showed no evidence for interdependence such as synergy (a total effect greater than the sum of the separate effects), super-additivity, antagonism, or competitive action (p<0.58).

### Sequencing of PTPN22 in psoriasis patients

By sequencing PTPN22 exons we were able to identify two coding variants: an amino acid change R263Q in one patient heterozygous for the risk haplotype C-4 and a synonymous change in position L247 in another patient homozygous for C-4. Testing of these two polymorphisms in the exploratory case-control study revealed very low minor allele frequencies for R263Q in patients (0.019) and controls (0.017), while the minor allele of L247L was only identified in one of the patients sequenced, but not in any other psoriasis patient or control. Fisher’s exact test indicated no significant difference between cases and controls. Therefore, these polymorphisms do not explain the association findings at haplotype level.

### Extension of study samples

We selected haplotype blocks B and C for extension of the study due to their association findings in the exploratory case-control study and their proximity to PTPN22. The range of statistical parameters indicating association with haplotypes B-3 and C-4 was similar to that in the exploratory case-control study, and their proximity to PTPN22 due to their association findings in the exploratory study samples.

Recalculation of the regression analysis for the combined data set confirmed the results of the exploratory study, while the effects were stronger for both main effects (table 3): we observed significant Wald $\chi^2$ values for both main effects, again as expected with the effect of rhHCR ($p<6.55 \times 10^{-35}$) much stronger than that of rhPTPN22 ($p<0.0014$). Both effects also acted in the same direction (estimates of −0.66 for rhHCR and −0.17 for rhPTPN22). Again, there was no evidence for an interaction effect.

### DISCUSSION

To investigate whether the previously described variant R620W within gene PTPN22* might also be a relevant susceptibility factor for a further T cell mediated disease, we tested this hypothesis for population based samples of controls and patients with psoriasis vulgaris. The variant was not found to be associated with psoriasis. This is in concordance with earlier data: Criswell et al. reported no evidence for association with R620W with this phenotype in 51 affected individuals of families with multiple autoimmune diseases, while Nistor et al. came to the same conclusion after investigating their large cohort of 517 psoriasis families. Another small study of 279 single patients with psoriasis described similar findings. In a group of 375 patients with psoriatic arthritis, we observed association with PTPN22*620W after stratification for sex with a higher ratio of male patients in the carriers than in the non-carriers of the risk allele. Also Orozco et al. showed a similar trend in a group of rheumatoid arthritis patients after stratification for sex and extra-articular manifestation. Thus, we were interested whether sex or different ages of onset (manifestation in childhood/early adolescence versus onset between 16 and 40 years of age) might influence association with PTPN22*620W. Due to lack of association within subgroups and similar results in the extended case-control study, we assume that this polymorphism does not contribute to susceptibility to psoriasis.

### Table 2

<table>
<thead>
<tr>
<th>Block-haplotype</th>
<th>Allele combination</th>
<th>Frequency</th>
<th>Psoriasis vulgaris controls</th>
<th>p value</th>
<th>p value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A-G-C-A-A-A</td>
<td>0.183</td>
<td>0.232</td>
<td>5.362</td>
<td>0.027</td>
<td>1.35 (1.05 to 1.74)</td>
</tr>
<tr>
<td>B</td>
<td>A-C-A-G</td>
<td>0.203</td>
<td>0.265</td>
<td>8.216</td>
<td>0.004</td>
<td>1.42 (1.12 to 1.81)</td>
</tr>
<tr>
<td>C</td>
<td>A-G-C-A</td>
<td>0.137</td>
<td>0.095</td>
<td>6.657</td>
<td>0.010</td>
<td>NS</td>
</tr>
<tr>
<td>D</td>
<td>A-C-A-G</td>
<td>0.189</td>
<td>0.243</td>
<td>6.433</td>
<td>0.011</td>
<td>1.38 (1.07 to 1.76)</td>
</tr>
<tr>
<td>A</td>
<td>A-G-C-A-A-A</td>
<td>0.137</td>
<td>0.100</td>
<td>11.435</td>
<td>0.001</td>
<td>1.26 (1.08 to 1.47)</td>
</tr>
<tr>
<td>B</td>
<td>A-C-A-G</td>
<td>0.106</td>
<td>0.162</td>
<td>10.505</td>
<td>0.005</td>
<td>1.26 (1.08 to 1.47)</td>
</tr>
<tr>
<td>C</td>
<td>A-G-C-A</td>
<td>0.199</td>
<td>0.239</td>
<td>7.733</td>
<td>0.005</td>
<td>1.26 (1.07 to 1.48)</td>
</tr>
</tbody>
</table>

A: exploratory case-control study (375 cases and 376 controls); B: combined study cohorts (793 cases and 937 controls). CI, confidence interval; NS, not significant; OR, odds ratio; $p_c$, corrected p value.

### Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>Model</th>
<th>Parameter</th>
<th>Estimate</th>
<th>Standard error</th>
<th>Wald $\chi^2$ (df = 1)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exploratory case</td>
<td>Reduced model</td>
<td>Intercept</td>
<td>0.06</td>
<td>0.08</td>
<td>0.50</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Saturated model</td>
<td>Intercept</td>
<td>-0.60</td>
<td>0.08</td>
<td>60.69</td>
<td>6.70 x 10^{-15}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rhHCR</td>
<td>-0.06</td>
<td>0.08</td>
<td>5.33</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rhPTPN22</td>
<td>-0.22</td>
<td>0.08</td>
<td>8.02</td>
<td>2.3 x 10^{-4}</td>
</tr>
<tr>
<td>Combined case</td>
<td>Reduced model</td>
<td>Intercept</td>
<td>-0.17</td>
<td>0.05</td>
<td>9.48</td>
<td>0.0021</td>
</tr>
<tr>
<td></td>
<td>Saturated model</td>
<td>Intercept</td>
<td>-0.17</td>
<td>0.05</td>
<td>10.3511</td>
<td>0.0013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rhHCR</td>
<td>-0.66</td>
<td>0.05</td>
<td>157.93</td>
<td>3.21 x 10^{-36}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rhPTPN22</td>
<td>-0.17</td>
<td>0.05</td>
<td>9.37</td>
<td>0.0022</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rhHCR</td>
<td>-0.17</td>
<td>0.05</td>
<td>151.93</td>
<td>6.55 x 10^{-35}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rhPTPN22</td>
<td>-0.17</td>
<td>0.05</td>
<td>10.21</td>
<td>0.0014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rhHCR</td>
<td>0.02</td>
<td>0.05</td>
<td>0.12</td>
<td>0.73</td>
</tr>
</tbody>
</table>

*Statistical parameters are presented for a reduced and a saturated model in both studies.
To further explore our hypothesis that a PTPN22 variant other than R620W might be relevant in the pathogenesis of psoriasis, we extended the analyses to haplotypes based on LD patterns, that is, haplotype blocks. When we used HapMap genotyping data from SNPs with minor allele frequencies of more than 0.15 around PTPN22, we detected a clear pattern of high LD in the proximity of this gene. Using 18 SNPs we were able to construct robust haplotypes extending over a genomic region of about 335 kb and covering PTPN22 and some neighbouring genes. Surprisingly, association with one haplotype each within two of the three sub-blocks of the large LD block was detected in the exploratory case-control study, suggesting this genomic region on 1p13.2 has a role in the pathogenesis of psoriasis. This was confirmed in the regression analysis where we could show that the PTPN22 haplotype C-4 confers risk of psoriasis. Although association for one further haplotype (A-3) of the third sub-block within the large LD block was weaker (not significant after Bonferroni correction), this sub-block can not be excluded as a region containing potential susceptibility factor(s) due to its high LD with haplotype blocks B and C.

Even though we were not able to confine the association findings to PTPN22 itself, we considered it the most suitable candidate gene for psoriasis. Therefore, we checked this gene for other coding variants in a subset of psoriasis patients carrying the PTPN22 risk haplotype C-4. We identified two very rare coding polymorphisms that could not explain the association found.

The HLA region on the short arm of chromosome 6 has long been known as the major psoriasis susceptibility factor (PSORS1) and the results of numerous genetic studies indicate that the HLA-Cw6 allele or a variant in strong disequilibrium with HLA-Cw6 represents the major risk factor especially for early onset psoriasis. There has been much controversy on the precise nature of the factor at PSORS1 that would influence immunological functions and predispose to psoriasis. The region exhibits an extraordinary high degree of LD, which has hampered the identification of this factor until today. We used the haplotype HCR*WWCC, which is in strong LD with the PSORS1 risk allele, but known to be only a rough estimate of this risk factor, to analyse possible interactions between the risk haplotype at PTPN22 and PSORS1. We observed negative estimates for both risk haplotypes even though no evidence for interaction between was observed. These results do not exclude effects of those two risk factors within the same pathway while a direct interaction between HLA-C risk allele and the genetic factor within PTPN22 or its proximity can be ruled out. Regression analysis also revealed that the contribution of the genetic factor(s) within PTPN22 or its genomic region play(s) a minor role as compared to the risk haplotype of the CCHCR1 gene. This is not unexpected and in concordance with studies of psoriasis susceptibility regions other than PSORS1 that confer only a comparably modest susceptibility.

In the extended case-control study we could confirm association findings for the two haplotypes B-3 and C-4, although we did not observe a real strengthening of the results compared to the exploratory study. But when comparing the regression analyses in the exploratory case-control study and the combined data set, the results indicated a slightly stronger effect of the PTPN22 risk haplotype in the larger study. The resulting odd ratios for the risk haplotypes in PTPN22’s genomic region are in the range that has been detected at other psoriasis loci and for susceptibility factors identified in other complex disorders like Crohn’s disease. Therefore, these results underline the potential existence of a genetic susceptibility factor for psoriasis vulgaris within this genomic region. Due to the relatively small effect of this potential variant our results will need to be independently confirmed, and a very large group of patients will be required. If relevance of this genetic factor is confirmed, it will be interesting to determine if a non-coding variant within PTPN22 is causal, since sequencing of our patients makes a further coding variant unlikely, or if a variant in another gene in LD constitutes the risk factor.

In summary, we exclude the possibility that R620W or another variant within the coding regions of PTPN22 plays a role in susceptibility to psoriasis vulgaris in the German patients investigated but suggest that other variant(s) within PTPN22 gene or in linkage disequilibrium with it are contributory susceptibility determinant(s) for psoriasis vulgaris in patients of German origin.

ACKNOWLEDGEMENTS

We are grateful to all patients and controls for participating in this study. We thank Verena Popp and Olga Zwengen for excellent technical assistance.

Authors’ affiliations

U Höffmeier, J Lascorz, A Reis, Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany
M Steffens, T F Wienker, Institute of Medical Biometry, Informatics and Epidemiology, University of Bonn, Bonn, Germany
H Burkhardt, Institute for Clinical Immunology and Rheumatology, Department of Internal Medicine III, University of Erlangen-Nuremberg, Erlangen, Germany
F Schürmeier-Horst, H Traupe, Department of Dermatology, University of Münster, Münster, Germany
M Ständer, Psoriasis Rehabilitation Hospital, Bad Bentheim, Erlangen, Germany
R Kelsch, C Baumann, Institute for Transfusion Medicine, University of Münster, Münster, Germany
W Küster, TOMESA Clinics, Bad Salzschlirf, Erlangen, Germany
R Mässner, K Reich, Department of Dermatology, University of Göttingen, Göttingen, Germany

This work was supported in part by grants from the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG, Tr 228/5-4 and Re 679/10-4) and from the Interdisciplinary Centre for Clinical Research (IZKF B32/1A) of the University of Erlangen-Nuremberg with a grant from The German Federal Ministry of Education and research grant no. 01 KS 0002

Competing interests: none declared

REFERENCES

1. Nickoloff BJ, Nestle FO. Recent insights into the immunopathogenesis of psoriasis provide new therapeutic opportunities. J Clin Invest 2004;113(12):1664-75


