Replication of LCE3C–LCE3B CNV as a Risk Factor for Psoriasis and Analysis of Interaction with Other Genetic Risk Factors

Ulrike Hüffmeier1,11, Judith G.M. Bergboer2,11, Tim Becker3, John A. Armour4, Heiko Traupe5, Xavier Estivill6,7, Eva Riveira-Munoz6, Rotraut Mössner8, Kristian Reich9, Werner Kurrat10, Thomas F. Wienker3, Joost Schalkwijk2, Patrick L.J.M. Zeeuwen2 and André Reis1

Recently, a deletion of two late cornified envelope (LCE) genes within the epidermal differentiation complex on chromosome 1 was shown to be overrepresented in 1,426 psoriasis vulgaris (PsV) patients of European ancestry. In this study, we report a confirmation of this finding in 1,354 PsV patients and 937 control individuals of German origin. We found an allele frequency of the deletion of 70.9% in PsV patients and of 64.9% in control individuals ($\chi^2 = 17.44, P = 2.97 \times 10^{-5}$, odds ratio (95% confidence interval) = 1.31 (1.15–1.48)). The overall copy number of the two LCE genes had no influence on the age of onset, but we observed a dosage effect at the genotype level. There was no evidence of statistically significant interaction with copy number of the $\beta$-defensin cluster on 8p23.1 or with an IL-23R pathway variant in a combined data set of German and Dutch individuals, whereas evidence for interaction with the PSORS1 risk allele in German individuals was marginal and did not remain significant after correction for multiple testing. Our study confirms the recently published finding that the deletion of the two LCE genes is a susceptibility factor for PsV with dosage effect, while, because of power limitation, no final conclusion regarding interaction with other PsV risk factors can be made at this stage.

Journal of Investigative Dermatology (2010) 130, 979-984; doi:10.1038/jid.2009.385; published online 17 December 2009

INTRODUCTION

Psoriasis vulgaris (PsV) is a common inflammatory skin disorder characterized by epidermal hyperproliferation, altered keratinocyte differentiation, and inflammation. An HLA-C allele or a variant in strong linkage disequilibrium (LD) with it is the major risk allele, especially in PsV patients manifesting before the age of 40 years. This finding probably corresponds to the most evidentiary psoriasis susceptibility locus (PSORS1). Besides this risk factor and PSORS4 (see below), replicated associations with candidate genes (RAPTOR and SLC12A8) have been reported so far at PSORS2 and PSORS5 (Hewett et al., 2002; Helms et al., 2003; Capon et al., 2004; Hufmmeier et al., 2005). In addition, variants in the IL-23R pathway have been identified by two genome-wide association studies for PsV (Capon et al., 2007; Cargill et al., 2007) and could be confirmed by further independent studies (Chang et al., 2007; Nair et al., 2008, 2009; Hufmmeier et al., 2009b). Finally, copy number variation (CNV) of a genomic segment on chromosome 8p23.1 harboring a cluster of genes encoding $\beta$-defensins (DEFB) small antimicrobial peptides, was identified to be associated with psoriasis in a Dutch and a German case-control cohort (Hollox et al., 2008) and variants of the NF-kB-pathway were recognized to be risk factors for PsV (Nair et al., 2009).

PSORS4 is a susceptibility locus on chromosome 1 initially identified in an Italian genome-wide linkage analysis of Italian families (Capon et al., 1999). This locus is of special interest for PsV, because it comprises the epidermal differentiation complex, a group of genes expressed in the upper strata of the epidermis. Although several genes at PSORS4—e.g., LOR, LCE1C, PGLYRP, SPRR genes, PRR9 genes, and IVL—have been suggested to account for psoriasis susceptibility (Giardina et al., 2006; Chen et al., 2009; Liu et al., 2008; Kainu et al., 2009), very recently, a CNV...
within the late cornified envelope (LCE) gene cluster was identified by a genome-wide scan using pooled DNAs. The deletion of two LCE genes (LCE3C and LCE3B) was shown to be at higher frequency in 1,426 psoriasis patients from several European countries than in controls and to be associated with psoriasis in a large family-based cohort (de Cid et al., 2009). Expression data in the same study suggested that carriers of the deletion may have a compromised repair response after barrier disruption of the skin (de Cid et al., 2009). In addition, in an independent genome-wide association study of a Chinese cohort, single-nucleotide polymorphisms (SNPs) in strong LD with the deletion were identified as risk factors for psoriasis (Zhang et al., 2009).

This study investigates the contribution of LCE3C-LCE3B-del to PsV susceptibility in German patients and also whether this contribution to disease is independent of three other known genetic risk factors. We, therefore, analyzed a large case-control cohort comprising 1,354 PsV patients and 937 control individuals for the presence of LCE3C-LCE3B-del and also genotyped three SNPs known to be in strong LD with it (rs10888502, rs4112788, and rs4845456). In addition, we also genotyped three SNPs known to be in strong LD with it known genetic risk factors. We, therefore, analyzed a large distribution of this contribution to disease is independent of three other haplotypes consisting of rs4112788 and LCE CNV: risk haplotype “G-deletion” was significantly associated with a P-value of 0.0010 (Table 3). The P-value indicating the distribution of P-values was one order of magnitude higher in the two-marker haplotypes analysis compared with four-marker analysis. To determine whether the risk alleles of the three SNPs have independent effects from the LCE CNV, we performed a conditional analysis. Thereby, we identified marginal evidence for the independent effects of rs4112788 (P = 0.044), but not for the other two SNPs (P-values not significant). The P-value for rs4112788 remained not significant after correction for multiple testing.

No significant differences in age of disease onset were observed between the three LCE CNV genotypes. This was also true when the analysis was restricted to patients with an age of onset before 40 years (also known as type I psoriasis), which represented the majority of our cases. The OR for psoriasis patients homozygous for LCE3C-LCE3B-del was 1.61 (1.21–2.14), which was higher than the OR for heterozygous patients (1.16 (0.87–1.55)), indicating a dosage effect.

As only part of the German cohort had been previously genotyped for copy number of β-defensin cluster, we performed a joint analysis with the Dutch study group to

### Table 1. Allele and genotype frequencies (absolute number (percentage)) in 1,354 PsV patients and 937 control probands, results of χ²-statistics, and odds ratios (95% confidence intervals) for the LCE CNV

#### (A)

<table>
<thead>
<tr>
<th>Allele</th>
<th>CON</th>
<th>PsV</th>
<th>χ²</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCE3C-LCE3B-del</td>
<td>1,215</td>
<td>1,899</td>
<td>17.44</td>
<td>2.97 × 10⁻⁵</td>
<td>1.31 (1.15–1.48)</td>
</tr>
<tr>
<td>Non-deletion</td>
<td>657</td>
<td>785</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### (B)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CON</th>
<th>PsV</th>
<th>χ²</th>
<th>P</th>
<th>OR (95% CI)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCE3C-LCE3B-del/LCE3C-LCE3B-del</td>
<td>391 (41.8)</td>
<td>678 (50.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCE3C-LCE3B-del/non-deletion</td>
<td>433 (46.3)</td>
<td>543 (40.5)</td>
<td>18.010</td>
<td>1.228 × 10⁻⁴</td>
<td>1.37 (1.05–1.80)</td>
</tr>
<tr>
<td>Non-deletion/non-deletion</td>
<td>112 (12.0)</td>
<td>121 (9.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; CNV, copy number variation; CON, control probands; LCE, late cornified envelope; LCE3C-LCE3B-del, deletion of the two LCE genes (LCE3C and LCE3B); OR, odds ratio; PsV, psoriasis vulgaris.

¹Carriers—homozygous or heterozygous—of LCE3C-LCE3B-del compared to non-carriers.
increase the statistical power for interaction analyses. Logistic regression analysis on data from 1,073 individuals did not reveal any evidence for interaction \((P = 0.22, \text{Table 4})\). In a combined German and Dutch case-control study consisting of 1,567 patients and 1,272 individuals, no evidence for interaction of the \(LCE3C-LCE3B-CN\) with rs6887695 (SNP
We present the first replication of LCE3C (Table 4).

Pon possible interactions revealed no significant differences in homogeneity of ORs for the PSORS1 risk allele between both cohorts, we analyzed the interaction of PSORS1 with other risk factors only in the subset of 2,291 German individuals. We observed a tendency toward interaction with LCE3C-LCE3B-CN, while the P-value did not remain significant after correction for the number of interaction analyses performed. Further calculations on possible interactions revealed no significant P-values (Table 4).

**DISCUSSION**

We present the first replication of LCE3C-LCE3B—del’s association with psoriasis, which was previously reported by de Cid et al. (2009). The frequency of the LCE3C-LCE3B-del allele in our German case-control cohort was very similar to that in the Dutch and US case-control samples (de Cid et al., 2009). The single SNPs were all associated at allele level, while effects were strongest for rs4112788. This variant also shows strongest LD to LCE CNV, and haplotype analyses revealed the stronger effects of a haplotype consisting of these two variants than those of all four variants. Testing for independence of LCE CNV and rs4112788 was inconclusive. Currently, we cannot exclude that rs4112788 might modify the risk at this locus, while the LCE CNV seems to be the more plausible risk factor. Further functional studies and maybe the upcoming meta-analysis (see below) will hopefully elucidate the role of the two variants in the pathogenesis of PsV.

Several independent studies (see, e.g., Gudjonsson et al., 2002) have observed that the PSORS1 risk factor is strongly associated with early age of disease onset. We performed a similar analysis for LCE3C-LCE3B-del, which indicated that it is not associated with early disease onset. But confirming the previous results from the European study (de Cid et al., 2009), we observed a dosage effect of the LCE3C-LCE3B-del at genotype level.

The major risk allele for psoriasis has been found to be epistatic to LCE3C-LCE3B-del in the subgroup of Dutch individuals (de Cid et al., 2009), but not in the other subgroups. We observed only marginal evidence for interaction in the German cohorts, which remained not significant after correcting for testing of multiple interactions by Bonferroni. A large multicenter meta-analysis on the possible interaction between PSORS1 risk allele and LCE3C-LCE3B-del is underway (Riveira et al., in preparation). The meta-analysis will provide more generalizable data regarding this possible interaction and might show differences in various European populations. We did not observe evidence for interaction of LCE3C-LCE3B-del with the β-defensin CNV nor with a variant of the IL12B locus, whereas we did not test the interaction with newly identified risk alleles in the genes TNIP1 and TNFAIP3 (Nair et al., 2009). Regarding possible interaction(s), we have to consider limited power because of study size, and this limit does not allow a final conclusion. In general, no evidence for interaction is not a wholly unexpected finding, as interaction in Crohn’s disease—a disease in which many more loci/risk factors have been identified in total—has been observed for only single risk factors (Hampe et al., 2007; Barrett et al., 2008) and evidence for the replication of these findings is scarce.

**MATERIALS AND METHODS**

**Study groups**

The German case-control study consisted mainly of 1,114 PsV patients and the 937 control probands, previously described in Huffmeier et al. (2009b). A minority of the 1,354 cases were index patients of 240 trios of a family-based cohort (Huffmeier et al., 2009a). The studies were approved by the ethical committees of the University of Erlangen-Nuremberg and of the University of Münster. The Dutch case-control group consisted of 213 patients and 335 control individuals and has been described earlier (Hollox et al., 2008). Permission for these studies was obtained from the local medical ethics committee (Commissie Mensegebonden Onderzoek).

Written informed consent was obtained from each patient and control proband before enrollment. The investigations were conducted according to the Declaration of Helsinki principles.

**Genotyping**

**Late cornified envelope variants.** We genotyped the CNV in German samples with a protocol modified from that used by de Cid et al. (2009): a multiplex assay of two fluorescently marked PCR products detected on an ABI3730 DNA sequencer (Applied Biosystems, Foster City, CA). Briefly, we used 40 ng DNA and the Amplitaq Gold polymerase (Applied Biosystems) to amplify a breakpoint-spanning PCR product of 351 bp (F: 5'-GGATACTAAGAAGTCTCAG-3', R: 5'-GTGGTGAGAGGGCATCT-3') for deletion alleles and a second amplicon for wild-type alleles (primers within the deleted region, product size 561 bp (F: 5'-CATTAGCTGAGCTTTTGC-3', R: 5'-ACAAGGTGATAACATTGTCAGGAGG-3'). The multiplex reaction was diluted 1:20; a volume of 5μl was

---

### Table 4. Uncorrected results (P-values) of interaction analyses in case-control cohorts

<table>
<thead>
<tr>
<th></th>
<th>(A) Dutch or German individuals</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LCE3C_3B-CN</td>
<td>IL12B</td>
<td>DEFB-CN</td>
<td></td>
</tr>
<tr>
<td>LCE3C_3B-CN</td>
<td>—</td>
<td>0.55</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>IL12B</td>
<td>0.55</td>
<td>—</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>DEFB-CN</td>
<td>0.22</td>
<td>0.80</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>(B) German individuals</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LCE3C_3B-CN</td>
<td>PSORS1</td>
<td>IL12B</td>
<td>DEFB-CN</td>
</tr>
<tr>
<td>LCE3C_3B-CN</td>
<td>—</td>
<td>0.021</td>
<td>0.49</td>
<td>0.32</td>
</tr>
<tr>
<td>PSORS1</td>
<td>0.021</td>
<td>—</td>
<td>0.98</td>
<td>0.66</td>
</tr>
<tr>
<td>IL12B</td>
<td>0.49</td>
<td>0.98</td>
<td>—</td>
<td>0.56</td>
</tr>
<tr>
<td>DEFB-CN</td>
<td>0.32</td>
<td>0.66</td>
<td>0.56</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: LCE, late cornified envelope; PSORS, psoriasis susceptibility locus.

1 P-value did not remain significant when corrected for number of interaction analyses performed.

P-values for interaction of two risk factors can be found in a square, with the corresponding risk factors above and on the left-hand side.
analyzed using size standard LIZ600 (Applied Biosystems) on the capillary sequencer. Genotypes passing quality control showed peak intensities >2,000 fluorescent units, and in putative heterozygote individuals, ratios of peak heights of LCE3C-LCE3B-del to those of the non-deletion alleles were >0.5 and <3. To estimate the error rate of genotyping, we performed duplicate genotyping of six 96-well-microtitre plates. In all, 515 DNAs yielded an amplification in both runs and were used to compare between genotypes. Within these, 449 (87.2%) passed both quality criteria (see above) and were concordant in both experiments. Seven DNAs (1.4%) passed quality control in both runs, but showed divergent genotypes. We therefore have to assume a genotyping error rate of approximately 1.4%. For genotyping failures of the LCE CNV, we performed duplicate genotyping of six 96-copy number of the LCE CNV, we used the three primers LCE3C (5'-TCACCTGAAGATAGCTCA-3'), LCE3CR (5'-TCCTCA ACCACTGTGTCTTCTCA-3'), and LCE3CR2D (5'-CATCCCCAGGGA TGCTGCATG-3') in a multiplex reaction as previously described (de Cid et al., 2009) and performed agarose gel electrophoresis to separate the deletion allele (199 bp) and non-deletion allele (240 bp). Using this method, we also confirmed the genotypes of 37 individuals who had been successfully genotyped by the mainly used method.

Single-nucleotide polymorphisms were genotyped with TaqMan assays (Applied Biosystems) in the German study groups. In addition, we sequenced 24 randomly selected individuals for the three variants as described earlier (Huffmeier et al., 2009b) and could verify their genotypes.

Genotyping of the LCE CNV in Dutch patients and controls was performed as described in de Cid et al. (2009). rs6887695, the variant of the IL12B gene, was successfully genotyped in Dutch cases and controls with a genotyping rate of 96.1%.

**DEFB CNV and available genotypes for interaction analyses**

Copy numbers of the β-defensin cluster were determined in subgroups of 317 German PsV patients and 305 control individuals, as well as in 179 Dutch psoriasis patients and 272 control probands as described by Hollox et al. (2008). The copy number of β-defensin cluster was not available for the rest of the cohort. For interaction analyses, we had data for the LCE copy number variant and the IL12B variant rs6887695 in all German and Dutch individuals. For the main risk factor for psoriasis (PSORS1), we had information on carriers/non-carriers of the HLA-Cw0602 allele in Dutch individuals and used an estimation in German individuals as described earlier (Huffmeier et al., 2009b).

**Statistical and interaction analyses**

To determine allele frequency differences between cases and controls, we used chi-square statistics, with one degree of freedom for comparisons of allele frequencies and two degrees of freedom for comparisons of genotypes. For comparisons resulting in significant statistical values, an OR with 95% confidence interval was calculated (OR [confidence interval]). The LD between the four variants was calculated with the software Haploview vs 4.0, Broad Institute, Harvard, Massachusetts (Barrett et al., 2005). Haplotypes were calculated with FAMHAP (Herold and Becker, 2009).

An unpaired t-test statistic was calculated to test whether PsV patients carrying one or two LCE3C-LCE3B-del alleles develop disease earlier than non-carriers/carriers of one LCE3C-LCE3B-del allele.

To test for a dosage effect of LCE3C-LCE3B-del, logistic regression analysis was performed using as reference homozygotes for the LCE3C_LCE3B non-deletion allele.

To adjust for the effect of LCE3C_3B-del, we followed the logistic regression framework described in Cordell and Clayton (2002). Significant P-values were corrected by Monte-Carlo simulation for three markers.

For stratification for other psoriasis risk factors, we used genotypes of the strongest associated variant of the IL-23R pathway (rs6887695 of IL12B gene) in the German cohort (Huffmeier et al., 2009b). To stratify for single risk factors, carriers of the common risk allele “G” of variant rs6886795 and carriers of more than four copies of the β-defensin cluster were regarded as risk groups versus non-risk groups.

To test whether the German and Dutch cohorts could be combined in one logistic regression model, the Cochran–Mantel–Haenszel statistical analyses were performed. For LCE and IL12B data, the test of homogeneity of the OR showed no significant difference ($P = 0.526$ for a recessive model and $P = 0.186$ for a dominant model), whereas it showed significant differences regarding the PSORS1 risk allele.

To test for interaction, we followed the logistic regression framework described in Cordell and Clayton (2002). Our model contained one variable (risk/non-risk) for each potential interaction partner and an interaction term to test for deviation from a multiplicative model. The significance of the interaction term was evaluated using a chi-square distribution with one degree of freedom. Significant P-values were corrected using the Bonferroni method, i.e., $P$-values were multiplied by 6 (for the number of interaction analyses performed).

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

**ACKNOWLEDGMENTS**

We are grateful to all patients and control probands for their participation in this study. We thank Anne M Bowcock (Human Genetics, Washington University School of Medicine) for helpful discussions. We also thank Petra Badorf and Claudia Danzer for their excellent technical assistance. This work was supported in part by a grant from the Interdisciplinary Centre for Clinical Research (IZKF B32/A8) of the University of Erlangen-Nuremberg. Research of the laboratory of XE is supported by the Spanish Ministry of Science and Innovation (SAF2008-00357) and by the Generalitat de Catalunya.

**REFERENCES**


Capon F, Di Meglio P, Szaub J et al. (2007) Sequence variants in the genes for the interleukin-23 receptor (IL23R) and its ligand (IL12B) confer protection against psoriasis. Hum Genet 122:201-6


Gudjonsson JE, Karason A, Antonsdottir AA et al. (2002) HLA-Cw6-positive and HLA-Cw6-negative patients with psoriasis vulgaris have distinct clinical features. *J Invest Dermatol* 118:162–5


