Deletion of the Late Cornified Envelope Genes LCE3C and LCE3B Is Associated with Psoriasis in a Chinese Population

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A common deletion comprising LCE3B and LCE3C, members of the late cornified envelope (LCE) gene cluster, has been shown to be significantly associated with psoriasis in several Caucasian populations. The expression of LCE can be induced by skin barrier disruption, leading to psoriatic lesions. To identify whether deletion of genes in the LCE region is a genetic risk factor in the pathogenesis of psoriasis, we genotyped the LCE3C and LCE3B deletion and single-nucleotide polymorphism rs4112788, which is in strong linkage disequilibrium with LCE3B deletion and single-nucleotide polymorphism LCE3B. Deletion of the two LCE genes was associated with psoriasis (odds ratio = 1.917; 95% confidence interval = 1.291-2.847, P = 0.001), a conclusion that was similar to that of another independent Chinese cohort study. The deletion was not significantly associated with the age of disease onset, and there was no significant epistatic interaction between deletion and PSORS1 risk allele on 6p21.3. Our study confirms an association between the deletion of LCE3C and LCE3B and psoriasis in a Chinese population.

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INTRODUCTION

Psoriasis vulgaris is a chronic inflammatory and hyper-proliferative skin disease that affects ~2% of the world’s population (Christophers, 2001). Generally regarded as an immunologically mediated disorder, it is characterized by vascular changes, abnormal keratinocyte proliferation and differentiation, and mixed infiltration of immunocompetent cells in the epidermis and dermis (Krueger and Bowcock, 2005). It is widely accepted that psoriasis is caused by a combination of environmental and genetic factors (Bowcock and Cookson, 2004). Genetic analyses have identified several psoriasis-susceptibility loci; the most strongly associated one (PSORS1) is within the major histocompatibility complex region, on chromosome 6p21 (Nair et al., 2006).

Copy number variation is a major source of human genetic diversity (Redon et al., 2006; Kidd et al., 2008), and it has been associated with susceptibility or resistance to several diseases, particularly autoimmune and inflammatory disorders (Estivill and Armengol, 2007). Recent studies have focused on the deletion of two genes of the late cornified envelope (LCE) gene family, LCE3B and LCE3C, which are located in the epidermal differentiation complex. The LCE cluster encoding stratum corneum proteins has a wide range of expression in various epithelia, responds to different physiological stimuli, leading to psoriatic lesions (de Cid et al., 2009). The deletion of LCE genes was first identified as being significantly associated with psoriasis in individuals of western European descent (de Cid et al., 2009). Expression data in the same study suggested that either carriers of the deletion had a compromised repair response or the absence of LCE3C and LCE3B genes induced an inappropriate repair response following barrier disruption, triggering compensatory expression by other LCE genes (de Cid et al., 2009). This finding was replicated in a study of psoriasis patients in Germany (Huffmeier et al., 2009a), although another study by one of the same authors found that the deletion did not contribute to psoriatic arthritis (Huffmeier et al., 2009b). A study of Spanish subjects found no association of allele and genotype
frequencies with patients versus controls, but did confirm that homozygous \textit{LCE3C}_{3B}-del (deletion of \textit{LCE3C} and \textit{LCE3B} genes) is a risk factor for developing psoriasis without psoriatic arthritis (Coto et al., 2010). Very recently, a meta-analysis confirmed an association between \textit{LCE3C/LCE3B} deletion and psoriasis in several ethnic groups and detected its interaction with \textit{HLA-Cw6} (Rivera-Munoz et al., 2011). Finally, a pleiotropic effect of \textit{LCE3C}_{3B}-del was verified by ELISA as a susceptible genetic risk factor for rheumatoid arthritis in a Spanish population (Docampo et al., 2010), but the deletion was found not to be associated with atop dermatitis in a cohort study of other Europeans (Bergboer et al., 2010). In addition, single-nucleotide polymorphisms (SNPs) in the vicinity of the deletion were shown to be associated with psoriasis in an independent Chinese population (Zhang et al., 2009).

Although psoriasis is found worldwide, the incidence in Western populations, at 2–5% (Cargill et al., 2007), is much higher than that in East Asian populations, in which the incidence is only 0.1–0.3% (Yip, 1984). Most of the reported studies have been in European populations; little has been published about the relationship between \textit{LCE3C}_{3B}-del and psoriasis in Asian populations. We therefore aimed to use a case-control cohort to investigate whether \textit{LCE} deletion contributes to susceptibility to psoriasis and assessed its potential epistatic interaction with \textit{PSORS1} in the Chinese population. We also investigated the distribution of rs4112788, a SNP that is in strong linkage disequilibrium (LD) with the deletion.

**RESULTS**

We identified the \textit{LCE} region using PCR amplification and direct sequencing to define the deletion break points and confirmed that in the Chinese study population there was also a deletion encompassing 32.2 kb (chr1: 150,822,167–150,854,364; hg18, build NCBI36), similar to the deletion region reported for psoriasis patients in European populations (de Cid et al., 2009).

Both \textit{LCE3C}_{3B}-del and rs4112788 were in Hardy–Weinberg equilibrium in the control groups (the \(P\)-value of Hardy–Weinberg equilibrium is 0.46 and 0.65). Genotyping rates were 99.5% for the \textit{LCE} copy number variant (CNV) and 98.4% for rs4112788.

We assessed the genetic effect of \textit{LCE3C}_{3B}-del on psoriasis risk using regression analysis and found \textit{LCE3C}_{3B}-del to be significantly associated with psoriasis, in both the dominant model (odds ratio (OR) = 1.917; 95% confidence interval (CI) = 1.291–2.847, \(P = 0.001\)) and the additive model (OR = 1.268; 95% CI = 1.045–1.538, \(P = 0.016\)) after adjusting for gender and age (Table 1).

When we examined the data for only patients with type I psoriasis with onset before 40 years of age, no significant differences in age at disease onset were observed among the three \textit{LCE} genotypes. We compared the controls and patients who did not develop arthritis (\(n = 457\)). No significant change of OR was detected given that most (84.6%) of the patients in our study had psoriasis vulgaris.

There was a significant association of the SNP rs4112788 with psoriasis in both the dominant model (OR = 1.688; 95% CI = 1.150–2.478, \(P = 0.007\)) and the additive model (OR = 1.213; 95% CI = 1.004–1.466, \(P = 0.045\)), adjusting for gender and age (Table 1). The association for rs4112788 did not remain significant after correction for multiple testing. The LD between \textit{LCE} CNV and rs4112788 was relatively high \((D' = 0.893, \text{\(r^2\)} = 0.782)\), yet lower than in a previous study in a Chinese population \((\text{\(r^2\)} = 0.91)\) (Rivera-Munoz et al., 2011).

We also tested the interaction between deletion of \textit{LCE} genes and \textit{PRORS1} in our sample. We chose rs1576 and rs1265078 as proxy markers for the \textit{PSORS1} locus; both were in strong LD with \textit{HLA-Cw6} as well (Asumalhti, 2002). These SNPs were closely related to psoriasis as expected (Supplementary Table S2 online); however, the \(P\)-value derived from the log-likelihood ratio test was 0.0856, showing no evidence of interaction between the \textit{PSORS1} and \textit{LCE3C/LCE3B}-del loci in our samples.

**DISCUSSION**

\textit{LCE3C}_{3B}-del has previously been reported in case-control studies in several populations (de Cid et al., 2009; Coto et al., 2010; Rivera-Munoz et al., 2011), but most such studies have been based on European samples. The prevalence of psoriasis varies from 0.5 to 4.6% between countries and races

**Table 1. Association of \textit{LCE3C} CNV and rs4112788 with psoriasis in cases and controls**

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Subjects</th>
<th>Risk allele frequency (%)</th>
<th>Genotype distribution, n (%)</th>
<th>Additive model</th>
<th>Dominant model</th>
<th>Recessive model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(OR (95% CI))</td>
<td>(P^1)</td>
<td>(OR (95% CI))</td>
</tr>
<tr>
<td>\textit{LCE3C}_{3B}-del</td>
<td>D(^2) &gt; N</td>
<td>Case</td>
<td>62.9</td>
<td>49 (10.6)</td>
<td>246 (53.0)</td>
<td>169 (36.4)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>59.0</td>
<td>122 (16.0)</td>
<td>380 (49.9)</td>
<td>259 (34.0)</td>
<td>1.045–1.538</td>
</tr>
<tr>
<td>rs4112788</td>
<td>C(^2) &gt; T</td>
<td>Case</td>
<td>63.7</td>
<td>52 (11.4)</td>
<td>228 (49.9)</td>
<td>177 (38.7)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>59.9</td>
<td>124 (16.5)</td>
<td>355 (47.3)</td>
<td>272 (36.2)</td>
<td>1.004–1.466</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; CNV, copy number variation; D, deletion; \textit{LCE3C}_{3B}-del, deletion of \textit{LCE3C} and \textit{LCE3B} genes; N, nondeletion; NR, non-risk allele; OR, odds ratio; R, risk allele.

\(^1\)Calculated \(P\)-values were adjusted for age and gender.

\(^2\)Risk allele.
We defined the deletion break points of LCE3C_3B-del in a Chinese population and observed strong evidence for association between the deletion and psoriasis, in line with the studies in European populations (de Cid et al., 2009; Huffmeier et al., 2009a). The absence of LCE3C and LCE3B may trigger skin barrier function in epidermis and then induce expression of the other LCE genes in the cluster, thus affecting global transcription of the epidermal differentiation complex via cis-regulatory elements such as conserved noncoding elements (de Guzman Strong et al., 2010). This then results in abnormal differentiation and hyperproliferation of epidermis, as previously reported (de Cid et al., 2009).

A previous study reported a negative association of LCE3C_3B-del between psoriatic arthritis and controls in individuals of German origin (Huffmeier et al., 2009b), but because of the limited number of psoriatic arthritis patients in our study (n=11, 2.4%), we were unable to investigate a deletion-frequency difference between patients with psoriasis vulgaris and those with psoriatic arthritis.

The SNP rs4112788, which is in strong LD with LCE3C_3B-del, was also genotyped and its association was found to be in the same direction as that of the deletion, with a slightly lower significance. In contrast to the study in a German population (Huffmeier et al., 2009b), a recent study found that rs4112788 was strongly associated with both psoriasis and psoriatic arthritis (Bowes et al., 2010), but it did not genotype LCE CNV for technical reasons.

Because of the relatively strong LD between LCE CNV and rs4112788, we cannot rule out that rs4112788 might have a marginal possibility of capturing the association signal with psoriasis. However, considering that rs4112788 is a nonfunctional variant according to NCBI DCOSE evolutionary conservation data, the deletion of LCE3B and LCE3C might be a more plausible candidate for the association detected here. Fine mapping of the region is needed to identify causal variation(s), and further functional studies would be required to confirm the mechanism underlying psoriasis predisposition.

One limitation of this investigation was the discrepancy of age and gender between our psoriasis patients and controls. Because of the difficulty of recruiting age- and gender-matched controls, we calculated the logistic regression analysis with gender and age as covariates to exclude their influence on the risk assessment. The association of LCE3C_3B-del and rs4112788 with psoriasis was significant in dominant and additive models with or without any adjustment; therefore, we could still conclude that deletion of the two LCE genes and rs4112788 are risk factors for psoriasis. The analysis results between cases and controls without any adjustment is shown in the Supplementary Material online (Supplementary Table S3).

The existence of potential epistasis of LCE3C_3B-del with HLA-Cw6 for psoriasis was found in Dutch and US (Michigan) samples (de Cid et al., 2009), but not in the study’s other subgroups. We also evaluated the relationship between LCE3C_3B-del and PSORS1; however, no significant epistatic effect was observed. Similarly, no interaction between LCE3C_LCE3B and HLA_Cw06 was reported in a previous study in some Asian populations including Chinese (Riveira-Munoz et al., 2011). It seems that there is no significant epistasis of the two loci in the Chinese population, and further work is needed to investigate whether the positive interaction is a population-specific effect or the product of methodological analysis.

In conclusion, this study confirms an association between the LCE region and psoriasis and, in an independent investigation of deletion of LCE_LCE3B and rs4112788 in a Chinese population, evidences their contributions to susceptibility to psoriasis. No significant differences in the epistatic interaction of deletion with PSORS1 were detected. Similar studies, using large sample sizes, need to be extended to other ethnic populations.

**MATERIALS AND METHODS**

**Study population**

A total of 468 psoriasis patients (mean age 40.27 ± 15.70 years; 58.8% male) were recruited by dermatologists from Ruijing Hospital and Huashan Hospital in Shanghai. Additional clinical information, such as age and family history, was collected by questionnaire. The psoriasis was considered “early onset” if the condition appeared at ≤40 years of age; the other patients were categorized as “late onset”. Patient characteristics are shown in more detail in Table 2. As controls, 768 healthy individuals (mean age 52.00 ± 15.91 years; 41.3% male) without psoriasis were recruited from the same communities, representing the same geographic area and ethnic population. The study protocols were approved by the institutional review board of the ethics committee of the Shanghai Institute for Biological Sciences and conducted according to the Declaration of Helsinki Principles. Written informed consent was obtained from all subjects before enrollment. High-molecular-weight genomic DNA was prepared from venous blood using the QuickGene 610L Automatic DNA/RNA Extraction System (Fujifilm, Tokyo, Japan).

| Table 2. Main characteristics of the 468 patients with psoriasis |
|---------------------------------|-----------------|
| Gender (male/female)            | 275 (58.8%)/193 (41.2%) |
| Mean age (years ± SD)           | 40.27 ± 15.70 (9-84) |
| **Type of psoriasis**           |                  |
| Vulgaris                        | 396 (84.6%)     |
| Guttate                         | 29 (6.2%)       |
| Arthritis                       | 11 (2.4%)       |
| Erythroderma                    | 9 (1.9%)        |
| Other                           | 23 (4.9%)       |
| AFFECTED RELATIVES (FAMILIAL PSORIASIS) | 28 (17%)   |
| Early-onset psoriasis¹          | 315 (67.3%)     |
| Mean age (years ± SD)           | 33.67 ± 12.81   |
| Late-onset psoriasis¹           | 153 (32.7%)     |
| Mean age (years ± SD)           | 58.30 ± 9.03    |

¹Early-onset psoriasis, age ≤ 40 years; late-onset psoriasis, age > 40 years.
Genotyping

CNV genotyping. We genotyped the LCE3C_3B-del region using direct sequencing encompassing 32,198bp (chr1: 150,822,167–150,854,364). To amplify the break-point-spanning PCR product of 419bp, we used LCE3C-LCE3B-del primers in the presence of the deletion (Supplementary Table S1 online). The cycling conditions were 95°C for 10 minutes, 40 cycles of three steps (95°C for 30 seconds, 61°C for 30 seconds, and 72°C for 40 seconds), followed by 72°C for 10 minutes, and 4°C thereafter. The region of LCE3B and LCE3C was detected with LCE3C-LCE3B primers (Supplementary Table S1 online) to specifically amplify 207-bp product within the deletion region. The cycling conditions were 95°C for 10 minutes, 35 cycles of three steps (95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds), followed by 72°C for 10 minutes, and 4°C thereafter. Purified PCR products were then sequenced using a BigDye Terminator Cycle Sequencing Kit and ABI PRISM 3730XL DNA capillary sequencers (Applied Biosystems, Foster City, CA). The genotyping success rate did not differ significantly between cases and controls (98.9 and 99.7%, respectively). To estimate the error rate of genotyping, 150 control and patient samples were replicated and genotyped with 100% concordance.

SNP genotyping. The SNP rs4112788 (T/C) was genotyped using direct sequencing with SNP rs4112788 primers (Supplementary Table 1 online). The cycling conditions were 95°C for 10 minutes, 35 cycles of three steps (95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 45 seconds), followed by 72°C for 10 minutes, and 4°C thereafter. Purified PCR products were analyzed on an ABI 3730xl DNA Analyzer (Applied Biosystems). Genotype data were obtained in 98.4% of the DNA samples. Forty randomly selected replicated samples were included and genotyped with 100% concordance.

The SNPs rs1576 (G/C) and rs1265078 (G/C) were genotyped using the TaqMan SNP genotyping assay on an ABI7900 system (Applied Biosystems). The standard 5-μl PCRs were carried out using TaqMan Universal PCR Master Mix reagent kits according to the manufacturer’s guidelines. Genotype data were obtained in 99.8 and 99.6% of the samples for each SNP, respectively. Forty replicated quality control samples were included and genotyped with 100% concordance.

Statistical analysis

We used SHEsis (Shi and He, 2005) to perform the Hardy-Weinberg equilibrium tests and to compare the differences in allele and genotype frequencies between cases and controls, as well as to calculate LD coefficients (D’ and r2) in control individuals. We used the homozygote of the LCE3C_3B allele as a reference and 0, 1, and 2 copies of the LCE3C_3B allele. Logistic regression analysis was used to calculate ORs, 95% CI values, and corresponding P-values, with gender and age as covariates, assuming additive, dominant, and recessive models based on previous reports (de Cid et al., 2009). In the additive model, homozygotes for the deleted allele (−/−), heterozygotes (−/+), and homozygotes for the nondeleted allele (+/+) were coded as an ordered categorical variable for the genotype (2, 1, and 0). The dominant model was defined as 1/1 + 1/0 versus 0/0 and the recessive model as 1/1 versus 1/0 + 0/0. The best genetic model was selected using the Akaike information criterion (AIC) value, and the lowest AIC value was considered the best-fitting model for the fitted variant. We used Bonferroni corrections for the multiple-testing corrections. All data management and statistical analysis were carried out using SPSS software (release 17.0; SPSS, Chicago, IL).

To test for interaction, we followed the methods described by Rafael de Cid et al. (2009), and we used the genotype-specific model to assess the epistasis effect. Potential interaction between LCE3C_3B-del or rs4112788 and rs1576 or rs1265078 was evaluated with a log-likelihood ratio test between models, including only the additive effect against both additive effect and the full interaction terminology, and all models were adjusted by gender and age.

CONFLICT OF INTEREST

The authors state no conflict of interest.

Acknowledgments

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Supplementary material

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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