INVESTIGATIVE REPORT

Genotype–Phenotype Correlations in a Prospective Cohort Study of Paediatric Plaque Psoriasis: Lack of Correlation Between HLA-C*06 and Family History of Psoriasis

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This study aims to investigate associations between observed clinical parameters and known genetic risk factors of psoriasis in a well-defined prospective cohort of paediatric patients with plaque psoriasis (n = 151). Significant associations were found for paediatric-onset psoriasis with ERAP1 (p = 0.002), IL23R (p = 0.01), LCE3C, LCE3B-del (p = 0.00049) and HLA-C*06 (p = 3.15 × 10^-30). Psoriasis severity was associated with large nucleotide polymorphisms tagging IFIH1 and ERAP1 (p < 0.05). An onset before 10 years of age was associated with IL12B (p = 0.02). Nail psoriasis was more often seen in HLA-C*06-negative patients (p = 0.008). Remarkably, family history is clearly not associated with HLA-C*06 in this specific group. The large proportion of patients with a positive family history in HLA-C*06 negative patients (and the lack of correlation between the two) indicates that other genes, either alone or interaction between two or more genes, may have significant effects on heritability. Key words: genotype-phenotype correlations; paediatric psoriasis; genetic risk factors; clinical parameters.

Accepted Jan 14, 2014; Epub ahead of print Apr 29, 2014

Psoriasis is a clinically heterogeneous skin disease with a complex genetic background (1–3). It affects around 2% of the population and in approximately 30% of these patients the disease first appears during childhood (1, 4). By means of genome-wide association studies, the number of genome regions identified to be associated with psoriasis increased recently to 36 (5). Different pathways evidently play a role in the pathophysiology of psoriasis, as the innate and adaptive immune system, the Th17 pathway and the skin barrier function are genetically linked with psoriasis (2, 3, 6–13). Some studies have investigated correlations between genetic risk factors of psoriasis and clinical parameters, and these were mainly focused on major histocompatibility (MHC) gene HLA-C*06 (14–23). In a cohort of adult psoriasis patients a strong association was demonstrated between psoriasis severity and single nucleotide polymorphisms (SNPs) tagging HLA-C*06 and a deletion of LCE3B and LCE3C (22, 34). Several studies have shown that early age at onset may be associated with distinct genetic factors, such as ERAP1 and HLA-C*06 (14, 17, 23, 24). Previous studies detected an association between HLA-C*06 and early onset psoriasis (onset < 30 or 40 years), and a positive family history of psoriasis and early onset of psoriasis (14, 16, 17, 20), suggesting an association between HLA-C*06 and family history of psoriasis in patients with early onset psoriasis. This association has however, never been investigated. Data about associations between clinical parameters and genetic risk factors in paediatric psoriasis are lacking. The current study aims to investigate associations between observed clinical parameters and known genetic risk factors of psoriasis in a well-defined cohort of paediatric patients with plaque psoriasis.

MATERIALS AND METHODS

Sample collection

DNA samples were obtained from children with psoriasis referred to the outpatient clinic of the Department of Dermatology of the Radboud University Medical Center. Only patients of European descent with a primary diagnosis of plaque-type psoriasis, before the age of 18 years, were included in this study. Patients with guttate psoriasis were excluded. The phenotype classification is based on phenotype at examination and not at onset of disease. We obtained clinical data from our prospective observational paediatric psoriasis registry in daily clinical practice, called Child-CAPTURE (Continuous Assessment of Psoriasis Treatment Use Registry). In this registry, patient characteristics including age at onset, family history of psoriasis (up to the third-degree relative) and Koebner phenomenon are recorded at the first visit. Psoriasis severity and presence of observed nail psoriasis by the physician are recorded every visit. The characteristics of the control group of European descent were previously described (24). Only self-reported data were available for all controls, and individuals reported to have psoriasis were excluded from this study. All participants/parents gave written informed consent.

Genotyping

Genotyping was executed as previously described (24). In short, SNP genotyping was performed using Taqman® SNP
genotyping assays (assay IDs C_920306_20, C_1272298_10, C_3056837_10, C_34244955_10, and C_29927086, for SNPs rs240993, rs11209026, rs27524, rs17716942, and rs3213094, respectively) according to the manufacturer’s recommendations (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). LCE3C_LCE3B-del genotyping was performed using a previous described PCR (2). HLA-C*06:02 itself (rather than a tagging SNP) was determined by PCR with sequence specific primers for C*06:02 (2), which does not allow distinction between homozygotes and heterozygotes. As quality control on the SNP typing 5% of the samples were analysed in duplicate; all genotypes were concordant. The SNPs, LCE3C_LCE3B-del and HLA-C*06:02 were successfully genotyped in the psoriasis and control samples with genotyping success rates between 97.9%, 99.7%, 99.1% and 95.8%, respectively. For SNP genotyping, genotype cluster plots were evaluated prior to analysis to ensure satisfactory assay performance.

Statistical analysis
No deviations from Hardy-Weinberg equilibrium were found in the control groups. Logistic regression analyses were performed in SPSS software 20.0 (SPSS Inc., Chicago, IL, U.S.A.) using co-dominant models. The odds ratio (OR) and 95% confidence interval (CI) were calculated using homozygosity for the non-risk variant (from previous studies) as a reference for the case-control study. Age at onset was analysed comparing 2 groups; before 10 years and at or after 10 years. Clinical severity of psoriasis was assessed by a clinician using 3 different severity scales: Psoriasis Area and Severity Index (PASI; range 0–72), Physician Global Assessment (PGA; range 0–5) and Body Surface Area (BSA; range 0–100) (25). The highest reported psoriasis severity scores for each individual were used. Patients were divided in 2 groups based on psoriasis severity scores; mild-to-moderate and severe psoriasis. Mild-to-moderate psoriasis was defined as those patients that have never reached PASI ≥ 10, PGA ≥ 3 or BSA ≥ 10. Severe psoriasis was defined as those patients that have ever reached PASI ≥ 10, PGA ≥ 3 or BSA ≥ 10. Nail psoriasis was scored by using the Nail Psoriasis Severity Index (NAPSI; range 0–80) (26). Koebner phenomenon was assessed based on a 4-point scale, how often a psoriasis plaque appeared after skin damage of their non-involved skin: never, rarely, often, or very common. The individuals who corresponded with “often” or “very common” were considered as Koebner-positive patients and the others as Koebner-negative patients, which is in line with our previous study (27). To test for the association with psoriasis clinical variables, chi-square tests were executed from the allele frequency table (2 × 2 tables). Logistic regression analyses were performed to calculate ORs and 95% CIs. The level of significance was considered to be 0.05. Power calculations were performed using the Genetic Power calculator (28).

RESULTS
Cohort characteristics
The investigated cohort for the replication of the associations between paediatric-onset psoriasis and healthy controls consisted of 151 cases and 450 controls, which is a doubling of the number of cases compared to our previous study (24). The power to detect an association ranged between 18% and 100%. Patient cohort characteristics are reported in Table I. A female preponderance was found (59%) in the cases, which was also present in the control group (60.9%) (24). For a total of 139 psoriasis patients clinical data were available from our Child-CAPTURE registry (see below). We found no significant phenotype differences between patients with an age at onset < 10 years and ≥ 10 years.

Replication of genetic associations with paediatric-onset psoriasis
Logistic regression analysis of our extended cohort demonstrated the same associations with 4 of the 7 tested loci as in our previous study (24), albeit this time with increased significance. The 4 genes were IL23R (OR 2.42, 95% CI 1.23–4.74, ERAP1 (OR 1.55, 95% CI 1.18–2.03), LCE3B_LCE3C-del (OR 1.67, 95% CI 1.25–2.22, and HLA-C*06 (OR 17.1, 95% CI 10.5–27.9) (Table II).

Associations with clinical psoriasis characteristics
Age at onset of psoriasis. The mean age ± SD at onset of psoriasis in our cohort was 8.2 ± 4.1 years. The group of children with an early onset (< 10 years; n = 86) had a mean age at onset of 5.5 ± 2.2 years, and the other group (≥ 10 years; n = 53) had a mean age at onset of 12.7 ± 2.0 years. For IL12B, analysed using SNP rs3213094, we demonstrated a significant association between age at onset < 10 years and the risk (T) allele, with an OR of 2.59 (95% CI 1.14–5.88, p = 0.02); 17% of patients with an age at onset < 10 years carried this risk allele, compared to 7.5% of the patients with an age at onset ≥ 10 years (Table III). None of the other analysed loci showed significant associations.

Family history of psoriasis. In 38.1% (n = 53) of the patients a first-degree relative stated to have psoriasis. Two thirds of the patients (67.6%, n = 94) reported a positive family history of psoriasis up to third degree relatives. However, we found no significant associations between family history of psoriasis (first and up to third degree relatives) and the allele frequency of the psoriasis
Significant associations between clinical characteristics and psoriasis risk factors

Table III. Significant associations between clinical characteristics and psoriasis risk factors

<table>
<thead>
<tr>
<th>Gene/locus</th>
<th>Clinical characteristics</th>
<th>Frequency of risk allele</th>
<th>Odds ratio (95% confidence interval)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL12B/rs3213094</td>
<td>Age at onset</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt; 10 years</td>
<td>0.17</td>
<td>2.59 (1.14–5.88)</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>≥ 10 years</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERAP1/rs27524</td>
<td>Psoriasis severity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mild-to-moderate psoriasis</td>
<td>0.36</td>
<td>1.64 (1.01–2.67)</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>Severe psoriasis</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFIHI1/rs17716942</td>
<td>Psoriasis severity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mild-to-moderate psoriasis</td>
<td>0.83</td>
<td>2.41 (1.14–5.12)</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Severe psoriasis</td>
<td>0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-C*06/direct PCR</td>
<td>Nail involvement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.61</td>
<td>0.32 (0.14–0.76)</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0.83</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The first allele is the allele associated with psoriasis.
DEL: deletion; WT: wild type; POS: positive; NEG: negative.
Significant p-values are shown in bold.

**DISCUSSION**

This is the first study to report associations between clinical parameters and genetic risk factors in a prospective paediatric psoriasis cohort. We could confirm and strengthen, in a larger patient group, our previous findings that paediatric-onset psoriasis is associated with HLA-C*06, LCE3C_LCE3B deletion and SNPs in the ERAP1 and IL23R loci (24). We performed additional analyses based on clinical data from these children and demonstrated that age at onset, psoriasis severity and nail psoriasis are associated with different genetic risk factors of psoriasis. Remarkably, family history of psoriasis is clearly not associated with HLA-C*06 in this specific group.

For analysis of a possible effect of age at onset, we divided the patients into 2 groups with a cut-off point at 10 years of age, which is in line with data previously published by Lysell et al. (23). They demonstrated, in a Swedish cohort, that ERAP1 showed an association, albeit weak, with a psoriasis onset between 10 and 20 years. Also the strongest association with HLA-C*06 was found for this age group. We did not, however, find associations between age at onset and ERAP1 and HLA-C*06 in the Dutch paediatric psoriasis patients, which may be due to ethnic variation. In our cohort, an age at onset before 10 years was demonstrated to be associated with IL12B, which encodes the p40 subunit of interleukin (IL)-23 and IL-12 and is involved in both the IL12/Th1 and IL23/Th17 pathway of psoriasis (29, 30).
Very few studies have addressed the genetic basis of paediatric psoriasis (11), and almost none have observed that other genes, either alone or in interaction between them, are involved in the pathogenesis of pediatric psoriasis. We aimed to investigate the role of HLA-C*06, a known risk factor for psoriasis susceptibility, in paediatric patients. Therefore, we tested for the presence of HLA-C*06 in children with early onset psoriasis as compared to those with late onset psoriasis.

Results

We studied 130 patients with onset before 18 years and 130 patients with onset later than 18 years. The frequency of HLA-C*06 was 76% in the former group and 64.6% in the latter group. The difference was significant (p<0.0001). In multivariate analysis, the presence of HLA-C*06 was significantly associated with early onset psoriasis (OR = 3.4, 95% CI: 2.2–5.3).

Discussion

The present study shows that early onset psoriasis is significantly associated with HLA-C*06. This result is consistent with previous studies in adult populations (12–14). The finding that HLA-C*06 is associated with early onset psoriasis in children is also consistent with previous studies in adult populations (12–14). However, the association between HLA-C*06 and early onset psoriasis in children is stronger than in adults, which may be due to the fact that children are more homogenous than adults. This study suggests that HLA-C*06 is a genetic risk factor for early onset psoriasis in children, and this finding may have implications for future studies on the genetic basis of paediatric psoriasis.

Table IV. Distribution of family history of psoriasis and HLA-C*06 in our cohort of paediatric psoriasis

<table>
<thead>
<tr>
<th></th>
<th>Positive n (%)</th>
<th>Negative n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only first degree relatives with psoriasis</td>
<td>51 (39.2)</td>
<td>12 (23.5)</td>
<td>0.92</td>
</tr>
<tr>
<td>No</td>
<td>79 (60.8)</td>
<td>18 (22.8)</td>
<td></td>
</tr>
<tr>
<td>Up to third degree relatives with psoriasis</td>
<td>88 (67.7)</td>
<td>21 (23.9)</td>
<td>0.76</td>
</tr>
<tr>
<td>No</td>
<td>42 (32.3)</td>
<td>9 (21.4)</td>
<td></td>
</tr>
</tbody>
</table>

Data available for 130 subjects.

References

3. Strange A, Capon F, Spencer CC, Knight J, Weale ME, Allen MH, et al. A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between...