reaction with 20 μM fluorescein-5-thiosemicarbazide in 0.1 M MES buffer (2-morpholinoethane sulfonic acid-Na, pH 5.5) [8]. Quantification of carbonylated proteins in the SC was carried out in terms of image analysis using fluorescence microscopic photos [9]. The mean value of carbonylated proteins in the SC around dark skin pores was 28.6 ± 4.3 AU, and was significantly higher than that around light skin pores, 24.0 ± 3.6 AU (p < 0.05, Students t-test). The sum of these results suggest that the stacking of corneocytes accumulating carbonylated proteins is a cause of dark skin pores, because corneocytes around skin pores have an opportunity for protein carbonylation due to their continuous exposure to sebum. Then, to seek involvement of Propionibacterium acnes on skin pore darkness, the fluorescence which is originated from coproporphyrin, was observed with the microscope (UV2way microscope INT100). However, it was failed to find involvement of the fluorescence and the darkness in skin pores in the case of volunteers. Thus, it remains unknown how dark skin pores and light skin pores are regulated and whether the frequency of dark skin pores depends on age. As one clue for that regulation, it could be thought to be attributed to the capability for antioxidation in keratinocytes or corneocytes such as superoxide dismutase or catalase. To resolve those questions, further examinations are required. We will continue this study and will report the results in the future.

Conflict of interest

The authors have declared no conflicting interests

References


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Letter to the Editor

Genetically defined variants of toll-like receptors 3, 7 and 9 as phenotype and risk modifier factors for psoriasis

To the Editor,

In psoriasis, a chronic inflammatory skin disease with a complex pathophysiology, multiple epidermal differentiation and immune-related genes interact with environmental factors [1]. Despite recent findings on pathogenic related-immune cellular subsets and cytokines (e.g. Th17 and Th22 pathways), the ultimate factors that trigger and preserve inflammation remain unknown. TLRs are germ line-encoded pattern-recognition receptors that recognize pathogen-associated and molecular patterns (PAMPs) and tissue damage-associated molecular patterns (DAMPs) such as self-nucleic acids. These receptors, expressed by immune and non-professional immune cells (i.e. keratinocytes), constitute a main immune sensing system that induces rapid inflammatory responses. Abnormal TLR function is related to chronic inflammatory and autoimmune diseases [2]. In psoriasis, TLRs are involved in i) triggering psoriasis-related cytokine secretion on keratinocytes after PAMP/DAMP-induced activation, ii) dendritic cell (DC) activation elicited by self-DNA-LL-37 complexes via TLR7, TLR8 and TLR9, breaking immune-tolerance [3], and iii) IL-23/Th17 axis promotion inducing IL-23 promotion in DCs and macrophages [4].

To explore the role of TLRs in psoriasis, we analysed 11 single nucleotide polymorphisms (SNPs) (Table 1) in 304 Spanish psoriasis patients and 305 healthy controls. Inclusion criteria were age ≥18 years and a follow-up of ≥6 months. The ethics committee approved the study protocol and all patients gave written informed consent before inclusion and blood extraction for TLR genotyping. Patients who received ≥1 systemic treatment, biological agents or phototherapy, were included in the moderate-to-severe group, according to previous studies [5]. The age at disease onset was dichotomized as <40 years (early-onset psoriasis) vs. ≥40 years (late onset psoriasis), a cut-off used in previous studies [6]. Table 1 shows the clinical and demographic characteristics of the study population. Clinical features were compared according to
Table 1
Toll-like receptor polymorphisms analysed and clinical and demographic characteristics of study population.

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Gene</th>
<th>Chr</th>
<th>Location</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Functional impact on receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs121917864</td>
<td>TLR2</td>
<td>4</td>
<td>Exon 2</td>
<td>c.2029C &gt; T</td>
<td>p.R677W</td>
<td>Hypomorphic</td>
</tr>
<tr>
<td>rs5743708</td>
<td>TLR3</td>
<td>4</td>
<td>Exon 4</td>
<td>c.1234C &gt; T</td>
<td>p.L412F</td>
<td>Hypomorphic</td>
</tr>
<tr>
<td>rs3775296</td>
<td>TLR7</td>
<td>1</td>
<td>UT c.-1486T</td>
<td>c.554T &gt; A</td>
<td>untranslated</td>
<td>Amorphic</td>
</tr>
<tr>
<td>rs4986790</td>
<td>TLR8</td>
<td>9</td>
<td>Exon 4</td>
<td>c.396A &gt; G</td>
<td>p.D299G</td>
<td>Hypomorphic</td>
</tr>
<tr>
<td>rs5744168</td>
<td>TLR8</td>
<td>1</td>
<td>Exon 6</td>
<td>c.1174C &gt; T</td>
<td>p.R392 &gt; Stop</td>
<td>Amorphic</td>
</tr>
<tr>
<td>rs179008</td>
<td>TLR9</td>
<td>3</td>
<td>Exon 2</td>
<td>c.2848G &gt; A</td>
<td>p.P454P</td>
<td>Hypomorphic</td>
</tr>
<tr>
<td>rs5743836</td>
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<td>3</td>
<td>UT c.-12377</td>
<td>c.12377T &gt; C</td>
<td>untranscribed</td>
<td>Increase transcriptional activity</td>
</tr>
<tr>
<td>rs187084</td>
<td>TLR9</td>
<td>3</td>
<td>UT c.-1486T</td>
<td>c.1486T &gt; C</td>
<td>untranscribed</td>
<td>Increase transcriptional activity</td>
</tr>
</tbody>
</table>

Table 2
Intracellular nucleic-sensing TLR3, TLR7 and TLR9 genotype analysis with respect to PsA and age of disease onset.

** Adjusted parameters included sex, family history and psoriatic arthritis.
** Comparisons of continuous variables between groups were performed using the Kruskal-Wallis test: the chi square test was used to compare frequencies.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP ID</th>
<th>No PsA (n = 164)</th>
<th>PsA (n = 117)</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>TLR7</td>
<td>rs179008</td>
<td>AA</td>
<td>183</td>
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<td>T9</td>
<td>9</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

** Abbreviation:** PsA = psoriatic arthritis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP ID</th>
<th>No PsA (n = 164)</th>
<th>PsA (n = 117)</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>TLR9</td>
<td>rs187084</td>
<td>CC</td>
<td>83</td>
<td>43</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>90</td>
<td>47</td>
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<td></td>
<td></td>
<td>TT</td>
<td>20</td>
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<td>4</td>
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<td></td>
<td></td>
<td>T9</td>
<td>9</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

** Abbreviation:** PsA = psoriatic arthritis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP ID</th>
<th>Pooled genotype</th>
<th>Early onset (n = 164)</th>
<th>Late onset (n = 117)</th>
<th>Univariate</th>
<th>Multivariate</th>
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<tr>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>OR (95% CI)</td>
</tr>
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<td>rs352140</td>
<td>CC</td>
<td>83</td>
<td>43</td>
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<td></td>
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<td>10</td>
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<tr>
<td></td>
<td></td>
<td>T9</td>
<td>9</td>
<td>1</td>
<td>3</td>
<td>15</td>
</tr>
</tbody>
</table>

** Abbreviation:** PsA = psoriatic arthritis.
psoriasis severity. Genetic data analysis showed all SNPs analysed were in HWE (p > 0.05) except TLR9 rs5743836, which was in disequilibrium only in the patient group (p = 0.02), suggesting a possible association with psoriasis. Linkage disequilibrium (LD) analysis was made between SNPs of the same gene and found high LD between TLR3, TLR4 and TLR9 SNPs.

We investigated whether any TLR SNP studied acted as a psoriasis susceptibility genetic factor by comparing genetic data of psoriasis patients and controls. There were significant differences for TLR9 rs5743836; specifically, the homozygous CC genotype was more frequent in patients (2.3% vs. 0%; p = 0.0278), in agreement with the disequilibrium observed in the HWE analysis and suggesting a potential association between the CC genotype and psoriasis (Table 2).

Potential associations between allelic and genotypic frequencies of all TLR SNPs and psoriasis severity, the presence of PsA and age at disease onset were assessed. Only TLR7 rs179008 showed a trend to greater disease severity (p = 0.0440) but was not significant in the multivariate analysis. The T allele and the pooled AT + TT genotype of TLR7 rs179008 SNP were significantly associated with PsA in the univariate analysis and multivariate analyses adjusted for sex, age at disease onset, disease duration, family history and PASI values (p = 0.0121 and p = 0.0344, respectively) (Table 2). According to age of disease onset, significant associations were found between the TLR3 rs3775291 polymorphism and early-onset psoriasis (Table 2). Heterozygous genotypes of TLR9 rs187084 and rs352140 SNPs were significantly associated with early-onset psoriasis in a dominant inheritance pattern (p = 0.0067 and p = 0.0346, respectively) (Table 2). Despite the high LD between TLR9 SNPs, haplotype analysis showed no relevant results.

We examined TLR polymorphisms as susceptibility and disease-modifying genetic factors in psoriasis. Few recent articles have investigated this issue [7–9]. Smith et al. and Shi et al. focused on TLR2 and TLR4 and showed contradictory results [7–9]. Zablota et al. reported similar results although few TLR2 and TLR9 SNPs were analysed [9]. We expanded the analysis to multiple SNPs of many relevant TLRs, specifically including SNPs of intracellular nucleic acid-sensing TLRs (TLR3, TLR7 and TLR9) believed to play a key role in psoriasis, and the most-studied cell-surface TLRs (TLR2 and TLR4). We found no relevant role for the TLR2 and TLR4 SNPs studied in psoriasis susceptibility, in contrast to previous reports [7–9]. Genetic differences in the populations studied might explain the contradictory results [7–9]. We also found a significant association between the TLR9 rs5743836 polymorphism and psoriasis. Surprisingly, Zablota et al. linked TLR9 rs5743836 with a more severe and earlier psoriasis onset [9]. To our knowledge, this is the first study linking TLR9 rs5743836 SNP to psoriasis susceptibility.

As a disease-modifying genetic factor, the hypomorphic TLR7 rs179008 polymorphism was significantly associated with psoriasis severity, but not in the multivariate analysis. However, it was significantly associated with PsA, despite a very high confounding variable adjustment. Although TLR7 is known to play a role in DC activation, which drives the inflammatory cascade in psoriasis, no specific role has been postulated in PsA, as it has in rheumatoid arthritis [10]. The most notable findings were regarding the age at onset of psoriasis. First, there was an association between TLR3 rs3775291 and early-onset psoriasis in the univariate and multivariate analyses. This SNP has been related to a hypomorphic form that down-regulates receptor inflammatory activity although how it contributes to an earlier onset of psoriasis is not clear and is beyond the scope of this work. Secondly, the mutated alleles of the TLR9 rs187084 and rs352140 polymorphisms were associated with early-onset psoriasis in a dominant pattern of inheritance. Zablota et al. observed a significant association between the TLR9 rs5743836 polymorphism and more severe and earlier disease onset. These three mutated alleles of TLR9 have been shown to up-regulate receptor transcription activity.

In conclusion, our results strengthen the concept that acid nucleic-sensing TLRs play a key role in psoriasis and showed significant associations between functional TLR3, TLR7 and TLR9 SNPs and PsA, earlier disease onset and a greater risk for psoriasis.

Conflict of interest

The authors declare no conflict of interest that may inappropriate influence the representation or interpretation of reported research results.

References


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Letter to the Editor

A novel vitamin D receptor polymorphism associated with leprosy

Letter to the Editor

Leprosy is a chronic infectious disease that primarily affects peripheral nerves, and the skin, but can also compromise functions of the eyes and other organs. *Mycobacterium leprae* (*M. leprae*) is the etiological agent and an obligate intracellular parasite [1]. Leprosy remains a serious public health problem in developing countries, such as India, Brazil and Indonesia, and it is endemic in Africa, Asia and Latin America [2].

Clinically, leprosy is a spectral disease presenting the tuberculoid pole with cell-mediated response and the lepromatous form, often with Th2 response. There is also a transition group between both forms, denominated as borderline. The World Health Organization (WHO) guidelines classify leprosy patients into two categories: Paucibacillary (PB), which includes tuberculoid leprosy and some borderline tuberculoid leprosy; and Multibacillary (MB), which includes lepromatous, mid borderline, borderline lepromatous and lepromatous leprosy [3].

Several aspects related to host, pathogen and environment contribute to maintain epidemiological rates. Individual genetic susceptibility is a determining factor, and various genes and their polymorphisms may play a critical role in leprosy.

A previous study reported interaction between the VDR (TT genotype and negative Mitsuda response) were 5.23 times more chance (OR = 5.23; CI95%:2.25–12.16; P = 0.0002) to develop leprosy. This polymorphism genotypes between PB patients is under selection (P = .004), and the mutant allele may still be incorporated in this group (Table 1). There was a significant difference in the allelic frequency in PB patients as demonstrated previously [7].

In our cohort study, samples were randomly selected. The disease affected both sexes, however it is more incident in males, as demonstrated previously [7]. Moreover, genetic polymorphisms may vary depending on the population [8]. Our study was performed in a miscegenated population, and our results may be a molecular signature in a high endemic country.

Our analysis revealed that A61894G in PB patients is under selection (P = .004), and the mutant allele may still be incorporated in this group (Table 1). In (GG + AG)x(AA) analysis comparing HC and PB groups (P = .047). In (GG + AG)x(AA) analysis comparing HC and PB groups (P = .004), we have described, for the first time, the A61894G polymorphism (also in the 3′UTR region), evaluated through LIS-SSCP. Genotypes were also compared using a Mitsuda test for each patient, and the polymorphisms were confirmed by sequencing. Statistical analyses were performed using R (2017, R Foundation for Statistical Computing, Vienna, Austria) and BioEstat 5.0 software. Significant differences for allele frequency, association of the VDR genotypes with age and Mitsuda and Hardy-Weinberg equilibrium (HWE) values were tested using Chi-square. Leprosy outcome was predicted through odds ratio (OR). P < .05 was considered significant.

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