



## ORIGINAL ARTICLE

# Association of polymorphous light eruption with NOD-2 and TLR-5 gene polymorphisms

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## Abstract

**Background** Polymorphous light eruption (PLE) is a common, immunologically mediated, photosensitive skin disease. After ultraviolet-B (UV-B) irradiation, patients with PLE show reduced Langerhans cell (LC) depletion in the epidermis, which results in a non-suppressive microenvironment in the skin. Interestingly, severe acute graft-versus-host disease (aGvHD) occurred in stem cell transplanted patients that showed no or incomplete depletion of LCs after UVB irradiation. Genetic variation in nucleotide-binding oligomerization domain 2 (NOD-2) and toll-like receptor 5 (TLR-5) genes also confers susceptibility to aGvHD.

**Objectives** We hypothesized that PLE is associated with genetic variation in the NOD-2 and TLR-5 genes.

**Methods** We investigated single-nucleotide polymorphisms (SNPs) of NOD-2 (R702W, G908R, 3020Cins) and TLR-5 (A592S, P616L, N392STOP) in skin biopsies of patients with PLE ( $n = 143$ ) and in healthy controls ( $n = 104$ ) using restriction fragment length polymorphism analysis.

**Results** The frequency of NOD-2 alleles with the SNP R702W was significantly higher in PLE than in controls (31.8% vs. 6.3%;  $P < 0.0001$ ), and homozygous carriers of this mutation were more common in PLE (27.9% vs. 0%;  $P < 0.0001$ ). For SNP 3020Cins, the allele frequency (7.3% vs. 0.7%;  $P = 0.0025$ ) and the number of heterozygotes (14.7% vs. 1.3%;  $P = 0.0019$ ) were higher in PLE. The frequency of alleles with the N392STOP SNP of the TLR5 gene, which is associated with a truncated, non-functional receptor, was significantly higher in PLE (21% vs. 5%; 7% vs. 1% homozygotes, 28% vs. 8% heterozygotes;  $P < 0.0001$ ). The other SNPs did not differ significantly.

**Conclusions** This study yielded a high frequency of functional SNPs in the NOD-2 and TLR-5 genes in PLE. The same SNPs are associated with aGvHD and there are similarities in the reaction of LCs after UVB irradiation between aGvHD and PLE. This leads to the hypothesis that patients with PLE may be more susceptible to developing GvHD after stem cell transplantation, an assumption that needs to be investigated further.

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## Conflicts of interest

The authors declare no conflicts of interest.

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## Introduction

Polymorphous light eruption (PLE) is an ultraviolet-A (UV-A) and ultraviolet-B (UV-B) light-induced photodermatosis with an estimated prevalence of up to 10–20% in Western Europe and the United States.<sup>1–3</sup> PLE most commonly affects women in the third and fourth decades of life but varies among skin types and ethnic racial groups.<sup>1,4–7</sup> Pruritic, urticarial, or erythematous papules, plaques, or vesicles appear mainly in spring within hours to days after intensive sun exposure and then fade within a few days after the discontinuation of sun exposure.<sup>3</sup> PLE lesions have highly variable morphology but generally occur

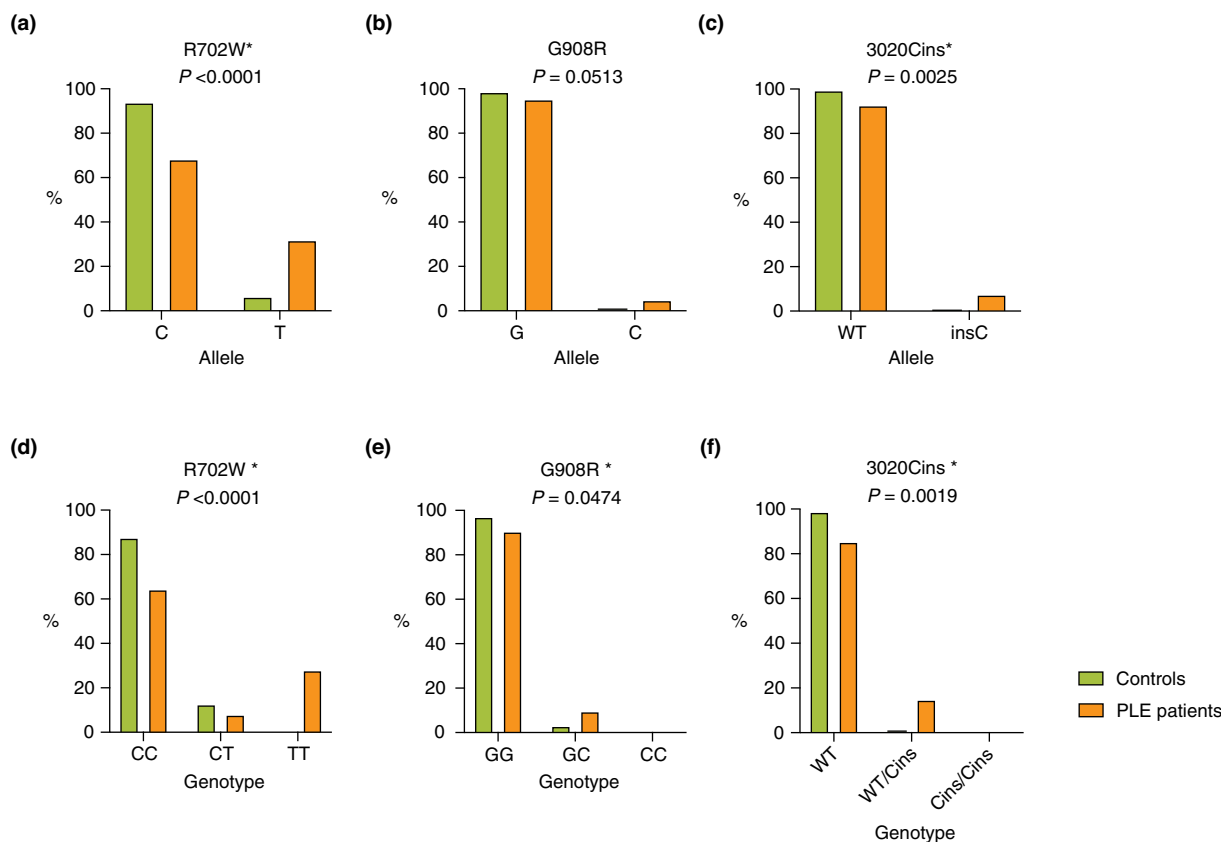
monomorphically in the individual patient.<sup>8</sup> In summer, a ‘hardening’ phenomenon can be observed, resulting in the tolerance of more intensive sunbathing. Hardening can also be achieved artificially by prophylactic desensitization with phototherapy in spring.<sup>4,7</sup> Areas that are continuously sun-exposed throughout the year such as the face are often unaffected by PLE.

The pathogenesis of PLE is not yet fully understood. A genetic predisposition is considered to play a role.<sup>9</sup> The concordant prevalence of PLE was shown to be 21% in monozygotic and 18% in dizygotic twins.<sup>10</sup> Another study demonstrated familial

clustering with a prevalence of photosensitivity in first-degree relatives of patients with PLE of 20.9% compared with that of 13.6% in the general population.<sup>11</sup> Besides genetics, decreased UV-induced immunosuppression is also assumed to play a role. The pathogenesis of PLE involves the production of UV-induced neo-antigens, which in combination with the lack of cutaneous immunosuppressive capacity results in a delayed-type hypersensitivity reaction in the skin.<sup>7</sup> Normally, UV radiation induces cytokine production and the influx of several cell types into the dermis and epidermis.<sup>12</sup> In contrast to healthy people, however, patients with PLE have decreased neutrophil infiltration into the skin after exposure to UV radiation. This lack of neutrophil infiltration is associated with impaired cytokine release, suppressed macrophage infiltration, and Langerhans cell (LC) resistance.<sup>13–15</sup> As bone marrow-derived dendritic cells are located within the epidermis, LCs represent the primary antigen-presenting cells in

the skin, which play an important role in local defence, contact sensitivity reactions, autoimmunity, and cancer surveillance. LCs have the capacity to initiate the development of effector T-cell responses to foreign antigens encountered in the skin by translocating from the epidermis to the regional lymph nodes. In healthy people, LCs disappear from the epidermis after UV-B exposure and migrate to draining lymph nodes to activate T cells located in the skin.<sup>16</sup> In patients with PLE, however, the capacity to deplete LCs from the skin after UV exposure is reduced<sup>17</sup> (Fig. 1). These immunological changes result in a non-suppressive microenvironment in the skin of patients with PLE.<sup>13,18</sup>

Besides their role in PLE, LCs are also important in the pathogenesis of graft-versus-host disease (GvHD). Persistent recipient LCs that survive the conditioning therapy contribute to the development of acute GvHD (aGvHD) after allogeneic



**Figure 1** Frequency of R702W, G908R, and 3020Cins mutations of the NOD-2 gene in patients with polymorphous light eruption. (a–c) Allele frequency and (d–f) genotype of patients with polymorphous light eruption and controls in % of SNPs R702W, G908R, and 1007 fs/3020Cins in the NOD-2 gene. The chi-square or Fisher’s exact test was used to determine the differences in genotype and allele frequencies. Tests of statistical significance were two-sided and considered significant when the *P*-value was <0.05. Significant differences in alleles between patients with polymorphous light eruption and healthy controls are marked with \*. A: adenine; G: guanine; C: cytosine; T: thymine; WT: wild-type; insC: cytosine insertion.

haematopoietic cell transplantation (allo-HCT) by activating donor T cells.<sup>19</sup> To prevent the development of aGvHD by depleting epidermal LCs, patients were irradiated with UV-B at the time of transplantation. After UV-B irradiation, patients with complete depletion of LCs in the skin had less severe or no aGvHD than patients with incomplete or no LC depletion.<sup>19</sup>

This finding led to our hypothesis that patients with PLE who are unable to deplete LCs after UV exposure may have a higher risk of developing GvHD after allo-HCT. Certain polymorphisms of the nucleotide-binding oligomerization domain 2 (NOD-2) and the toll-like receptor 5 (TLR-5) genes have also been associated with more severe aGvHD after allo-HCT. NOD-2 and TLR-5 belong to the pattern recognition receptors (PRRs), which recognize microbes trying to break through the surface barrier of the innate immune system.<sup>20</sup>

NOD-2, previously known as caspase recruitment domain 15 (CARD-15), is an intracellular pattern recognition receptor, which is important for immune defence against intracellular microbes.<sup>21,22</sup> Single-nucleotide polymorphisms (SNPs) in NOD-2-like R702W, G908R, and 3020Cins have been linked to aGvHD<sup>23–25</sup> and to inflammatory bowel disease.<sup>26,27</sup> SNPs in NOD-2 are associated with the inhibition of nuclear factor  $\kappa$  B (NF- $\kappa$ B), reducing the production of cytokines, such as interleukin IL-4, IL-10, tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), or tumour necrosis factor beta (TNF- $\beta$ ).<sup>28–30</sup> The expression of these cytokines is also reduced in patients with PLE, which led to our assumption that SNPs in NOD-2 may also play a role in PLE.<sup>13</sup>

Toll-like receptors (TLRs) on immunocompetent cells such as epidermal LCs or other dendritic cells, macrophages, and endothelial or epithelial cells recognize pathogen-associated molecular patterns present on the surface of both pathogens and commensal microorganisms.<sup>31,32</sup> TLRs are also germline-encoded sensors of the innate immune system associated with the expression of inflammatory mediators, major histocompatibility complex proteins, and costimulatory molecules by the above-mentioned cells.<sup>33</sup> The SNP N392STOP within TLR-5 is linked to severe GvHD.<sup>34</sup>

We hypothesized that genetic NOD-2 and TLR-5 polymorphisms may also play a pathogenic role in patients with PLE. For this reason, we investigated SNPs in the NOD-2 receptor (R702W, G908R, and 3020Cins) and TLR-5 (A592S, P616L, and N392STOP) in patients with PLE and compared the frequency of these SNPs with that in healthy controls.

## Materials and methods

### Study population

We retrospectively used paraffin-embedded skin biopsies that had been taken consecutively between 1997 and 2016 from 143 patients with PLE for DNA isolation. We used only biopsies from patients with a clinically suspected diagnosis of PLE (based on patient history and clinical presentation) which had been

confirmed histologically by a dermatohistopathologist. The routine biopsies of patients with PLE were used anonymously. Frequency of genotypes in PLE samples was compared with that of 104 healthy controls that had already been described in previous studies.<sup>35</sup> DNA from healthy controls had been isolated from blood samples. The total number of PLE and control samples used for the analysis of each SNP varied and is indicated as (n) for each SNP in Tables 1 and 2.

### Genomic DNA isolation

Genomic DNA was extracted from paraffin-embedded skin biopsies from patients with PLE using the blackPREP FFPE DNA kit (Analytik Jena GmbH, Jena, Germany) or from peripheral blood of healthy donors using the NucleoSpin FFPE DNA kit (Machery-Nagel®, Düren, Germany) according to the manufacturer's protocol. Since formalin fixation and paraffin extraction may lead to fragmented DNA, we had excluded in advance paraffin-embedded samples that had failed to generate technically flawless results for further restriction fragment length analysis.

### Analysis of restriction fragment length polymorphism (RFLP)

Polymorphisms were analysed using polymerase chain reaction (PCR) followed by nested PCR reaction (nPCR) with subsequent analysis of restriction fragment length polymorphism (RFLP) of three SNPs within the NOD-2 gene (R702W, G908R, and 3020Cins) and of three SNPs within the TLR-5 gene (A592S, P616L, and N392STOP) of patients with PLE and healthy controls. PCR was performed with a total reaction volume of 50  $\mu$ L containing 10 ng of genomic DNA under conditions and with PCR primers (Sigma-Aldrich GmbH; Taufkirchen, Germany) as indicated in Table S1. For DNA enrichment, a nested PCR with nPCR primers (Sigma-Aldrich GmbH; Taufkirchen, Germany) as indicated in Table S1 was supplemented with 2  $\mu$ L of polyethylene glycol (PEG) precipitated PCR product. Consequently, 10  $\mu$ L of the nested PCR amplified product was digested by restriction enzymes<sup>36–40</sup> as indicated in Table S2 and analysed *via* electrophoresis on a 12% polyacrylamide gel. Length of PCR products for determining the genotype of indicated SNPs in NOD-2/TLR-5 are indicated in Table S2.

### Statistical analysis

The observed genotype frequencies in cases and controls were in agreement with Hardy–Weinberg equilibrium, suggesting no population stratification. Frequencies of alleles and genotypes are reported with their group percentages. The chi-square or Fisher's exact tests were used to determine differences in allele and genotype frequencies. Tests of statistical significance were two-sided and were considered significant when the *P*-value was <0.05. All statistical analyses were performed with the GraphPad prism software package (GraphPad Software Inc. 9.01, San Diego, CA, USA).

**Table 1** Allele distribution and genotype of NOD-2 in patients with polymorphic light eruption and control subjects

Gene/SNP	Genotype/Allele	Patients with PLE <i>n</i> = genotype (%) / <i>n</i> = allele (%)	Controls <i>n</i> = genotype (%) / <i>n</i> = allele (%)
R702W C > T		<i>n</i> = 140 (%) / <i>n</i> = 280 (%)	<i>n</i> = 104 (%) / <i>n</i> = 208 (%)
	CC	90 (64.2)	91 (87.5)
	CT	11 (7.9)	13 (12.5)
	TT	39 (27.9)	0
	C	191 (68.2)	195 (93.7)
G908R G > C		<i>n</i> = 115 (%) / <i>n</i> = 230 (%)	<i>n</i> = 102 (%) / <i>n</i> = 204 (%)
	GG	104 (90.4)	99 (97.1)
	GC	11 (9.6)	3 (2.9)
	CC	0	0
	G	219 (95.2)	201 (98.5)
3020Cins		<i>n</i> = 143 (%) / <i>n</i> = 286 (%)	<i>n</i> = 75 (%) / <i>n</i> = 150 (%)
	WT/WT	122 (85.3)	74 (98.7)
	WT/3020Cins	21 (14.7)	1 (1.3)
	3020Cins/3020Cins	0	0
	WT	265 (92.7)	149 (99.3)
	Cins	21 (7.3)	1 (0.7)

**Table 2** Allele distribution and genotype of TLR-5 in patients with polymorphic light eruption and control subjects

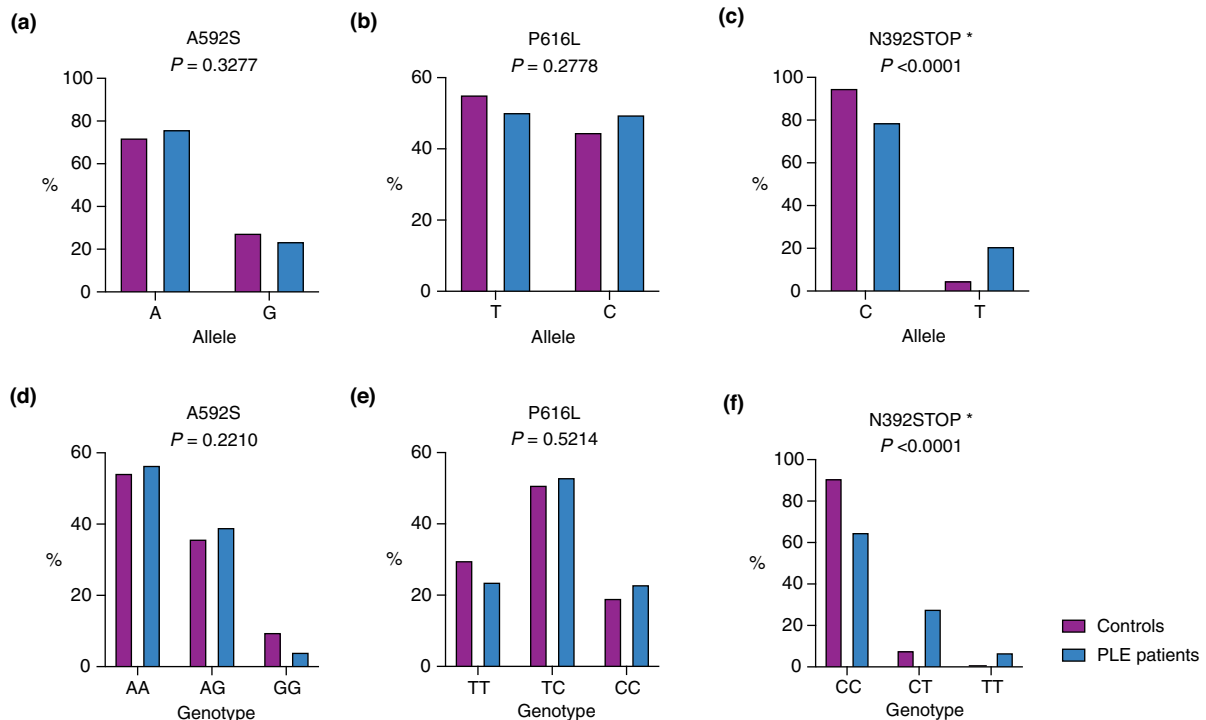
Gene/SNP	Genotype/Allele	Patients with PLE <i>n</i> = genotype (%) / <i>n</i> = allele (%)	Controls <i>n</i> = genotype (%) / <i>n</i> = allele (%)
A592S rs2072493 A > G		<i>n</i> = 143 (%) / <i>n</i> = 286 (%)	<i>n</i> = 103 (%) / <i>n</i> = 206 (%)
	AA	81 (56.6)	56 (54.4)
	AG	56 (39.2)	37 (35.9)
	GG	6 (4.2)	10 (9.7)
	A	218 (76.2)	149 (72.3)
P616L rs5744174 T > C		<i>n</i> = 143 (%) / <i>n</i> = 286 (%)	<i>n</i> = 104 (%) / <i>n</i> = 208 (%)
	TT	34 (23.8)	31 (29.8)
	TC	76 (53.1)	53 (51)
	CC	33 (23.1)	20 (19.2)
	T	144 (50.3)	115 (55.2)
N392STOP rs5744168		<i>n</i> = 100 (%) / <i>n</i> = 200 (%)	<i>n</i> = 100 (%) / <i>n</i> = 200 (%)
	CC	65 (65)	91 (91)
	CT	28 (28)	8 (8)
	TT	7 (7)	1 (1)
	C	158 (79)	190 (95)
	T	42 (21)	10 (5)

## Results

### Frequency of R702W, G908R, and 3020Cins mutations of the NOD-2 gene

The allele frequency of the R702W mutation (T-allele) differed significantly between patients with PLE (31.8%) and controls (6.3%) ( $\chi^2 = 47.07$ , 1;  $P < 0.0001$ ) (Fig. 1a;

Table 1). The allele frequency of the G908R mutation (C-allele) did not differ significantly between patients with PLE (4.8%) and controls (1.5%) ( $\chi^2 = 3.799$ , 1;  $P = 0.0513$ ) (Fig. 1b; Table 1). The allele frequency of the 3020Cins mutation was significantly higher in patients with PLE (7.3%) than in controls (0.7%) ( $\chi^2 = 9.153$ , 1;  $P = 0.0025$ ) (Fig. 1c; Table 1).



**Figure 2** Frequency of A592S, P616L, and N392STOP mutations of the TLR-5 gene in patients with polymorphic light eruption. (a–c) Allele frequency and (d–f) genotype of patients with polymorphic light eruption and controls in % of SNPs A592S, P616L, and N392STOP in the TLR-5 gene. The chi-square or Fisher's exact tests were used to determine the differences in genotype and allele frequencies. Tests of statistical significance were two-sided and considered significant when the *P*-value was <0.05. Significant differences in alleles between patients with polymorphic light eruption and healthy controls are marked with \*. A: adenine; G: guanine; C: cytosine; T: thymine; WT: wild-type; insC: cytosine insertion.

Analysis of the genotype of the **R702W** SNP mutation with the Chi-square test yielded significant differences between the two groups ( $\chi^2 = 34.61$ , 2;  $P < 0.0001$ ). Of the patients with PLE, 27.9% had a homozygous genotype and 11% a heterozygous genotype in contrast to controls (0% = homozygous; 12.5% heterozygous) (Fig. 1d; Table 1). We also found significant differences in the **G908R** SNP ( $\chi^2 = 3.930$ , 1;  $P = 0.0474$ ): None of the two groups had a homozygous genotype, but 9.6% of the patients with PLE ( $n = 115$ ) had a heterozygous genotype in contrast to only 2.9% of the controls ( $n = 102$ ) (Fig. 1e; Table 1). Neither of the two groups had a homozygous genotype in the SNP **3020Cins**, but 14.7% of the patients with PLE ( $n = 143$ ) had a heterozygous genotype in contrast to 1.3% of the controls ( $n = 75$ ). Again, the difference between the two groups was significant ( $\chi^2 = 9.666$ , 1;  $P = 0.0019$ ) (Fig. 1f; Table 1).

#### Incidence of A592S, P616L, and N392STOP mutations of the TLR-5 gene

The allele frequency of the **A592S** mutation (G-allele) was lower in patients with PLE (23.8%) than in controls (27.7%)

( $\chi^2 = 0.9579$ , 1;  $P = 0.3277$ ) (Fig. 2a; Table 2). In contrast, the allele frequency of the **P616L** mutation (C-allele) was higher in patients with PLE (49.7%) than in controls (44.7%), but the result was not statistically significant ( $\chi^2 = 1.178$ , 1;  $P = 0.2778$ ) (Fig. 2b; Table 2). The allele frequency of the **N392STOP** mutation (T-allele) was significantly higher in patients with PLE (21%) than in controls (5%) ( $\chi^2 = 22.63$ , 1;  $P < 0.0001$ ) (Fig. 2c; Table 2).

The genotypes of the **A592S** SNP showed no significant difference between the two groups ( $\chi^2 = 3.020$ , 2;  $P = 0.2210$ ). The homozygous genotype was more common in controls (9.7%) than in patients with PLE (4.2%). The heterozygous genotype, however, was more common in patients with PLE than in controls (39.2% vs. 35.9%) (Fig. 2d; Table 2). The genotypes of the **P616L** SNP showed no significant difference between the two groups ( $\chi^2 = 1.302$ , 2;  $P = 0.5214$ ), but, again, more patients with PLE had a homozygous (53.1%) and heterozygous (23.1%) genotype than controls (51% and 19.2%). Overall, the heterozygous genotype was more frequent in patients with PLE (53.1%) and controls (51%) than the wild type (WT) (PLE 23.8%;

controls 29.8%) (Fig. 2e; Table 2). The N392STOP SNP also differed significantly between the two groups ( $\chi^2 = 19.94$ , 2;  $P < 0.0001$ ). The homozygous and heterozygous genotypes were statistically more frequent in patients with PLE (7% and 28%) than in controls (1% and 8%) (Fig. 2f; Table 2).

## Discussion

This is the first study to examine the SNPs in the NOD-2 and TLR-5 genes in patients with PLE. We hypothesized that SNPs in the NOD-2 receptor (R702W, G908R, 3020Cins) and TLR-5 (A592S, P616L, N392STOP) in individuals with PLE respectively, may differ from those in control subjects.

In accordance with this hypothesis, the mutated allele frequencies in NOD-2 differed significantly in the SNP R702W between patients with PLE and controls. In addition, all investigated SNPs (R702W, G908R, and 3020Cins) differed in terms of the genotype. We showed that patients with PLE were more likely to have mutated genotypes than controls. The percentage distribution of the genotypes of SNPs in NOD-2 in patients with PLE was similar to the distribution of these SNPs in patients with inflammatory bowel disease in Caucasians and in patients with severe aGvHD after stem cell transplantation (SCT).<sup>40–42</sup>

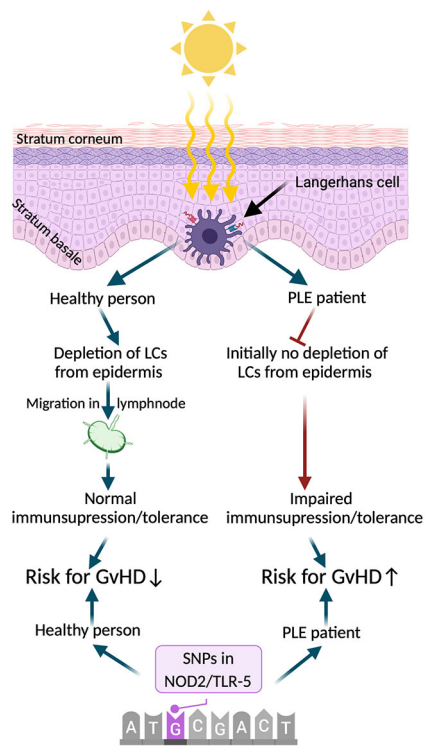
Many studies refer to NOD-2 polymorphisms. For instance, the SNPs in NOD-2, especially the 3020Cins SNP, have been shown to be associated with the activation or inhibition of nuclear factor  $\kappa$  B (NF- $\kappa$ B). NOD-2 activates NF- $\kappa$ B after intracellular stimulation by bacterial products and mitogen-activated protein kinase signalling pathways, leading to a series of immune responses.<sup>29,43,44</sup> NF- $\kappa$ B is also activated by UV radiation and regulates genes involved in a variety of inflammatory and immune processes, including cytokine production, induction of inflammatory enzymes, and activation of adhesion molecules.<sup>45</sup> Cells with SNPs in NOD-2 do not activate NF- $\kappa$ B when stimulated with the NOD-2 ligand muramyl dipeptide. The SNP 3020Cins results in a shortened protein lacking large parts of its muramyl dipeptide-binding portion.<sup>30,46</sup> Therefore, this defect in the NOD-2 protein structure may impair NF- $\kappa$ B activation and reduce or enhance the production of pro-inflammatory cytokines.<sup>29</sup> Netea *et al.*<sup>28</sup> showed that in patients with Crohn's disease, the 3020Cins frameshift mutation in the NOD-2 gene is associated with impaired release of interleukin 10 (IL-10) from blood mononuclear cells after stimulation with the TLR-2 ligands peptidoglycan and Pam3Cys-KKKK. The SNP generates a loss of function phenotype at this point. Patients with PLE irradiated with UV-B also show reduced expression of IL-10 as well as of interleukin 4 (IL-4) and TNF $\alpha$ . This reduction may be caused by a lack of neutrophils or be a consequence of reduced LC migration and T-helper-2 displacement.<sup>13</sup> Yet, this reduction may also be due to the SNP 3020Cins, which disrupts NF- $\kappa$ B activation and may thus reduce the expression of IL-10, IL-4, and TNF $\alpha$ . In the study by Netea *et al.*, the release of transforming growth factor beta (TGF- $\beta$ ) was also impaired after

mononuclear cells of patients with Crohn's disease carrying the 3020Cins mutation had been stimulated with peptidoglycan.<sup>28</sup> Patients with PLE showed decreased TGF- $\beta$ 1 immunoreactivity in lesional epidermis, which might have been influenced by the 3020Cins SNP.<sup>47</sup> Based on these findings, it is conceivable that among other factors, defective NF- $\kappa$ B regulation leads to an insufficient immune response or incorrect immune suppression. It would be interesting to investigate in further studies whether patients with PLE and such SNPs, in particular, the 3020Cins SNP mutation, show impaired NF- $\kappa$ B expression.

In the SNPs A592S and P616L of the TLR-5 receptor, neither the mutated allele frequency nor the genotype differed significantly between patients with PLE and controls. However, there was an increased frequency of mutant alleles in SNP N392STOP (21% vs. 5%) and the mutant genotype was more frequent in patients with PLE compared with controls (7% vs. 1% homozygotes and 28% vs. 8% heterozygotes). The average percentage distribution of the genotypes of the SNP N392STOP in TLR-5 in the analysed patients with PLE was similar to that in haematopoietic stem cell recipients with severe aGvHD.<sup>34</sup> Several SNPs within the TLR genes have been linked to infectious, inflammatory, and carcinogenic processes. The N392STOP SNP showed a trend toward a higher incidence of severe aGvHD in patients with SCT,<sup>34</sup> and the SNPs A592S and P616L have already been investigated in correlation with inflammatory bowel disease<sup>39,48</sup> and colorectal cancer.<sup>49</sup> We focused the discussion particularly on the N392STOP polymorphism due to the significant differences found between patients with PLE and controls. The stop codon polymorphism renders the truncated ligand-binding domain incapable of mediating flagellin signalling, thus increasing the susceptibility to infection. Flagellin has been shown to be the main stimulator of *Legionella pneumophila* for IL-8 production in lung epithelial cell lines.<sup>50</sup> In peripheral mononuclear blood cells of people who are heterozygous for TLR-5 N392STOP, IL-6 production is significantly reduced when stimulated with flagellin. PLE is characterized by lymphocyte-rich inflammatory infiltrates. Therefore, suction blister fluid samples from patients with PLE were analysed for the presence of cytokines, which induce peripheral blood lymphocyte migration *in vitro*. Norris *et al.*<sup>51</sup> demonstrated that IL-6 and IL-8 may be involved in the induction of peripheral blood lymphocytes in PLE. It is conceivable that people with N392STOP polymorphisms tend to have reduced IL-6 secretion.

Patients with PLE do not adequately deplete LCs after UV exposure. This reduced depletion of LCs after UV-B irradiation has been shown to increase the likelihood of aGvHD development in patients after allo-HCT. Since polymorphisms in the NOD-2 receptor and TLR-5 of the recipient and the donor are also made responsible for the development of aGvHD in SCT patients, we hypothesized that patients with PLE may have the same mutations. The risk factors for aGvHD are numerous, such as human leukocyte antigen (HLA) mismatch/unrelated





**Figure 3** Potential hypothesis of acute graft-versus-host reaction in patients with polymorphous light eruption. Patients with polymorphous light eruption irradiated with ultraviolet-B do not deplete Langerhans cells from the epidermis. Patients with incomplete Langerhans cell depletion have experienced severe acute graft-versus-host disease after haematopoietic stem cell transplantation. Patients with graft-versus-host disease have also a higher number of SNPs in NOD-2/TLR-5 than controls. Patients with polymorphous light eruption who have to undergo stem cell transplantation and have single-nucleotide polymorphisms in the NOD-2 and TLR-5 gene may have an increased likelihood of developing acute graft-versus-host disease.

donors,<sup>52</sup> or total body irradiation.<sup>53</sup> UV-B irradiation after allogeneic haematopoietic cell transplantation (allo-HSCT) has been shown to protect patients from developing aGvHD, but patients with incomplete LC depletion in the epidermis after UV-B irradiation developed a more severe form of aGvHD.<sup>19</sup> The NOD-2 SNPs investigated in our study are also discussed as risk factors for developing aGvHD.<sup>23–25,42,54,55</sup> The N392STOP SNP in TLR-5 showed a trend towards a more severe type of GvHD.<sup>34</sup> This SNP may also be a risk factor for invasive aspergillosis, which is particularly common in severe GvHD after SCT.<sup>56</sup> The SNPs A592S and P616L have not yet been studied in relation to aGvHD but in inflammatory bowel disease<sup>39,48</sup> and colorectal cancer.<sup>49</sup> In mice, pre-treatment with the TLR-5 ligand flagellin reduced the severity

of GvHD.<sup>57</sup> TLR-5 may be associated with attenuating the activation of antigen-presenting cells and hence be responsible for late LC depletion in patients with PLE.

The question arises whether patients with PLE in particular, those with defined SNPs in the NOD-2 and TLR-5 genes have a higher risk of developing aGvHD after allo-HCT (Fig. 3). PLE may thus be another risk factor for aGvHD. This question cannot be answered by means of our study results and has to be addressed in more detail in further studies. Because the SNPs in NOD-2 in particular are associated with chronic inflammatory bowel diseases, it would also be interesting to examine such patients for the presence of PLE or *vice versa*.

### Conclusion

Our study showed that mutant allele frequencies differed significantly in the NOD-2 SNPs R702W and 3020Cins as well as in the TLR-5 SNP N392STOP between patients with PLE and controls. Patients with PLE also showed significantly more homozygous and heterozygous genotypes in the NOD-2 SNPs R702W, G908R, and 3020Cins as well as in the TLR-5 SNP N392STOP than controls. These SNPs may alter the NF- $\kappa$ B signalling pathway in patients with PLE, resulting in changes in the immune response. The lack of LC depletion from the epidermis in UV-irradiated patients with PLE, which indicates failure of UV-induced immunosuppression, may be a sign of altered immune tolerance. These findings will inspire further studies in this under investigated field, which should aim at validating and extending these initial results.

### Limitations

This study did not investigate the clinical relevance of SNPs, and no genotype–phenotype correlation could be performed because of the lack of clinical data of patients with PLE (anonymized biopsies).

Further investigations, especially with regard to the depletion of LCs and the involvement of the NF- $\kappa$ B pathway and antimicrobial peptides, could not be carried out because of the lack of biopsy material. These questions should be the objective of further studies.

Another limitation of this study is the fact that no further clinical data were available from the patients with PLE since the biopsies were used anonymously. To overcome this limitation and to confirm the interesting results of this retrospective study, we are planning a prospective study using blood from patients with PLE that will include also all other relevant clinical patient data.

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## Data availability statement

The authors will make materials, data and associated protocols available upon request by mailing to the corresponding author.

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### Supporting information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** NOD-2/TLR-5 primers and conditions used for PCR and nested PCR reaction.

**Table S2.** Restriction enzymes and length of PCR products after enzyme cleavage for determining the genotype of indicated SNPs in NOD-2/TLR-5.