Clinical and genetic differences between pustular psoriasis subtypes

863 pustular psoriasis patients
- Generalised Pustular Psoriasis, n=251
- Acrodermatitis Continua of Hallopeau, n=28
- Palmoplantar Pustulosis, n=560
- Multiple diagnoses, n=24

Clinical features
whole cohort, n = 863

Genetic features
subset of cohort, n = 473

ACH: acrodermatitis continua of Hallopeau; GPP: generalised pustular psoriasis; PPP: palmoplantar pustulosis; PV: psoriasis vulgaris
Clinical and genetic differences between pustular psoriasis subtypes

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ABSTRACT

Background: The term pustular psoriasis indicates a group of severe skin disorders characterised by eruptions of neutrophil-filled pustules. The disease, which often manifests with concurrent psoriasis vulgaris (PV), can have an acute systemic (generalised pustular psoriasis, GPP) or chronic localised presentation (palmoplantar pustulosis, PPP; acrodermatitis continua of Hallopeau, ACH). While mutations have been uncovered in IL36RN and AP1S3, the rarity of the disease has hindered the study of genotype-phenotype correlations.

Objective: We sought to characterise clinical and genetic features of pustular psoriasis through the analysis of an extended patient cohort.

Methods: We ascertained a dataset of unprecedented size, including 863 unrelated patients (251 GPP, 560 PPP, 28 ACH, 24 multiple diagnoses). We undertook mutation screening in 473 cases.

Results: PV concurrence was lowest in PPP (15.8% vs. 54.4% in GPP and 46.2% in ACH, P<0.0005 for both), whereas mean age of onset was earliest in GPP (31.0 years vs. 43.7 in PPP and 51.8 in ACH, P<0.0001 for both). The percentage of females was higher in PPP (77.0%) than GPP (62.5%) (P=5.8x10^{-5}). The same applied to the prevalence of smokers (79.8% vs 28.3%, P<10^{-15}). While AP1S3 alleles had similar frequency (0.03-0.05) across disease subtypes, IL36RN mutations were less common in PPP (0.03) than GPP (0.19) and ACH (0.16) (P=1.9x10^{-14} and 0.002, respectively). Importantly, IL36RN disease alleles had a dose-dependent effect on age of onset, in all forms of pustular psoriasis (P=0.003).

Conclusions: The analysis of an unparalleled patient resource revealed key clinical and genetic differences between PPP and GPP.

Clinical implications: The association between IL36RN mutations and early-onset pustular psoriasis defines a patient group which should be prioritised for IL36RN screening and may particularly benefit from the development of IL-36 inhibitors.

Capsule summary: Standardised phenotyping and mutation screening of an extended pustular psoriasis resource demonstrated key differences between disease subtypes, highlighting clinical, demographic and genetic features that are unique to PPP.
**Keywords:** Generalised pustular psoriasis, palmoplantar pustulosis, acrodermatitis continua of Hallopeau, *IL36RN, AP1S3*, genotype-phenotype correlation.

**Abbreviations:** ACH, acrodermatitis continua of Hallopeau; ERASPN, European Rare and Severe Psoriasis Expert Network; GPP, generalised pustular psoriasis; PPP, palmoplantar pustulosis; PV, Psoriasis Vulgaris.
INTRODUCTION

The term pustular psoriasis refers to a group of severe inflammatory skin disorders manifesting with repeated eruptions of painful, neutrophil-filled pustules. These conditions can present with acute episodes of skin pustulation and systemic upset (generalised pustular psoriasis, GPP) or with chronic pustular eruptions that affect the palms and soles (palmoplantar pustulosis, PPP) or the tips of fingers and toes (acrodermatitis continua of Hallopeau). Of note all forms of the disease can be complicated by concurrent psoriasis vulgaris (PV)\(^1\).

We and others have shown that mutations of the gene encoding the IL-36 receptor antagonist (\textit{IL36RN}) are associated with GPP\(^2, 3\). While these defects are mostly observed in the homozygous or compound heterozygous state, a number of patients carrying single heterozygous changes have also been reported\(^4\).

Disease alleles associated with GPP have subsequently been identified in \textit{AP1S3} (encoding a sub-unit of the AP-1 adaptor complex)\(^5\) and \textit{CARD14} (encoding a keratinocyte NF-\(\kappa\)B adaptor protein)\(^6\). Of note, \textit{IL36RN}, \textit{CARD14} and \textit{AP1S3} mutations have also been described in PPP and ACH, demonstrating a shared genetic basis for pustular forms of psoriasis\(^5, 7, 8\). Patients harbouring disease alleles at two distinct loci (\textit{IL36RN} and \textit{AP1S3}; \textit{IL36RN} and \textit{CARD14}) have also been reported\(^9, 10\). Thus, an increasingly complex picture is emerging with evidence of substantial genetic heterogeneity, pleiotropy (the phenomenon whereby a single gene can influence more than one trait) and digenic inheritance.

In this context, the analysis of genotype-phenotype correlations would facilitate the stratification of patient cohorts and streamline the genetic diagnosis of disease subtypes. Rigorous studies, however, have been hindered by the rarity of pustular psoriasis, which has prevented the ascertainment and standardised phenotyping of sizeable patient resources.

Here, we sought to address this issue through the formation of a multi-centre consortium. We brought together eight tightly phenotyped patient cohorts through a collaboration with the European Rare and Severe Psoriasis Expert Network (ERASPEN). This enabled us to ascertain a unique clinical resource, including 863 unrelated cases and exceeding by nearly three-fold the size of any published dataset. The analysis of this extended cohort revealed very significant differences in the clinical and genetic features of pustular psoriasis subtypes. Specifically, it demonstrated that PPP differs from ACH and GPP in terms of patient demographics, disease presentation and underlying genetic abnormalities.
METHODS

Patient ascertainment

This research was carried out in accordance with the principles of the declaration of Helsinki and was approved by the ethics committees of participating institutions. Written informed consent was also obtained from all participants. The study aligned 8 patient cohorts (n=863) recruited in the reference centres listed in Table E1. The largest resource (n=255 British and Irish cases) was provided by St John’s Institute of Dermatology (London, UK) and combined an historical dataset (n=177) with patients ascertained prospectively (n=78) through the APRICOT clinical trial (Anakinra in Pustular psoriasis, Response In a Controlled Trial; EudraCT n. 2015-003600-23) and its sister mechanistic study PLUM (Pustular psoriasis, eLucidating Underlying Mechanisms). An additional 40 affected individuals (listed as “Others” in Table E1) were recruited outside the main reference centres, by clinicians who sent individual samples to the ERASPEN Consortium or St John’s Institute of Dermatology.

Pustular psoriasis was diagnosed by expert dermatologists based on direct clinical examination, with the ERASPEN consensus criteria\(^1\) used in at least 506 cases. The observation of primary, sterile, macroscopically visible pustules affecting non-acral skin (GPP), palms/soles (PPP) or the nail apparatus (ACH) was the main inclusion criterion. Conversely, the occurrence of pustules that were restricted to the edges of psoriatic plaques represented an exclusion criterion.

Mutation screening

IL36RN, AP1S3 and CARD14 mutations were screened by Sanger sequencing in 473 cases for whom DNA was available. Primer sequences and cycling conditions have been described elsewhere\(^3,5,6\). Nucleotide substitutions were identified using Sequencher 4.9 (Gene Codes). The deleterious effect of the newly identified c.115+5G>A mutation was confirmed using Spliceman and MaxEntScan\(^11,12\), whereas the pathogenic potential of CARD14 alleles was assessed with CADD\(^13\).

Statistics

The clinical and demographic characteristics of study participants were analysed using a binomial test (to establish the presence of a sex bias among pustular psoriasis patients), chi-
square test with Yates correction (to analyse differences in the prevalence of PV and proportion of affected females across disease types) and a Kruskal-Wallis test followed by Dunn’s multiple comparison test (to analyse differences in age of onset between PPP, ACH and GPP cases). The analysis of the genetic data was based on a chi-square test with Yates correction (to compare the frequency of disease alleles in PPP, ACH, GPP and the combined prevalence of \( IL36RN \) mutations across ethnic groups) and a one-tailed Fisher’s exact test (for the association between the \( IL36RN\)-pSer113Leu allele and PPP). Genotype-phenotype correlations were investigated by implementing logistic (for PV concurrence and sex ratios) and linear (for age of onset) regression analysis, using the disease subtype as a covariate. All tests were implemented in R\(^{14}\).

Patients with multiple diagnoses were excluded from all statistical analyses, as they could not be assigned to a single disease group.
RESULTS

Age of onset and PV concurrence rates vary significantly among disease subtypes

As members of the ERASPEN network, we previously defined consensus criteria for the diagnosis of pustular psoriasis. Here, we build on this work to describe the presentation of key disease features, as observed in clinical practice. We analysed 863 unrelated patients, the majority of which (823/863, 95.4%) were recruited through six European, one North-African and one Asian reference centres (Table 1, Table E1). Of note, key patient demographics (male to female ratios and mean age of onset for the various disease types) were comparable across these cohorts (Table E2).

While GPP (251/863, 29.1%) and PPP cases (560/863, 64.9%) accounted for most of the dataset, the ACH sample was substantially smaller (28/863, 3.2%), reflecting the extreme rarity of this condition. Of note, the concurrence of multiple disease forms (most notably GPP with ACH and GPP with PPP) was reported in a small percentage of affected individuals (24/863, 2.8%).

A number of co-morbidities were observed, with diabetes and hypertension figuring most prominently, regardless of patient ethnicity (Table E3). In keeping with published associations, we also found that 11/281 (3.9%) European PPP subjects suffered from autoimmune thyroid disease.

The mean age of onset differed considerably across disease types and was lower in GPP (31.0±19.7 years) compared to PPP (43.7±14.4; \( P=9.3\times10^{-19} \)) and ACH (51.8±20.4; \( P=1.2\times10^{-7} \)) (Fig 1, A and Table E2). Despite these marked differences, there was substantial heterogeneity within the individual disease cohorts, with very early (<10 years) and very late onset (>70 years) cases observed in all forms of pustular psoriasis.

While the prevalence of PV in the overall dataset (29.1%) was much higher than that reported for the general population (2-3%), concurrence rates varied among disease variants. In particular, the frequency of PV among individuals affected by PPP (15.8%) was significantly lower than that seen in ACH (46.2%) (\( P=0.0004 \)) and GPP (54.4%) (\( P=2.2\times10^{-16} \)) (Fig 1, B). While the latter result was partly driven by a very high prevalence of PV among Malaysian GPP patients (Table E2), the difference remained significant (\( P=0.01 \)) when the sizeable Malaysian cohort (n=138) was removed from the analysis. Thus, our investigations have demonstrated key differences between disease subtypes, highlighting PPP as a late-onset condition with low PV concurrence.
**PPP is the form of pustular psoriasis that is mostly influenced by sex and smoking status**

It has been reported that females and smokers are at higher risk of PPP than males and non-smokers\(^1^6\). Here, we observed a degree of sex bias in all forms of pustular psoriasis, as the female to male ratio was 1.5 in ACH, 1.7 in GPP and 3.5 in PPP. The distortion in sex ratios observed in GPP and PPP was statistically significant ($P<10^{-5}$ and $P<10^{-15}$, respectively) and readily recognizable in individual cohorts (Table E2). Of note, the difference between the PPP and GPP female to male ratios was also significant ($P=5.8\times10^{-5}$), highlighting PPP as the condition that is most influenced by sex-related factors (Fig 1, C).

In our dataset, 79.8% (249/312) of PPP sufferers for whom data was available were current or past smokers. Of interest, the rate of PV concurrence was much higher in PPP patients who smoked (or had done so in the past) compared to those who did not (12.4% vs. 1.6%, $P=0.009$), suggesting that cigarette smoking may modulate disease manifestations. In fact, smoking has a well-documented effect on aryl hydrocarbon receptor signalling\(^1^7\), a pathway which modulates the severity of inflammation in psoriatic skin\(^1^8\).

While the ACH sample was too small for analysis, the percentage of smokers in the GPP dataset (26/96, 28.3%) was significantly lower than that observed in PPP ($P<10^{-15}$), indicating that the adverse effect of cigarette smoking is specific to the latter condition.

**Definition of a patient subset for genetic analysis**

Having investigated the key clinical manifestations of pustular psoriasis, we sought to define their relationship with the patients’ genotypes. For this purpose, we examined the mutation status of 473 affected individuals for whom DNA was available (Table E1). We collated genetic data previously generated by our group (n=358)\(^4^6,^9\), while also examining 115 newly recruited cases. Importantly, Table E4 shows that the patient subset screened for mutations is representative of the broader dataset, suggesting that the findings obtained in this sample can be generalised to the whole resource.

**The frequency of IL36RN mutations differentiates PPP from ACH and GPP**

The *IL36RN* coding sequence and exon/intron junctions were screened in the entire patient resource, uncovering 66 individuals (4 ACH, 45 GPP, 12 PPP, 5 multiple diagnoses) with
disease alleles (Table 2, Table E5). Thirty-six of these subjects harboured bi-allelic (homozygous/compound heterozygous) changes, with the remaining 30 carrying mono-allelic (single heterozygous) variants. All the observed mutations had been previously described, except for a c.115+5G>A splicing variant uncovered in a North-American GPP case (Table E5).

*IL36RN* disease alleles were present in a variety of ethnic groups, with the highest prevalence observed among patients of European (34.7%) and East Asian descent (28.8%) (Fig 1, D). While we did not detect any rare changes in the 21 South-Asian cases we examined, a homozygous p.Leu21Pro mutation has been described in a Pakistani GPP pedigree\(^1\), suggesting that deleterious *IL36RN* alleles can also be found within the Indian subcontinent.

The proportion of individuals harbouring *IL36RN* disease alleles was higher in GPP and ACH (23.7% and 18.2%, respectively) compared to PPP (5.2%). GPP and ACH patients were also more likely to carry bi-allelic mutations compared to individuals affected by PPP (Table E5). As a result, the prevalence of *IL36RN* mutations was significantly increased in GPP (0.19) and ACH (0.16), compared to PPP (0.03) (\(P=1.9\times10^{-14}\) and 0.0018, respectively) (Table 2). Nonetheless, the association between *IL36RN* mutations and PPP, which has been recently questioned\(^2\), was statistically significant. In fact, an analysis of the recurrent p.Ser113Leu variant showed that its frequency in British patients was almost ten times higher than that observed in population-matched controls (\(P=9.3\times10^{-8}\); OR: of 10.8; 95% CI: 5.3-22.0) (Table 3).

We next sought to determine whether *IL36RN* alleles were associated with key features of pustular psoriasis across disease subtypes. We therefore implemented a regression analysis, using the clinical diagnosis as a covariate. While we did not observe a consistent effect of *IL36RN* mutations on PV concurrence (Table E6), we found a significant association with early age of onset (\(P=0.003\); Fig 1, E), which was observed in all three forms of the disease (Table E6). Thus, *IL36RN* alleles have shared genetic effects across pustular psoriasis subtypes, but occur at a very low frequency among PPP patients.

*CARD14* mutations are only observed in a small minority of cases

While a sizeable patient subset (n=106/473) was sequenced for the entire *CARD14* coding region, a targeted screening of exons 3 and 4 was undertaken in the rest of the sample,
given that the only disease alleles associated with pustular (p.Asp176His) or plaque (p.Gly117Ser) psoriasis map to this mutation hotspot\textsuperscript{6,20,21}. We found 3, previously described\textsuperscript{6} GPP individuals of Chinese descent bearing the p.Asp176His variant. We did not detect any CARD14 substitutions among European GPP patients, but observed 5 British PPP cases harbouring rare non-synonymous changes with deleterious potential (Table E7). While most the above subjects (6/8, 75\%) suffered from concurrent PV, the small size of the dataset prevented us from establishing genotype-phenotype correlations.

\textit{AP1S3 mutations occur with comparable frequency across disease types}

While a substantial patient subset (n=249) was screened for the entire coding region, the rest were only sequenced for exon 2, given that the only known \textit{AP1S3} mutations (p.Phe4Cys, p.Arg33Trp) map to this genomic segment \textsuperscript{25,9}. This revealed 24 European cases (2 ACH, 4 GPP, 14 PPP and 4 with multiple diagnoses) bearing the p.Phe4Cys or p.Arg33Trp changes (Table E8). No additional mutations were observed in the subjects who were screened for the entire coding region. Of note, three patients (2 GPP and 1 PPP) carried both \textit{AP1S3} and \textit{IL36RN} disease alleles (Table E9).

The prevalence of \textit{AP1S3} mutations was not significantly different across disease types (Table 2) and did not seem to influence PV concurrence or age of onset (Table E10). However, it was noteworthy that almost all individuals bearing \textit{AP1S3} disease alleles (23/24, 95.8\%) were females. While this observation was not statistically significant (\(P=0.06\)), a trend towards female over-representation was apparent in all clinical variants (Table E10), suggesting that the penetrance of \textit{AP1S3} mutations might be modified by sex-specific factors, such as hormone levels or X-linked modifiers.
DISCUSSION

The purpose of our study was to robustly define clinical and genetic features of pustular psoriasis, by investigating a patient cohort of unprecedented size.

We initially sought to define the presentation of the various clinical variants through a rigorous statistical analysis of key phenotypic features. This work, which builds on the definition of consensus diagnostic criteria by ERASPE1, underscores the importance of collaborative efforts in the analysis of rare diseases. Here, a common case report form was used in all prospectively recruited cases, enabling standardised patient phenotyping and robust data collection. The participation of multiple centres also allowed us to monitor the effects of ascertainment bias and show that key patient demographics were comparable across the various datasets.

Our analysis demonstrated novel and significant differences between disease subtypes. Specifically, it showed that PPP is associated with patient demographics (very high prevalence of females and smokers), clinical (low rates of PV) and genetic features (low prevalence of IL36RN mutations) that are clearly distinct from those observed in ACH and GPP. Given that abnormal IL-36 signalling has now been implicated in the pathogenesis of plaque psoriasis22, it is tempting to speculate that these observations may be correlated with each other and that the decreased prevalence of PV among PPP sufferers may be linked to the low frequency of deleterious IL36RN alleles in this patient group.

We also found that IL36RN mutations are associated with an earlier age of onset, across all variants of pustular psoriasis. This validates the results that we originally obtained in GPP4 and indicates that IL36RN should be prioritised for mutation screening when patients show disease symptoms before the age of 30 (40 in the case of ACH/PPP). Given that biologics that counter the effect of IL36RN mutations by blocking IL-36 signalling are now under development23, such targeted screening could have important implications for patient management.

Our study showed that IL36RN mutations are the most frequent genetic abnormality observed in pustular psoriasis. In fact, deleterious AP1S3 alleles were only found in 7-10% of patients and CARD14 variants were observed in a very small number of affected individuals. Importantly, our analysis demonstrated that known genes only account for a minority of disease cases. This is especially the case in PPP, where the combined frequency of AP1S3 and IL36RN mutations is less than 10%. Additional studies will therefore be needed to
illuminate the genetic landscape of this condition, facilitate its diagnosis and better understand the correlation between genotype and clinical phenotype. While the discovery of novel genetic determinants has so far been hindered by the rarity and heterogeneous nature of the disease, the ascertainment and rigorous phenotyping of our clinical resource lay a robust foundation for future gene identification studies.

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DECLARATION OF INTERESTS

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References


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$^1$Includes unknown ethnicity (n = 19), mixed ethnicity (n = 4), Middle Eastern (n = 4), Finnish (n = 2), Filipino (n = 1), Hispanic (n = 1), Jamaican (n = 1), Romani (n = 1);

Unkn: unknown; ACH: acrodermatitis continua of Hallopeau; GPP: generalised pustular psoriasis, PPP: palmoplantar pustulosis
Table 2. IL36RN and AP1S3 mutation frequency across disease types

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<td>4/22</td>
</tr>
<tr>
<td>count (frequency)</td>
<td>(0.05)</td>
<td>(0.05)</td>
<td>(0.03)</td>
<td>(0.18)</td>
</tr>
</tbody>
</table>

³Patients were classified as ‘Positive’ if they were carrying at least one mutation at the examined locus. ²The p.Phe4Cys and p.Arg33Trp mutations have no frequency in East-Asian populations and therefore were not screened in patients from this ethnic group. ACH: acrodermatitis continua of Hallopeau; GPP: generalised pustular psoriasis, PPP: palmoplantar pustulosis

Table 3 Association between IL36RN-p.Ser113Leu and PPP

<table>
<thead>
<tr>
<th></th>
<th>p.Ser113Leu</th>
<th>WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases¹</td>
<td>11 (3.6%)</td>
<td>291 (96.4%)</td>
</tr>
<tr>
<td>Controls²</td>
<td>26 (0.4%)</td>
<td>7402 (99.6%)</td>
</tr>
</tbody>
</table>

¹British patients only; ²Controls from publicly accessible cohorts (TWINSUK and ALSPAC)
Figure 1: Features of pustular psoriasis observed in the disease cohort. (A) The mean age of onset was compared across disease groups using a Kruskal-Wallis test followed by Dunn’s multiple comparison test. (B) Differences in PV concurrence were analysed with a chi-square test. (C) Differences in the proportion of affected females were assessed using a chi-square test. The dashed line indicates the percentage of females in general population. (D) Differences in the combined frequency of IL36RN mutations were assessed across ethnic groups, using a chi-square test. Pairwise comparisons were undertaken with Fisher’s exact test. The analysis was restricted to GPP cases, as this is only group for which data was available for multiple ethnicities. Other mutations: alleles seen only once in the cohort. The notation c.115+6T>C; p.Pro76Leu refers to patients carrying the two variants on the same haplotype. (E) The effects of IL36RN mutations on age of onset were assessed by means of linear regression. ACH: acrodermatitis continua of Hallopeau; GPP: generalised pustular psoriasis, PPP: palmoplantar pustulosis **P < 0.01; ***P < 0.001; ****P < 0.0001.