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IL-36-driven pustulosis: Transcriptomic signatures match between generalized pustular psoriasis (GPP) and acute generalized exanthematous pustulosis (AGEP)

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IL-36-driven pustulosis: Tra[Journal Pre-proof]neralized pustular psoriasis (GPP) and acute generalized exanthematous pustulosis (AGEP)

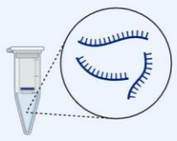
1 **Clinical data & sample collection**



Histopathologic analysis



2 **RNA isolation & quality control**

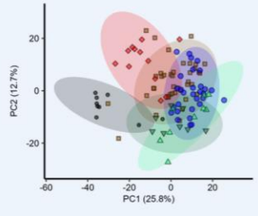


Library preparation & RNA-Seq



3 **Data analysis**

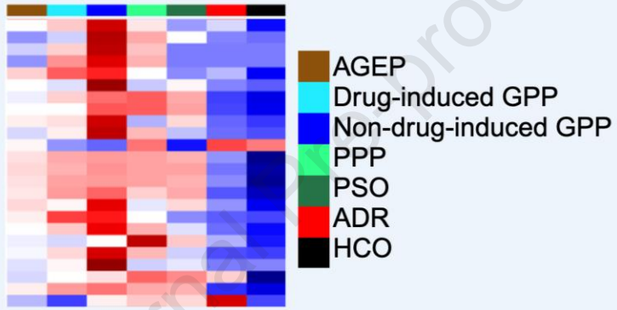




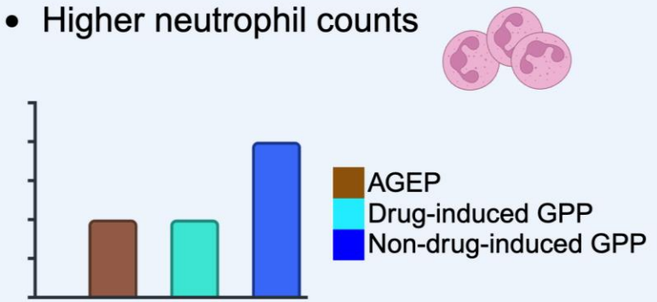
Drug-induced GPP vs. AGEP:
- No differentially expressed genes out of 13,000 analyzed genes
- Similar immune response pattern & immune cell composition

Non-drug-induced GPP vs. AGEP:

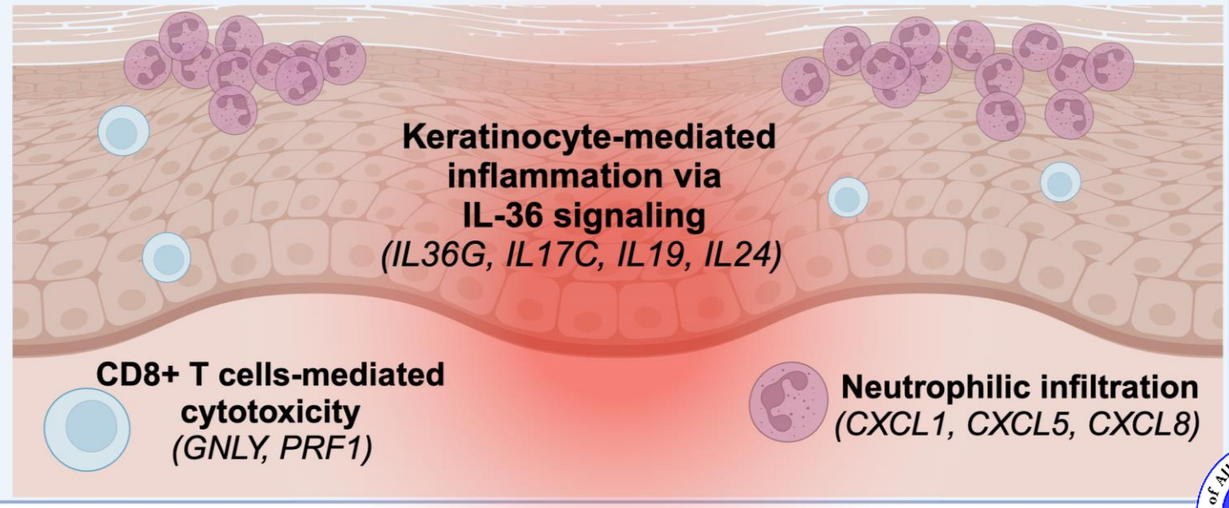
- Higher expression of Th17-related genes



- Higher neutrophil counts



Proposed model of the pathophysiological chain of drug-induced IL-36-driven pustulosis (AGEP and GPP):



AGEP, acute generalized exanthematous pustulosis; GPP, generalized pustular psoriasis; PPP, palmoplantar pustulosis; PSO, plaque-type psoriasis; ADR, other cutaneous adverse drug reactions; HCO, healthy control skin

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IL-36-driven pustulosis: Transcriptomic signatures match between generalized pustular psoriasis (GPP) and acute generalized exanthematous pustulosis (AGEP)

Short title: RNA-Seq profiles of GPP and AGEP

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Conflict of interest
The authors declare no competing financial interests.

Data availability
All data needed to evaluate the conclusion of the current manuscript are reported in the paper, supplementary data files or can be accessed through GEO Series accession number GSE280414 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE280414).

Ethics
All clinical trial procedures were approved by the Ethics Committee of the Medical University of Graz (protocol number 34-290 ex 21/22) or the Federal State of Carinthia, Austria (protocol number A23/15). All participants gave their written informed consent in accordance with the principles of the Declaration of Helsinki.

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Abstract

Background:

Due to similarities, the distinction between generalized pustular psoriasis (GPP) and acute generalized exanthematous pustulosis (AGEP) has been a matter of debate for long time.

Objective:

Our aim was to define the molecular features of GPP and AGEP.

Methods

We analyzed skin biopsy samples and clinical data from 125 patients with AGEP, GPP, palmoplantar pustulosis (PPP), plaque psoriasis (PSO), and non-pustular cutaneous adverse drug reactions (ADRs), as well as from healthy skin controls using RNA sequencing and blinded histopathological analyses.

Results

The transcriptome and histopathologic features of AGEP and GPP samples exhibited significant overlap (177 differentially expressed genes (DEGs) in GPP and AGEP compared to healthy skin, only 2 DEGs comparing AGEP and GPP), yet displayed marked differences from those of PPP, PSO, and ADR samples, with a notable number of DEGs (131 DEGs comparing AGEP and PSO, 75 DEGs comparing AGEP and PPP and 52 DEGs comparing AGEP and ADR) and pathways. A transcriptome profile subgroup evaluation of more than 13,000 analyzed genes did not reveal any differentially expressed genes in drug-induced GPP and AGEP. Moreover, the immune response pattern and immune cell composition did not differ between drug-induced GPP and AGEP, whereas non-drug-induced GPP had higher expression of Th17-related genes and a higher neutrophil count than AGEP.

Conclusion

We propose that AGEP is a drug-induced variant of GPP and therefore part of IL-36-related pustulosis. A key signature overarching this spectrum was identified, thereby opening the therapeutic approach of IL-36 inhibition to all subtypes of the disease.

Key words

IL-36, acute generalized exanthematous pustulosis, AGEP, generalized pustular psoriasis, GPP, palmoplantar pustulosis, PPP, plaque psoriasis, cutaneous adverse drug reactions

Abbreviations used:

GPP: generalized pustular psoriasis

AGEP: acute generalized exanthematous pustulosis

PPP: palmoplantar pustulosis

PSO: plaque psoriasis

ADR: cutaneous adverse drug reactions

HCO: healthy control subjects

RNA-Seq: RNA sequencing

DEGs: differentially expressed genes

IL-36RN: IL-36 receptor antagonist

Key Messages (2-3 independent statements that present the key findings and comment on their implications)

- We found a strong overlap in the transcriptomic profiles of AGEP and GPP, which differ from those of (other) cutaneous drug reactions, palmoplantar pustulosis and plaque psoriasis.
- In particular, drug-induced GPP and AGEP have matching transcriptome profiles that differ from non-drug-induced GPP.

Capsule Summary (1 sentence, 35 words max)

Since the transcriptome profiles of drug-induced GPP and AGEP strongly match, and no single parameter allows to distinguish between the two conditions, we propose that AGEP can be considered as a drug-induced variant of GPP.

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Introduction

Pustular psoriasis comprises a spectrum of chronic, persistent to intermittent or recurrent inflammatory skin conditions with different clinical phenotypes, including generalized pustular psoriasis (GPP), acrodermatitis continua of Hallopeau, GPP of pregnancy (impetigo herpetiformis), annular pustular psoriasis and palmoplantar pustulosis, or palmoplantar pustular psoriasis (PPP)(1,2). Although patients with pustular psoriasis may have concomitant plaque psoriasis, the two forms of psoriasis differ clinically, genetically, and phenotypically. Accurate differential diagnosis is important, as GPP flare-ups are potentially life-threatening (3). GPP, a rare, severe autoinflammatory systemic disease, is characterized by recurrent relapses with a sudden onset of an extensive rash with sterile pustules and widespread erythema on non-acral body sites. Supportive diagnostic features accompany the disease and include fever, fatigue, and various laboratory abnormalities such as elevated CRP serum levels, hypocalcemia and hypoalbuminemia (4–6).

Clinical and histopathological features of GPP considerably overlap with those of acute generalized exanthematous pustulosis (AGEP)(5,7). AGEP is defined by the presence of a drug- or infection-induced, scattered pustular rash accompanied by generalized symptoms of varying severity, which closely resemble those observed in GPP. It manifests rapidly and resolves quickly (4,8,9). Drugs that have been associated with AGEP include antibiotics, antimalarials, and antifungals (10,11). Surprisingly, some of the same medications trigger GPP The pathogenesis of AGEP and GPP is similar, including the presence of mutations in the IL-36 receptor antagonist (*IL36RN*) and the increased production and release of IL-36 and IL-17 in the skin and blood (6,7,11–13). Although the exact pathomechanisms for both GPP and AGEP are unknown, several hypotheses have been proposed that IL-1 and IL-36 trigger an inflammatory keratinocyte response leading to neutrophil recruitment via chemokine expression (9,14). The general efficacy of the anti-IL-36 receptor antibody spesolimab in patients with GPP, independent of the presence of *IL36RN* mutations, suggests that the IL-36 signaling pathway plays a crucial role in the pathogenesis of the disease (15) and that other mutations or polymorphisms of the *CARD14* and *AP1S3* gene may also enhance IL-36 signaling (5). Histologically, AGEP and GPP also share common features such as sub-corneal or intra-epidermal pustules and a perivascular infiltrate in the dermis (7). However, it is important to distinguish between AGEP and GPP given the differences in treatment and clinical course. While the skin lesions in patients with AGEP usually resolve spontaneously within two weeks, GPP has a longer course with repeated relapses, and can

be fatal if not treated adequately (16). According to the European Rare and Severe Psoriasis Expert Network (ERASPEN), GPP is not diagnosed until at least one relapse has occurred or the disease has persisted for more than three months (1). A history of flares supports the diagnosis, however the recently published International Consensus from the International Psoriasis Council states that GPP may be diagnosed on first presentation (4). Although GPP can be triggered by certain provoking factors, this is not considered a diagnostic criterion. When patients first present with pustular skin lesions and an association with medication is made, the "wait-and-watch" strategy typical of AGEP may result in the first episode of GPP being overlooked, which may lead to a worsening of the patient's condition.

Our analyses demonstrate that the transcriptome profiles of AGEP and GPP match and both display a dysregulation in genes related to neutrophils and IL-36-related inflammatory signaling. In turn, this indicates that AGEP is maybe a drug-induced variant of GPP and therefore part of IL-36-related pustulosis spectrum.

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Materials and Methods

Patients and biopsy samples

Clinical data and archived FFPE biopsy samples from lesional skin of patients with AGEP (n = 49) and GPP (n = 46) were collected. For detailed information regarding clinical data, see Supplemental Table 1. In addition, lesional skin samples of patients with palmoplantar pustulosis (PPP, n = 9), cutaneous adverse drug reactions (type IV hypersensitivity; ADR, n = 12), plaque psoriasis (PSO, n = 9), and skin of healthy control subjects (HCO, n = 9) were included. Detailed information about PSO and HCO samples can be found here (17).

Histopathologic analyses

FFPE blocks (GPP, n = 46; AGEP, n = 49; PPP, n = 9; PSO, n = 9; ADR, n = 12) were cut into 3.5µm sections, routinely processed, stained with hematoxylin and eosin (HE), and examined for the presence of parakeratosis, psoriasiform epidermal hyperplasia, spongiosis, necrotic keratinocytes, intracorneal/subcorneal non-follicular pustules, intraepidermal non-follicular pustules, large pustules (> 50 neutrophils), elongated dermal papillae, dilated tortuous vessels in dermal papillae, papillary dermal edema, neutrophils in the dermal infiltrate, and eosinophils in the dermal infiltrate. The two dermatopathologists (E.S. and L.C.) were blinded with regard to the group assignment of the samples. Of 125 sections, 3 were excluded due to insufficient tissue size or staining artifacts, resulting in n = 43 for the GPP group. Histopathologic features of AGEP and GPP samples were compared in R with Fisher's exact test with *rstatix:: pairwise_fisher_test()* and multiple test adjustment by false discovery rate (18).

RNA extraction, sequencing and data analysis

Total RNA was extracted from FFPE skin biopsies of lesional skin of patients and healthy control skin and libraries were prepared with the QuantSeq 3'mRNA-Seq FWD Library Preparation Kit (Lexogen, Vienna, Austria) and samples were sequenced on an Illumina NextSeq 2000 system with NextSeq 2000 at Lexogen GmbH (Vienna, Austria). A detailed description of RNA isolation, library preparation, sequencing and data analysis can be found in the Supplementary methods section.

Results

Study population

In a multi-center team effort, we worked together with members of the Central Eastern and Europe (CEE) GPP expert network (2) and collected clinical data and archived FFPE biopsy samples from the lesional skin of patients with AGEP (n = 49) and GPP (n = 46), from 12 sites in 8 countries. The diagnoses were confirmed based on evaluation of clinical data and the courses of disease provided by the investigators. In addition, lesional skin samples of patients with palmoplantar pustulosis (PPP, n = 9), cutaneous adverse drug reactions (ADR, n = 12), plaque psoriasis (PSO, n = 9), and for control purposes, from healthy skin (HCO, n = 9) were included. Clinical characteristics of the study population can be found in Supplementary Table 1.

Histopathologic patterns of AGEP and GPP

GPP and AGEP samples displayed widely overlapping histologic features, consistent with previous findings (8,19–21). Overall, dilated tortuous vessels in dermal papillae, elongated dermal papillae, intracorneal/subcorneal non-follicular pustules, large pustules (>50 neutrophils), and psoriasiform epidermal hyperplasia were present in more cases of GPP than AGEP samples, while the presence of eosinophils in the dermal infiltrate was less common in GPP than in AGEP samples (Fig. 1, Suppl. Table 2).

Transcriptomic profiles of AGEP and GPP

Out of 129 samples included in the RNA sequencing analysis, 21 samples had to be excluded due to insufficient quality of the sequencing data, resulting in the following number of samples for each group: AGEP (n = 40), GPP (n = 30), PPP (n = 9), PSO (n = 8), ADR (n = 12) and HCO (n = 9) (Fig. 2A, exclusions listed in Supplementary Data 1). Across all samples, approximately 13,000 genes had sufficiently good signals with a median count > 10 across all samples. These were included in the analyses of differentially expressed genes (DEGs). The mean age of the archived FFPE blocks of the included samples was 5.3 years, displaying a remarkable range of 0.1 to 20.2 years. However, a higher block age did not correlate with sample quality (Supplementary Fig. 1). The results of multivariate principal component analysis (PCA) show that the gene expression profiles for AGEP and GPP samples largely overlap, while these profiles show some differences to PPP and PSO and a clear difference to ADR and healthy skin (Fig. 2B). Furthermore, the PCA results show that the distribution of female and male patients had no effect

on the clustering of gene expression profiles for the different groups (Fig. 2C). Next, we examined the differentially expressed genes (DEGs) by carrying out 15 pairwise comparisons (Fig. 2D) and found that the GPP samples had the highest number of significantly altered genes, with 1547 DEGs in the GPP compared to the healthy skin group, followed by the AGEP group with 906 DEGs compared to HCO. In the ADR group, 757 DEGs were found, 647 DEGs in the PPP group, and 268 DEGs in the PSO group, compared to healthy skin (Fig. 2D). In agreement with a previous study performing microarray analyses of FFPE samples from GPP, PPP, and AGEP (22), we found that the GPP samples displayed the most unique gene expression profiles, with 494 DEGs (167 up-regulated, 327 down-regulated) uniquely found in the GPP group compared to healthy skin, followed by ADR (130 DEGs, 60 up-regulated, 70 down-regulated), PPP (122 DEGs, 45 up-regulated, 77 down-regulated), and AGEP (40 DEGs, 24 up-regulated, 16 down-regulated) (Fig. 3 A, B). However, we saw that 177 DEGs (92 upregulated, 85 downregulated) were found in both GPP and AGEP compared to healthy skin (Fig. 3 A, B). When comparing AGEP with GPP samples, we found only 2 DEGs, again indicating largely overlapping gene expression profiles, while 131 DEGs were found when comparing AGEP and PSO samples, 75 DEGs when comparing AGEP and PPP and 52 DEGs when comparing AGEP and ADR (Fig. 2D, Supplementary Fig. 2). In contrast, in GPP we found 191 DEGs when comparing with ADR, 70 DEGs when comparing GPP with PSO and only 17 DEGs when comparing GPP with PPP (Fig. 2D), consistent with their clinical classification as pustular psoriasis variants (1). Interestingly, we found 57 upregulated DEGs (Fig. 3A) and 13 downregulated DEGs (Fig. 3B) that co-occurred in the pairwise comparisons of AGEP, GPP, ADR, PSO, and PPP with HCO, which might represent an “inflammatory core signature”. These included genes associated with inflammation and psoriasis (e.g., *S100A12, IL1B, AREG*)(23), keratinization (e.g., *KRT6A, SPRR1B*)(24), cytotoxicity (e.g., *GZMB*)(25), and *DSG3,* which is associated with blister formation and programmed cell death (26,27) (data not shown). The common signaling pathways that were found to be significantly enriched in the pairwise comparisons between AGEP, GPP, ADR, PSO and PPP with HCO included mainly inflammation-related pathways (e.g., cytokine-mediated signaling pathway, immune response-regulating signaling pathway, immune effector process, immune response-activating signaling pathway) (Fig. 3C). The two significantly altered genes in AGEP vs. GPP were *PDK4* (pyruvate dehydrogenase kinase 4) and *ZBTB16* (zinc finger and BTB domain containing 16) (Fig. 4A, B). *PDK4* is a key enzyme of energy metabolism (28), and *ZBTB16* is a lineage-determining

transcription factor expressed by innate-like T cells (29). The comparison of their gene expression in all diagnostic groups revealed that this was downregulated in GPP, PPP and PSO, while AGEP and ADR samples displayed similar expression levels to healthy skin (Fig. 4A). When we looked at all significant genes in AGEP vs. HCO and GPP vs. HCO samples, we saw that 60% of all genes were significantly regulated in the same direction in both comparisons (i.e., either up- or downregulated). The remaining genes were all changed in the same direction, but significant in only one of the two comparisons, most often due to lower LFCs. This result underlines the very strong agreement between the transcriptomic profiles of AGEP and GPP (Fig. 4C, D, H). A gene set enrichment analysis (GSEA) indicated the presence of metabolic differences between AGEP and GPP samples, as significantly enriched pathways include mitochondrial ATP synthesis, oxidative phosphorylation and cytoplasmic translation, in addition to keratinocyte-associated pathways such as keratinization (Fig. 4E). When comparing the 15 most strongly enriched gene ontology (GO) terms between AGEP and HCO and GPP and HCO, these were again shown to largely overlap. In fact, 13 terms were shared by both comparisons (e.g., chemotaxis, cytokine-mediated signaling pathway, immune effector process, leukocyte chemotaxis and migration, and immune response-activating signaling pathway) (Fig. 4F, G).

Immune response pattern genes and anti-IL-36R responder genes in AGEP and GPP

To further characterize the different groups in terms of their expression of immune signatures, we examined the expression of marker genes for type 1/Th1-related, type 2/Th2-related, type 3/Th17-related, neutrophil-related, and cytotoxicity-related immune response patterns (Fig. 5A; marker genes adapted from (30–32)). We found that GPP samples had the highest expression of Th17- (e.g., *IL17A, IL23A, IL17C, IL36G, IL36B, S100A12*) and neutrophil-related genes (e.g., *CXCL1, CXCR1, CXCL8*), while AGEP showed a weaker expression of Th17-related genes, moderately high expression of neutrophil-related genes, but a higher expression of cytotoxicity-related genes (e.g., *GNLY, RIPK3, PRF1*) than GPP. ADR samples showed a high expression of type 1/Th1 (e.g., *TBX21, IL15*), type 2/Th2 (e.g., *GATA3, IL10, IL33*) and cytotoxicity-related transcripts (e.g., *FAS, GZMB, GNLY, RIPK3, CASP3, PRF1*). As expected, a moderately high expression of Th17-related genes was found in PSO samples, although the expression of known pro-inflammatory cytokines (e.g., *IL17A, IL23A, TNF*) was surprisingly low. PPP samples showed high expression of type 3/Th17-related transcripts, and a high expression of neutrophil chemoattractants (e.g., *CXCL6, CXCL8, CCL3, CCL4*) (Fig. 5A).

Subsequently, we conducted an analysis of marker genes that have recently been described as a means of rapidly improving aberrant expression in GPP skin lesions following treatment with spesolimab, with a view to establishing a correlation between this and disease improvement (30). In our dataset, genes related to keratinocyte-mediated inflammation (*IL17C, IL36G, IL19, IL20, IL24*), neutrophil recruitment and neutrophil transcripts (e.g., *CXCL1, CXCL5, CXCL8, CXCR2*) exhibited the highest expression in GPP, followed by slightly lower expression in PPP and AGEP. PSO, ADR, and healthy skin exhibited low expression levels, with the exception of *ELANE* (elastase expressed by neutrophils that inhibits neutrophil enzyme release and chemotaxis), which demonstrated high expression levels in HCO and PSO and lower expression levels in all other groups (Fig. 5B). The expression of pro-inflammatory mediator genes (*IL1B, IL6,* and *TNF*) exhibited heterogeneity across groups. *IL1B* demonstrated the highest expression in GPP, followed by PPP and AGEP. *IL6* exhibited similar expression in AGEP, GPP, ADR, and PPP. *TNF* exhibited the highest expression in ADR, followed by PPP and PSO. As anticipated, the expression of genes for proinflammatory mediators was minimal in healthy skin. In conclusion, our findings corroborate that anti-IL-36R responder genes are markedly expressed in GPP samples, while expression is only moderately reduced in PPP and AGEP (Fig. 5B). These results suggest that AGEP, GPP, and PPP exhibit a shared dysregulation in genes pertaining to neutrophils and IL-36-related inflammatory signaling.

Immune cell composition patterns in AGEP and GPP

To estimate the immune cell composition, we used CIBERSORTx, also called digital flow cytometry, a computational approach that allows the identification of the relative proportions of different cell types based on transcriptome data (33,34). Of the 22 different immune cell types represented in CIBERSORTx, we detected 10 cell types (CD8+ T cells, regulatory T cells, resting dendritic cells, activated NK cells, neutrophils, naïve B cells, plasma cells, monocytes, M1 macrophages, and M2 macrophages) in our bulk RNA-Seq dataset (Fig. 6). For the remaining 12 cell types (naïve CD4+ T cells, active and resting CD4+ memory T cells, follicular Th cells, γδ T cells, memory B cells, eosinophils, activated and resting mast cells, activated dendritic cells, resting NK cells, and M0 macrophages), the cell numbers or marker gene expression levels were too low to allow for deconvolution. Confirming our findings on immune response patterns, we found that neutrophil numbers were highest in GPP, followed by PPP and AGEP, and a significant difference was observed between AGEP and GPP. Consistent with cytotoxic marker genes being highly expressed in ADR, the number of CD8+ T cells was highest in ADR,

followed by AGEP, which had significantly higher levels than in GPP. The number of activated NK cells was also highest in ADR, followed by GPP and AGEP. When comparing AGEP and GPP, we found higher cell counts of CD8+ T cells, naïve B cells and M2 macrophages in AGEP, suggesting that AGEP may have a stronger cytotoxic profile than GPP. Resting dendritic cell numbers were significantly increased in ADR and lower in GPP than in HCO, while monocyte numbers were significantly higher in AGEP, GPP, and ADR than in healthy skin (Fig. 6).

To further investigate the subtle differences between AGEP and GPP, we divided the GPP samples into two groups, drug-induced and non-drug-induced GPP, and analyzed their gene expression profiles compared to AGEP. Of the 40 AGEP and 30 GPP samples initially included in the RNA-Seq data analysis, 20 were excluded due to the lack of information on flare triggers. Results of multivariate principal component analysis (PCA) reveal that the expression profiles of drug-induced GPP samples were more similar to AGEP than non-drug-induced GPP, which clustered separately (Fig. 7A). When comparing AGEP with drug-induced GPP, we found no evidence of differentially expressed genes (Fig. 7B), but when comparing AGEP with non-drug-induced GPP, we found 7 DEGs (3 upregulated, *TXNIP*, *IRX3*, *ZBTB16* and 4 downregulated genes, *FERMT1*, *IL1RN*, *PNP*, *ALOX12B*) (Fig. 7C, D). In addition, the CIBERSORTx results show that the number of neutrophils was significantly higher in non-drug-induced GPP than in AGEP and drug-induced GPP. The number of CD8+ T cells was slightly higher in AGEP than in non-drug-induced GPP, but did not reach significance (Fig. 7E, F, Supplementary Fig. 3). Furthermore, the expression of immune response patterns in drug-induced GPP was very similar to those in AGEP samples, with considerably lower expression of type 3 immune response- and neutrophil-related genes compared to non-drug-induced GPP (Fig. 7G). The analysis of histological features revealed no significant differences between AGEP, drug-induced and non-drug-induced GPP (data not shown).

Discussion

This multicenter study identified a strong overlap between the transcriptome profiles of AGEP and GPP, with only two significantly altered genes. Thirteen out of the 15 top significantly enriched gene ontology terms were present in both AGEP and GPP compared to healthy skin (e.g., cell chemotaxis, cytokine-mediated signaling pathway and immune effector process). Interestingly, the stark contrast of the GPP transcriptome compared to ADR (191 deregulated genes) was much smaller in AGEP vs. ADR (52 genes), as might have been expected given that AGEP is regarded a severe cutaneous drug reaction. Breaking down the expression of marker genes for type 1, type 2, type 3, neutrophil, and cytotoxicity-related immune response patterns, AGEP samples had a stronger cytotoxic signature than GPP, with higher expression of cytotoxicity marker genes (e.g., *PRF1, GZMB,* and *GNLY*), again explainable as a drug reaction. GPP, on the other hand, had the strongest Th-17/type 3 and neutrophil-related signature, while AGEP samples had weaker Th-17/type 3 signature expression and a slightly weaker expression of neutrophil-related transcripts. The cytotoxic signature in AGEP was confirmed by CIBERSORTx (34,35), revealing higher number of CD8+ T cells, naïve B cells and M2 macrophages in AGEP, while the number of neutrophils was higher in GPP. All these findings confirm in AGEP, drug-specific cytotoxic T cells, possibly together with NK cells and NKT cells, induce apoptosis of keratinocytes via cytotoxic proteins such as granzyme B, perforin and granulysin (7,36). IL-17 expression by neutrophils, mast cells and macrophages is increased in AGEP patients, suggesting that innate cells may also be involved in the pathogenesis (37). Drug-specific T cells from patients with AGEP were shown to have a predominant Th1 cytokine profile and an increased production of interferon (IFN)-γ and granulocyte/macrophage colony-stimulating factor (GM-CSF), which regulate neutrophilic inflammation through the production of CXCL-8 (38). Indeed, we saw that the expression of Th1/type 1-related immune response patterns was highest in ADR, while AGEP samples had moderately high expression and GPP samples showed lower expression.

The only two significant genes in AGEP compared to GPP were *PDK4* and *ZBTB16*, both of which were downregulated in GPP, PPP and PSO as compared to AGEP and ADR, both showing similar expression levels to healthy skin. *PDK4* (pyruvate dehydrogenase kinase 4) is a key mitochondrial enzyme that regulates energy metabolism (28) and is enriched in monocytes, eosinophils and myeloid DCs according to the human protein atlas (39,40). Furthermore, *PDK4*

expression is increased in wound healing (41) and negatively regulates IL-10 production by macrophages (42)). *ZBTB16* (zinc finger and BTB domain containing 16) is a lineage-determining transcription factor for innate-like T cells and is expressed in mucosa-associated invariant T (MAIT) cells, NKT- cells, NK cells and γδT cells (29,39,40), and has been shown to be expressed by γδT cells in psoriasis (43). However, we were unable to detect γδ T cells, and no significant difference was detected in NK cell numbers between AGEP and GPP samples, so the clinical relevance of these two significantly altered genes remains to be determined.

AGEP and GPP have a similar pathogenesis, such as mutations in IL-36 receptor antagonist (*IL36RN*) (12,13), aberrant IL-36 signaling (11,44,45), and increased expression of IL-17 (7,46). In GPP, IL-1 and IL-36 trigger an inflammatory response of keratinocytes via the expression of chemokines, leading to neutrophil recruitment. In our dataset, the expression of the key neutrophil chemoattractants *CXCL1* and *CXCL8* was highest in GPP and PPP, considerably lower in AGEP and PSO, and showed levels similar to healthy skin in ADR. Furthermore, *in vitro* models have shown that neutrophils regulate IL-36 activity in GPP (47), in addition to their recently described role as IL-26 producers (31), emphasizing the importance of neutrophils in pustular psoriasis (47). IL-26 induces overexpression of IL-1, leading to keratinocyte activation via IL-26R and increased neutrophil recruitment via CXCL-1 and CXCL-8 (31,48,49). We found that *IL26* expression was highest in GPP and PPP, whereas AGEP had levels similar to healthy skin, suggesting that the autoinflammatory circuit (leading to keratinocyte activation and neutrophil recruitment) may not yet be fully developed in AGEP. Unexpectedly, we found that the expression of *IL36G* (the gene encoding IL-36γ), was highest in PPP, followed by PSO and GPP, and only moderately expressed in AGEP. In addition, the expression of genes related to Th17 signaling and the number of neutrophils and neutrophil-related transcripts were slightly lower in AGEP than in GPP samples, suggesting that these two subtly differ and that important features of chronic autoinflammatory disease are not yet fully established in AGEP.

Consistent with previous studies, we found high expression of type 1 and type 2 signature genes (50,51) and a very strong cytotoxic pattern in ADR (50,52) with a high expression of genes for granulysin, known to be expressed by NK cells and cytotoxic T cells, and granzyme B and perforin, known to be highly expressed by cytotoxic T cells (36). The immune cell composition in ADR samples was characterized by the highest number of CD8+ T cells, activated NK cells, and macrophages. AGEP samples shared common features with ADR, as evidenced by only 52

significantly altered genes between the two, upregulation of genes related to cytotoxicity, and significantly increased numbers of CD8+ T cells and macrophages. In agreement with previous studies (22,53), we found that the transcriptome profiles of GPP were similar to those of PPP (only 17 DEGs), demonstrating that both share common modulated genes. The expression of immune response patterns was also similar, although the expression of most neutrophil transcripts was weaker in PPP. This is consistent with the CIBERSORTx results, which indicate similar immune cell compositions in GPP and PPP, with slightly more neutrophils, monocytes and M2 macrophages in GPP, consistent with their classification as variants of pustular psoriasis (5,54).

Although the gene expression analysis of FFPE tissues is associated with challenges such as formalin-induced RNA degradation, protein-coding transcripts of fresh-frozen and FFPE tissues strongly correlate (55), and FFPE-RNA-Seq is a valuable tool for analyzing archived skin biopsy samples (22,56). We have successfully analyzed samples with a remarkable age range of 0.1 to 20.2 years, making our approach particularly relevant for the study of rare skin diseases such as GPP and AGEP, where tissue availability is limited. In agreement with previous studies (57–59), a moderately high expression of Th17-related genes was observed in PSO, although the expression of known proinflammatory cytokines (e.g. *IL17A*, *IL23A*, *TNF*) was surprisingly low. In addition, the analysis of immune cell composition revealed generally low levels, which could be explained by the fact that the skin biopsy samples are usually from chronic skin lesions, in which the expression of immune cell transcripts could be lower than in the skin with acute inflammation present in AGEP, GPP and ADR.

In a recent study, Baum et al. investigated the molecular expression patterns after IL-36R blockade with spesolimab in patients with GPP and defined marker genes with expressions that changed rapidly after treatment and correlated with clinical improvement (53). The genes that responded to anti-IL-36R blockade were categorized into: keratinocyte-mediated inflammation, neutrophil recruitment, neutrophilic transcripts and pro-inflammatory mediators. When analyzing their gene expression in our dataset, we confirmed that these anti-IL-36R blockade responder genes are highly expressed in our GPP samples, and found that their expression pattern is similar, albeit slightly weaker, in AGEP and PPP, highlighting that AGEP, and GPP share a common dysregulation in genes related to neutrophils, neutrophil chemotaxis and inflammatory IL-36 signaling, which could be potentially targeted with spesolimab. Surprisingly however, spesolimab failed to demonstrate efficacy in the examined study populations in PPP (60,61).

To test the hypothesis that AGEP is a drug-induced variant of GPP, we compared the transcriptome profiles and histologic features and found them to be very similar. However, when comparing AGEP with non-drug-induced GPP, slight differences in the transcriptome profile were observed. Non-drug-induced GPP samples were clustered separately, (as evident in the PCA plot), and we found seven significantly changed genes. These included *IL1RN*, which encodes the receptor antagonist of IL-1 (62), *FERMT1*, which is associated with Kindler syndrome and epidermis development (63), and *TXNIP*, which regulates keratinocyte differentiation (64). Furthermore, the immune response signatures of non-drug-induced GPP differed from those of AGEP and drug-induced GPP, displaying a higher expression of type 3/Th17 and neutrophil-related genes and significantly higher numbers of neutrophils.

Conclusion

Taken together, the transcriptome profiles of drug-induced GPP and AGEP strongly matched, and not a single parameter examined allowed us to differentiate between the two conditions. Therefore, we propose that AGEP can be considered a drug-induced variant of GPP. This is consistent with a recent report that anti-IL-36 treatment with spesolimab (approved for GPP) has been clinically effective in AGEP (65). Together, these findings are particularly important for the clinical diagnosis of IL-36 spectrum diseases such as GPP (6), as the “wait-and-watch” strategy commonly used in AGEP may lead to the first episode of GPP being overlooked, in turn, leading to exacerbation and potential progression of the patient’s disease.

Language editing

InstaText Scholar (Ljubljana, Slovenia) was used for language editing.

Author contributions

Conceptualization: TB, NB, PW; Data curation: TB, NB, JW, KF, TG, ES, LC; Formal analysis: TB, NB, JW; Funding acquisition: TB, PW; Investigation: TB, NB, JW, KF, TG, ES, LC; Methodology: TB, NB; Project administration: TB, PW, MS, HZ; Resources: TG, DM, VM, AR, MKK, GR, JTM, BMS, RC, KP, MM, LP, AS, MS, HZ, PW; Software: NB; Supervision: PW;

Validation: TB, NB, PW; Visualization: TB, NB; Writing – original draft preparation: TB; Writing – review and editing: TB, NB, PW, AAN.

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References

1. Navarini AA, Burden AD, Capon F, Mrowietz U, Puig L, Köks S, et al. European consensus statement on phenotypes of pustular psoriasis. J Eur Acad Dermatology Venereol. 2017;31(11):1792–9.
2. Wolf P, Ceovic R, Conrad C, Falkensteiner K, Graier T, Kołt-Kamińska M, et al. Characteristics and management of generalized pustular psoriasis (GPP): Experience from the Central and Eastern Europe (CEE) GPP Expert Network. J Eur Acad Dermatology Venereol. 2024;(December 2023):1–12.
3. Zheng M, Jullien D, Eyerich K. The Prevalence and Disease Characteristics of Generalized Pustular Psoriasis. Am J Clin Dermatol. 2022;23(s1):5–12.
4. Choon SE, Van De Kerkhof P, Gudjonsson JE, De La Cruz C, Barker J, Morita A, et al. International Consensus Definition and Diagnostic Criteria for Generalized Pustular Psoriasis from the International Psoriasis Council. JAMA Dermatology. 2024;160(7):758–68.
5. Bachelez H. Pustular psoriasis and related pustular skin diseases. Br J Dermatol. 2018;178(3):614–8.
6. Marrakchi S, Guigue P, Renshaw BR, Puel A, Pei XY, Fraitag S, et al. Interleukin-36-receptor antagonist deficiency and generalized pustular psoriasis. N Engl J Med. 2011;365(7):620–8.
7. Feldmeyer L, Heidemeyer K, Yawalkar N. Acute generalized exanthematous pustulosis: Pathogenesis, genetic background, clinical variants and therapy. Int J Mol Sci. 2016;17(8).
8. Roujeau JC, Bioulac-Sage P, Bourseau C, Guillaume J-C, Bernard P, Lok C, et al. Acute generalized exanthematous pustulosis. Arch Dermatol. 1992;127(9):1333–8.
9. Tetart F, Walsh S, Milpied B, Gaspar K, Vorobyev A, Tiplica GS, et al. Acute generalized exanthematous pustulosis: European expert consensus for diagnosis and management. J Eur Acad Dermatology Venereol. 2024;(February):1–9.
10. Sidoroff A, Halevy S, Bavinck JNB, Vaillant L, Roujeau JC. Acute generalized exanthematous pustulosis (AGEP) - A clinical reaction pattern. J Cutan Pathol. 2001;28(3):113–9.
11. Meier-Schiesser B, Feldmeyer L, Jankovic D, Mellett M, Satoh TK, Yerly D, et al. Culprit Drugs Induce Specific IL-36 Overexpression in Acute Generalized Exanthematous Pustulosis. J Invest Dermatol. 2019;139(4):848–58.

12. Sugiura K, Takemoto A, Yamaguchi M, Takahashi H, Shoda Y, Mitsuma T, et al. The majority of generalized pustular psoriasis without psoriasis vulgaris Is caused by deficiency of interleukin-36 receptor antagonist. J Invest Dermatol. 2013;133(11):2514–21.
13. Navarini AA, Valeyrie-Allanore L, Setta-Kaffetzi N, Barker JN, Capon F, Creamer D, et al. Rare variations in IL36RN in severe adverse drug reactions manifesting as acute generalized exanthematous pustulosis. J Invest Dermatol. 2013;133(7):1904–7.
14. Furue K, Yamamura K, Tsuji G, Mitoma C, Uchi H, Nakahara T, et al. Highlighting interleukin-36 signalling in plaque psoriasis and pustular psoriasis. Vol. 98, Acta Dermato-Venereologica. 2018. p. 5–13.
15. Elewski BE, Lebwohl MG, Anadkat MJ, Barker J, Ghoreschi K, Imafuku S, et al. Rapid and sustained improvements in Generalized Pustular Psoriasis Physician Global Assessment scores with spesolimab for treatment of generalized pustular psoriasis flares in the randomized, placebo-controlled Effisayil 1 study. J Am Acad Dermatol. 2023;89(1):36–44.
16. Chang SL, Hu S, Hung SI, Huang YL, Hsiao WC, Chung WH. A comparison of Ki-67 antigen presentation in acute generalized exanthematous pustulosis and pustular psoriasis. Arch Dermatol Res. 2010;302(7):525–9.
17. Benezeder T, Painsi C, Patra V, Dey S, Holcmann M, Lange-Asschenfeldt B, et al. Dithranol targets keratinocytes, their crosstalk with neutrophils and inhibits the IL-36 inflammatory loop in psoriasis. Elife. 2020;9(e56991):1–31.
18. Kassambara A. Pipe-Friendly Framework for Basic Statistical Tests [R package rstatix version 0.6.0]. Comprehensive R Archive Network (CRAN). [Internet]. 2020.
19. Isom J, Braswell DS, Siroy A, Auerbach J, Motaparthi K. Clinical and histopathologic features differentiating acute generalized exanthematous pustulosis and pustular psoriasis: A retrospective series. J Am Acad Dermatol. 2020;83(1):265–7.
20. Kardaun SH, Kuiper H, Fidler V, Jonkman MF. The histopathological spectrum of acute generalized exanthematous pustulosis (AGEP) and its differentiation from generalized pustular psoriasis. J Cutan Pathol. 2010;37(12):1220–9.
21. Vyas NS, Charifa A, Desman GT, McNiff JM. Distinguishing pustular psoriasis and acute generalized exanthematous pustulosis on the basis of plasmacytoid dendritic cells and MxA protein. J Cutan Pathol. 2019;46(5):317–26.

22. Liang Y, Xing X, Beamer MA, Swindell WR, Sarkar MK, Roberts LW, et al. Six-transmembrane epithelial antigens of the prostate comprise a novel inflammatory nexus in patients with pustular skin disorders. J Allergy Clin Immunol. 2017;139(4):1217–27.
23. Krueger JG, Wharton KA, Schlitt T, Suprun M, Torene RI, Jiang X, et al. IL-17A inhibition by secukinumab induces early clinical, histopathologic, and molecular resolution of psoriasis. J Allergy Clin Immunol. 2019;144(3):750–63.
24. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of Psoriasis. Annu Rev Immunol. 2014;32:227–55.
25. Hay ZLZ, Slansky JE. Granzymes: The Molecular Executors of Immune-Mediated Cytotoxicity. Int J Mol Sci. 2022;23(3).
26. Schäbitz A, Hillig C, Mubarak M, Jargosch M, Farnoud A, Scala E, et al. Spatial transcriptomics landscape of lesions from non-communicable inflammatory skin diseases. Nat Commun. 2022;13(1):1–13.
27. Ellebrecht CT, Bhoj VG, Nace A, Choi EJ, Mao X, Cho MJ, et al. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. Science (80-). 2016;353(6295):179–84.
28. Zhang S, Hulver MW, McMillan RP, Cline MA, Gilbert ER. The pivotal role of pyruvate dehydrogenase kinases in metabolic flexibility. Nutr Metab. 2014;11(1):1–9.
29. Legoux F, Gilet J, Procopio E, Echasserieau K, Bernardeau K, Lantz O. Molecular mechanisms of lineage decisions in metabolite-specific T cells. Nat Immunol. 2019;20(9):1244–55.
30. Eyerich K, Eyerich S. Immune response patterns in non-communicable inflammatory skin diseases. J Eur Acad Dermatology Venereol. 2018;32(5):692–703.
31. Baldo A, Domizio J Di, Yatim A, Vandenberghe-Dürr S, Jenelten R, Fries A, et al. Human neutrophils drive skin autoinflammation by releasing interleukin (IL) -26. J Exp Med. 2024;221(5):e20231464.
32. Alsabbagh MM. Cytokines in psoriasis: From pathogenesis to targeted therapy. Hum Immunol. 2024;85(4):110814.
33. Steen CB, Liu CL, Alizadeh AA, Newman AM. Profiling Cell Type Abundance and Expression in Bulk Tissues with CIBERSORTx. Methods Mol Biol. 2020;Nov(2117):135–57.
34. Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, et al.

Determining cell type abundance and expression from bulk tissues with digital cytometry. Nat Biotechnol. 2019;37(7):773–82.

35. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods. 2015;12(5):453–7.
36. Schlapbach C, Zawodniak A, Irla N, Adam J, Hunger RE, Yerly D, et al. NKp46 + cells express granulysin in multiple cutaneous adverse drug reactions. Allergy Eur J Allergy Clin Immunol. 2011;66(11):1469–76.
37. Kakeda M, Schlapbach C, Danelon G, Tang MM, Cecchinato V, Yawalkar N, et al. Innate immune cells express IL-17A/F in acute generalized exanthematous pustulosis and generalized pustular psoriasis. Arch Dermatol Res. 2014;306(10):933–8.
38. Schaerli P, Britschgi M, Keller M, Steiner UC, Steinmann LS, Moser B, et al. Characterization of Human T Cells That Regulate Neutrophilic Skin Inflammation. J Immunol. 2004;173(3):2151–8.
39. Uhlen M, Karlsson MJ, Zhong W, Tebani A, Pou C, Mikes J, et al. A genome-wide transcriptomic analysis of protein-coding genes in human blood cells. Science (80-). 2019;366(6472).
40. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Tissue-based map of the human proteome. Science (80-). 2015;347(6220).
41. Ma Z, Ding Y, Ding X, Mou H, Mo R, Tan Q. PDK4 rescues high-glucose-induced senescent fibroblasts and promotes diabetic wound healing through enhancing glycolysis and regulating YAP and JNK pathway. Cell Death Discov. 2023;9(1):1–15.
42. Na YR, Jung D, Song J, Park JW, Hong JJ, Seok SH. Pyruvate dehydrogenase kinase is a negative regulator of interleukin-10 production in macrophages. J Mol Cell Biol. 2020;12(7):543–55.
43. Plužarić V, Štefanić M, Mihalj M, Tolušić Levak M, Muršić I, Glavaš-Obrovac L, et al. Differential Skewing of Circulating MR1-Restricted and γδ T Cells in Human Psoriasis Vulgaris. Front Immunol. 2020;11(December).
44. Johnston A, Xing X, Wolterink L, Barnes DH, Yin ZQ, Reingold L, et al. IL-1 and IL-36 are dominant cytokines in generalized pustular psoriasis. J Allergy Clin Immunol. 2017;140(1):109–20.
45. Arakawa A, Vollmer S, Besgen P, Galinski A, Summer B, Kawakami Y, et al. Unopposed IL-36 Activity Promotes Clonal CD4 + T-Cell Responses with IL-17A Production in

Generalized Pustular Psoriasis. J Invest Dermatol. 2018;138(6):1338–47.

46. Song HS, Kim SJ, Park TI, Jang YH, Lee ES. Immunohistochemical comparison of IL-36 and the IL-23/Th17 axis of generalized pustular psoriasis and acute generalized exanthematous pustulosis. Ann Dermatol. 2016;28(4):451–6.
47. Boehner A, Navarini AA, Eyerich K. Generalized pustular psoriasis – a model disease for specific targeted immunotherapy, systematic review. Exp Dermatol. 2018;27(10):1067–77.
48. Meller S, Di Domizio J, Voo KS, Friedrich HC, Chamilos G, Ganguly D, et al. TH17 cells promote microbial killing and innate immune sensing of DNA via interleukin 26. Nat Immunol. 2015;16(9):970–9.
49. Poli C, Augusto JF, Dauvé J, Adam C, Preisser L, Larochette V, et al. IL-26 Confers Proinflammatory Properties to Extracellular DNA. J Immunol. 2017;198(9):3650–61.
50. Pichler WJ, Yawalkar N, Britschgi M, Depta J, Strasser I, Schmid S, et al. Cellular and molecular pathophysiology of cutaneous drug reactions. Am J Clin Dermatol. 2002;3(4):229–38.
51. Brugnolo F, Annunziato F, Sampognaro S, Campi P, Manfredi M, Matucci A, et al. Highly Th2-skewed cytokine profile of beta-lactam-specific T cells from nonatopic subjects with adverse drug reactions. J Immunol. 1999;Jul 15;163(2):1053–9.
52. Fernandez TD, Mayorga C, Torres MJ, Cornejo-Garcia JA, López S, Chaves P, et al. Cytokine and chemokine expression in the skin from patients with maculopapular exanthema to drugs. Allergy Eur J Allergy Clin Immunol. 2008;63(6):712–9.
53. Baum P, Visvanathan S, Garcet S, Roy J, Schmid R, Bossert S, et al. Pustular psoriasis: Molecular pathways and effects of spesolimab in generalized pustular psoriasis. J Allergy Clin Immunol. 2022;149(4):1402–12.
54. Naik HB, Cowen EW. Autoinflammatory pustular neutrophilic diseases. Dermatol Clin. 2013;31(3):405–25.
55. Jacobsen SB, Tfelt-Hansen J, Smerup MH, Andersen JD, Morling N. Comparison of whole transcriptome sequencing of fresh, frozen, and formalin-fixed, paraffin-embedded cardiac tissue. PLoS One. 2023;18(3 March):1–15.
56. Kwong LN, De Macedo MP, Haydu L, Joon AY, Tetzlaff MT, Calderone TL, et al. Biological Validation of RNA Sequencing Data From Formalin-Fixed Paraffin-Embedded Primary Melanomas. JCO Precis Oncol. 2018;(2):1–19.
57. Suárez-Fariñas M, Li K, Fuentes-Duculan J, Hayden K, Brodmerkel C, Krueger JG.

Expanding the Psoriasis Disease Profile: Interrogation of the Skin and Serum of Patients with Moderate-to-Severe Psoriasis. J Invest Dermatol. 2012;132(11):2552–64.

58. Swindell WR, Johnston A, Voorhees JJ, Elder JT, Gudjonsson JE. Dissecting the psoriasis transcriptome: inflammatory- and cytokine-driven gene expression in lesions from 163 patients. BMC Genomics. 2013;14(1):527.
59. Krueger JG, Fretzin S, Suárez-Fariñas M, Haslett PA, Phipps KM, Cameron GS, et al. IL-17A is essential for cell activation and inflammatory gene circuits in subjects with psoriasis. J Allergy Clin Immunol. 2012;130(1):145–54.
60. Mrowietz U, Burden AD, Pinter A, Reich K, Schäkel K, Baum P, et al. Spesolimab, an Anti-Interleukin-36 Receptor Antibody, in Patients with Palmoplantar Pustulosis: Results of a Phase IIa, Multicenter, Double-Blind, Randomized, Placebo-Controlled Pilot Study. Dermatol Ther (Heidelb). 2021;11(2):571–85.
61. Burden AD, Bissonnette R, Navarini AA, Murakami M, Morita A, Haeufel T, et al. Spesolimab Efficacy and Safety in Patients with Moderate-to-Severe Palmoplantar Pustulosis: A Multicentre, Double-Blind, Randomised, Placebo-Controlled, Phase IIb, Dose-Finding Study. Dermatol Ther (Heidelb). 2023;13(10):2279–97.
62. Jensen LE. Targeting the IL-1 family members in skin inflammation. Curr Opin Investig Drugs. 2010;11(11):1211–20.
63. Has C, Chmel N, Levati L, Neri I, Sonnenwald T, Pigors M, et al. FERMT1 promoter mutations in patients with Kindler syndrome. Clin Genet. 2015;88(3):248–54.
64. Hsiao PF, Huang YT, Lu PH, Chiu LY, Weng TH, Hung CF, et al. Thioredoxin-interacting protein regulates keratinocyte differentiation: Implication of its role in psoriasis. FASEB J. 2022;36(5):1–14.
65. Russo G, Dumont S, Menzinger S, Pastor D, Toutous Trellu L, Kaya G, et al. Severe Acute Generalized Exanthematous Pustulosis Successfully Treated by Spesolimab. Acta Derm Venereol. 2024;104(September):adv41311.

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Figure legends

Figure 1: Histopathologic features of AGEP and GPP. HE-stained sections of AGEP (n = 49) and GPP (n = 43) samples were examined for the presence (black; "yes") or absence (gray; "no") of histologic features. Significance was set at $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ and tested with Fisher's exact test.

Figure 2: Transcriptome profiles of AGEP and GPP samples largely overlap, but differ from PPP, PSO, ADR, and HCO samples and the numbers of differentially expressed genes differ between pairwise comparisons of groups. Overview of approach and number of included samples for histological and transcriptomic analyses (A). PCA scores plot representing the transcriptome profile of each sample (n = 108 in total), where nearness signifies similarity in expression. The PCA was based on the top 500 genes (highest row variance) and the ellipses mark the 95% confidence interval for each group with sample numbers given in (B). PCA scores plot as in (B) colored by female (red; n = 61) and male patients (blue; n = 47) shows no notable trend by sex (C). The bar graph shows the number of significantly changed genes (*BH adjusted* $p < 0.05$, s-value (estimated false sign/small effect rate) < 0.005 and absolute LFC > 1 in each comparison (n = 15). Blue symbolizes significant downregulation and red upregulation (D). AGEP, acute generalized exanthematous pustulosis; GPP, generalized pustular psoriasis, ADR, cutaneous adverse drug reaction; PPP. palmoplantar pustulosis; PSO, plaque psoriasis; HCO, healthy control subjects.

Figure 3: Gene intersections and top 15 shared pathways representing an inflammatory core signature in AGEP, GPP, ADR, PPP and PSO samples. UpSet plots showing all possible overlaps of differentially expressed genes (DEGs) across all pairwise comparisons with intersections being visualized as connected dots. The number of overlapping DEGs is shown on the top of each column. The columns on the left show the number of DEGs for each pairwise comparisons. Significantly upregulated genes are shown in (A) and significantly downregulated genes are shown in (B). Pathway analysis showing the top 15 significantly enriched gene ontology terms that are shared in the pairwise comparisons of AGEP, GPP, ADR, PPP, and PSO with HCO (C).

Figure 4: Differentially expressed genes and pathways in AGEP and GPP samples compared to each other and to HCO samples. Heatmap showing the two significantly upregulated protein-coding genes comparing AGEP with GPP (A). Volcano plots showing differentially expressed genes (DEGs, BH adjusted $p < 0.05$, s-value < 0.005, $|LFC| > 1$) for the comparisons of AGEP vs. GPP (B), AGEP vs. HCO (C), and GPP vs. HCO (D). Blue symbolizes significant downregulation and red upregulation. Dots plot of GSEA (gene set enrichment analysis) results illustrating GO (gene ontology) biological processes in AGEP vs. GPP (E), AGEP vs. HCO (F) and GPP vs. HCO (G), with the top 15 significant positively or negatively enriched pathways based on co-expressed genes. The gene ratio is the percentage of genes that significantly correlated from the total number of genes associated to that process. Terms are ranked by decreasing gene ratio; NES, normalized enrichment score, reflecting enrichment of a gene set, adjusted for size and directionality. Comparison of DEG results for GPP vs. HCO with AGEP vs. HCO (H). The bar chart summarizes the overlap of up- and downregulated genes. Gray symbols mark genes that are not significant in both comparisons (cut-offs as in B-D). Red-pink hues mark genes that are significantly upregulated in both or only of the comparisons, while blue-yellow hues mark the downregulated genes. Note that none of the significantly regulated genes show an opposing regulation direction.

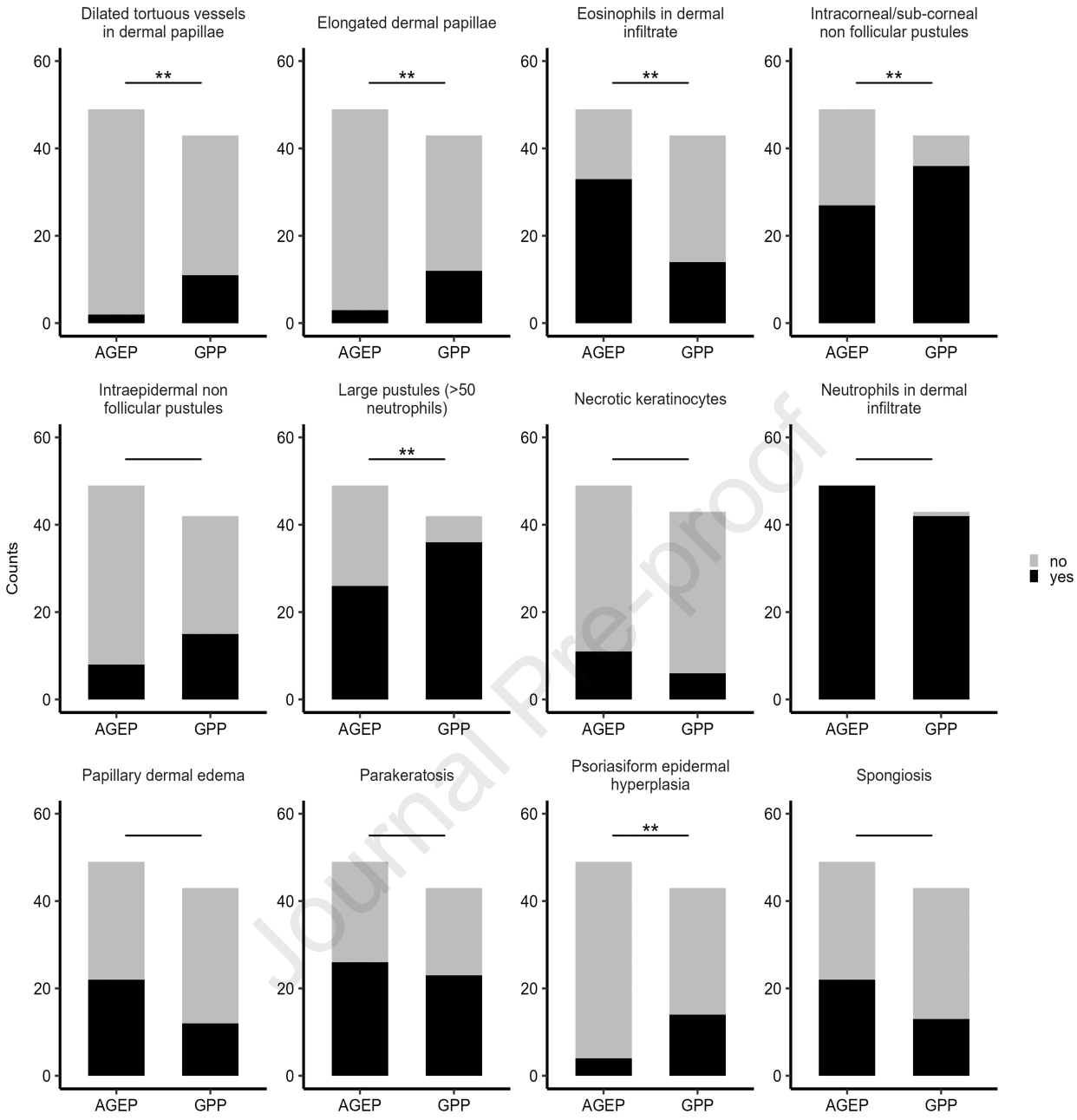
Figure 5: Expression of immune response patterns and anti-IL-36R blockade responder genes. Heatmap showing the average expression per group of pre-defined immune response patterns genes, grouped into type 1/Th1-, type 2/Th2, type 3/Th17-, neutrophil-, and cytotoxicity-related genes (A). Heatmap showing the average expression (*z*-scaled *vst* expression levels) per group of anti-IL-36R blockade responder genes grouped into keratinocyte-mediated inflammation, neutrophil recruitment, neutrophilic transcripts and pro-inflammatory mediator (marker genes adapted from (53)).

Figure 6: Immune cell infiltration differs slightly between AGEP and GPP skin biopsy samples shown by digital flow cytometry via a CIBERSORTx analysis of RNA-Seq data. Of the 22 immune cell types included in CIBERSORTx, ten cell types were detected and are shown here. Comparison of different immune cell types in AGEP vs. GPP and AGEP, GPP, ADR, PPP, and PSO vs. HCO pairwise comparisons. Bars represent mean ± SD; significance was set at $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ and determined with linear mixed effects models.

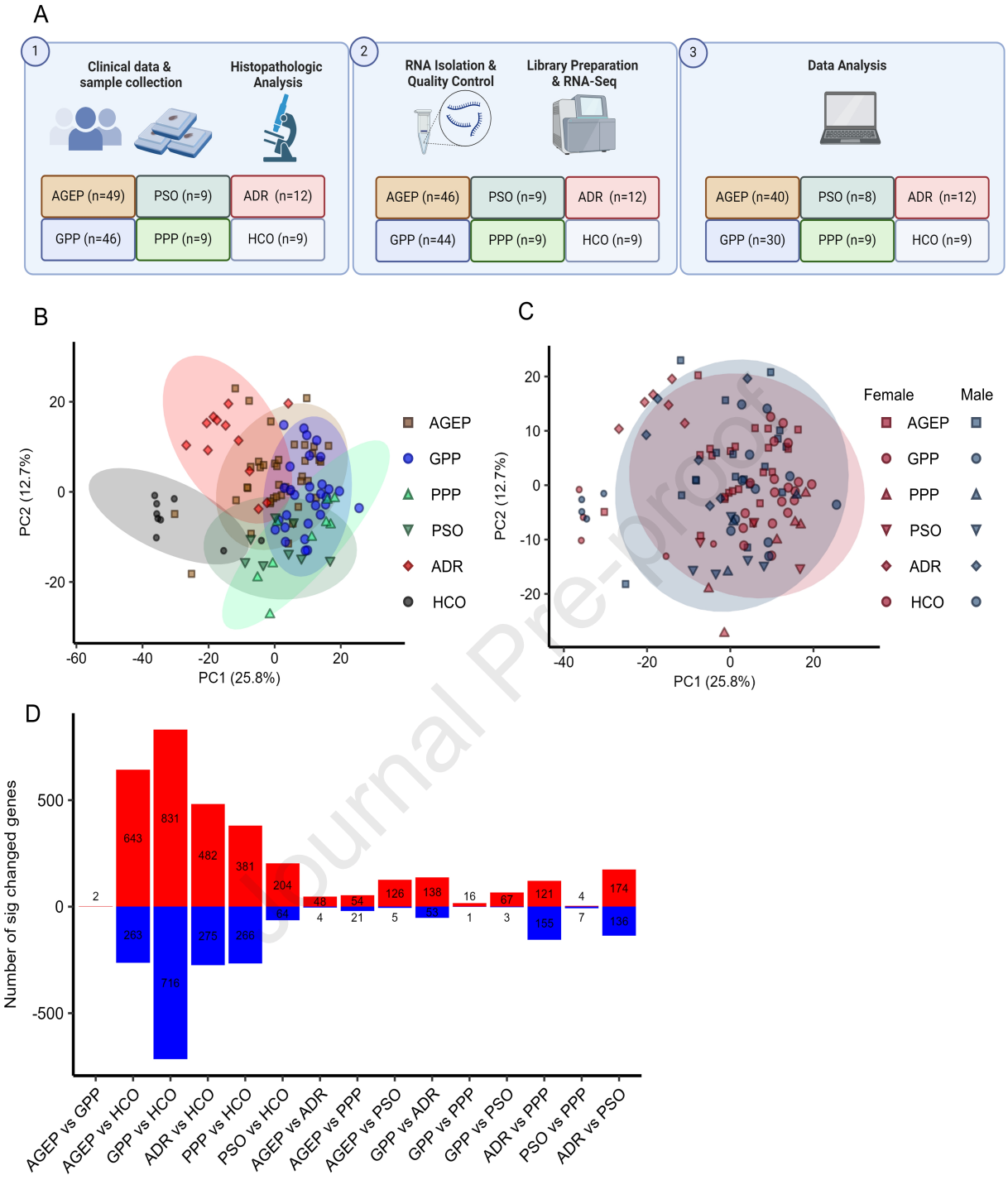
Figure 7: Transcriptome profiles of AGEP and drug-induced GPP samples largely overlap, but profiles of non-drug-induced GPP samples subtly differ from those of AGEP samples. PCA scores plot representing the transcriptome profile of each sample (n = 50 in total), where nearness signifies similarity in expression. PCA was based on the top 500 genes (highest row variance), the ellipses mark the 95% confidence interval for each group: AGEP (n = 35), drug-induced GPP (n = 8) and non-drug-induced GPP (n = 7) (A). Volcano plots showing differentially expressed genes (DEGs, BH adjusted $p < 0.05$, s-value < 0.005, |LFC| > 1) for the comparisons AGEP vs. drug-induced GPP (B) and AGEP vs. non-drug-induced GPP (C). Blue symbolizes significant downregulation and red upregulation. Heatmap showing the seven differentially expressed genes comparing AGEP with non-drug-induced GPP (D). Digital flow cytometry via a CIBERSORTx analysis of RNA-Seq data for neutrophils (E) and CD8+ T cells (F). Bars represent mean ± SD; significance was set at $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ and determined with linear mixed effects models (E, F). Heatmap showing the average expression (*z*-scaled *vst* expression levels) per group of pre-defined immune response patterns genes, grouped into type 1/Th1-, type 2/Th2, type 3/Th17-, neutrophil-, and cytotoxicity-related genes (G).

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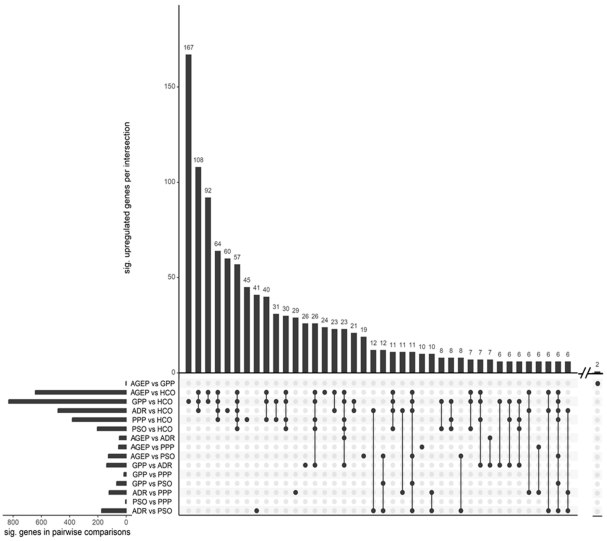
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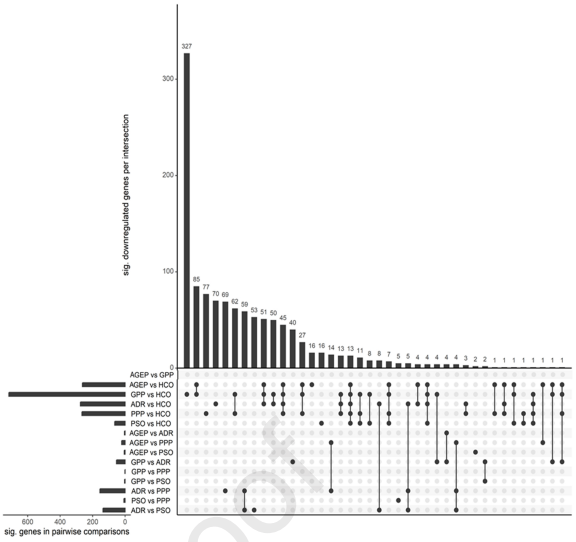
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A
1
Clinical data & sample collection
Histopathologic Analysis
AGEP (n=49)
PSO (n=9)
ADR (n=12)
GPP (n=46)
PPP (n=9)
HCO (n=9)
2
RNA Isolation & Quality Control
Library Preparation & RNA-Seq
AGEP (n=46)
PSO (n=9)
ADR (n=12)
GPP (n=44)
PPP (n=9)
HCO (n=9)
3
Data Analysis
AGEP (n=40)
PSO (n=8)
ADR (n=12)
GPP (n=30)
PPP (n=9)
HCO (n=9)
B
PC2 (12.7%)
PC1 (25.8%)
AGEP
GPP
PPP
PSO
ADR
HCO
C
PC2 (12.7%)
PC1 (25.8%)
Female
Male
AGEP
GPP
PPP
PSO
ADR
HCO
D
Number of sig changed genes
AGEP vs GPP
AGEP vs HCO
GPP vs HCO
ADR vs HCO
PPP vs HCO
PSO vs HCO
AGEP vs ADR
AGEP vs PPP
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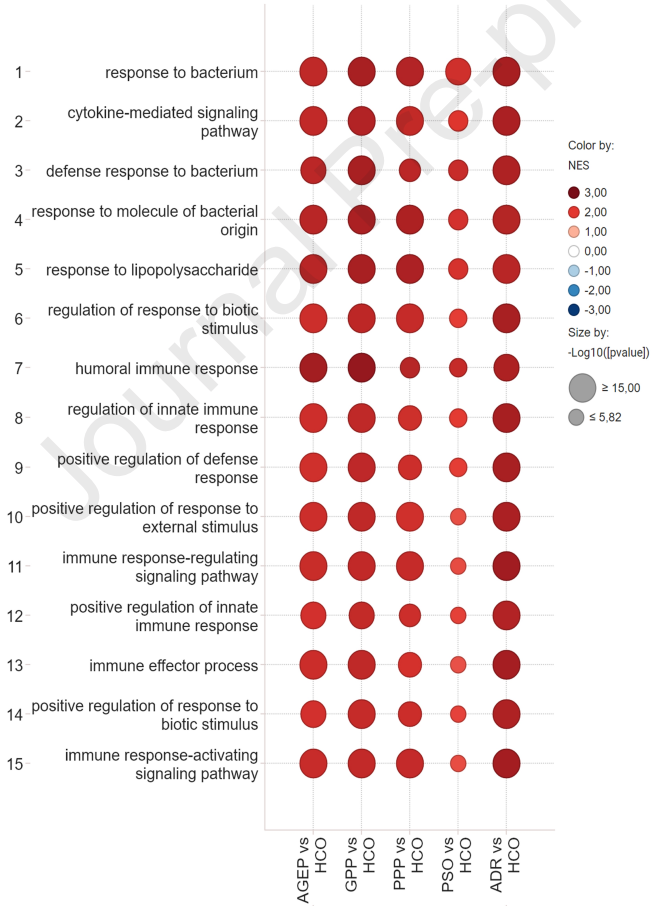
A



B

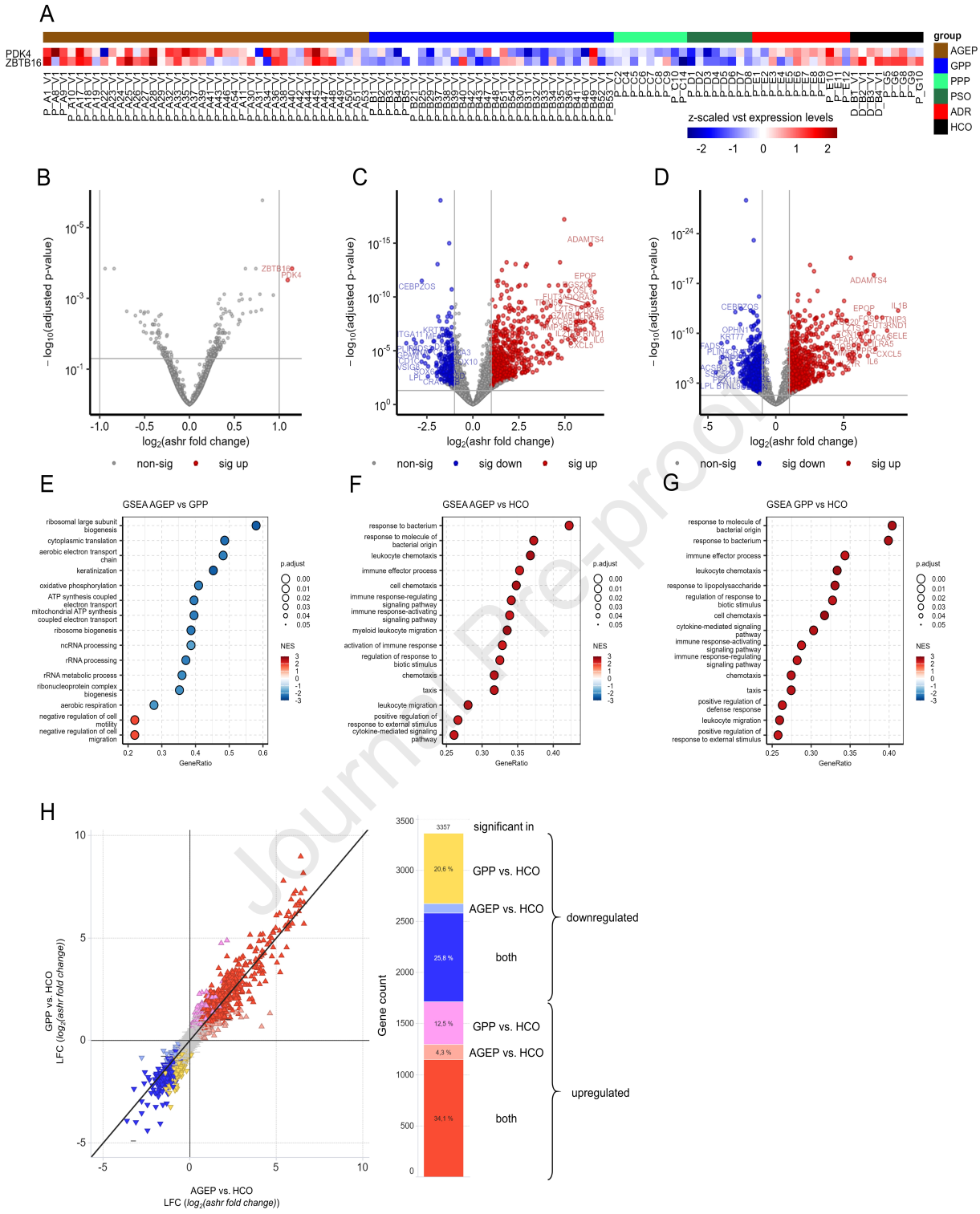


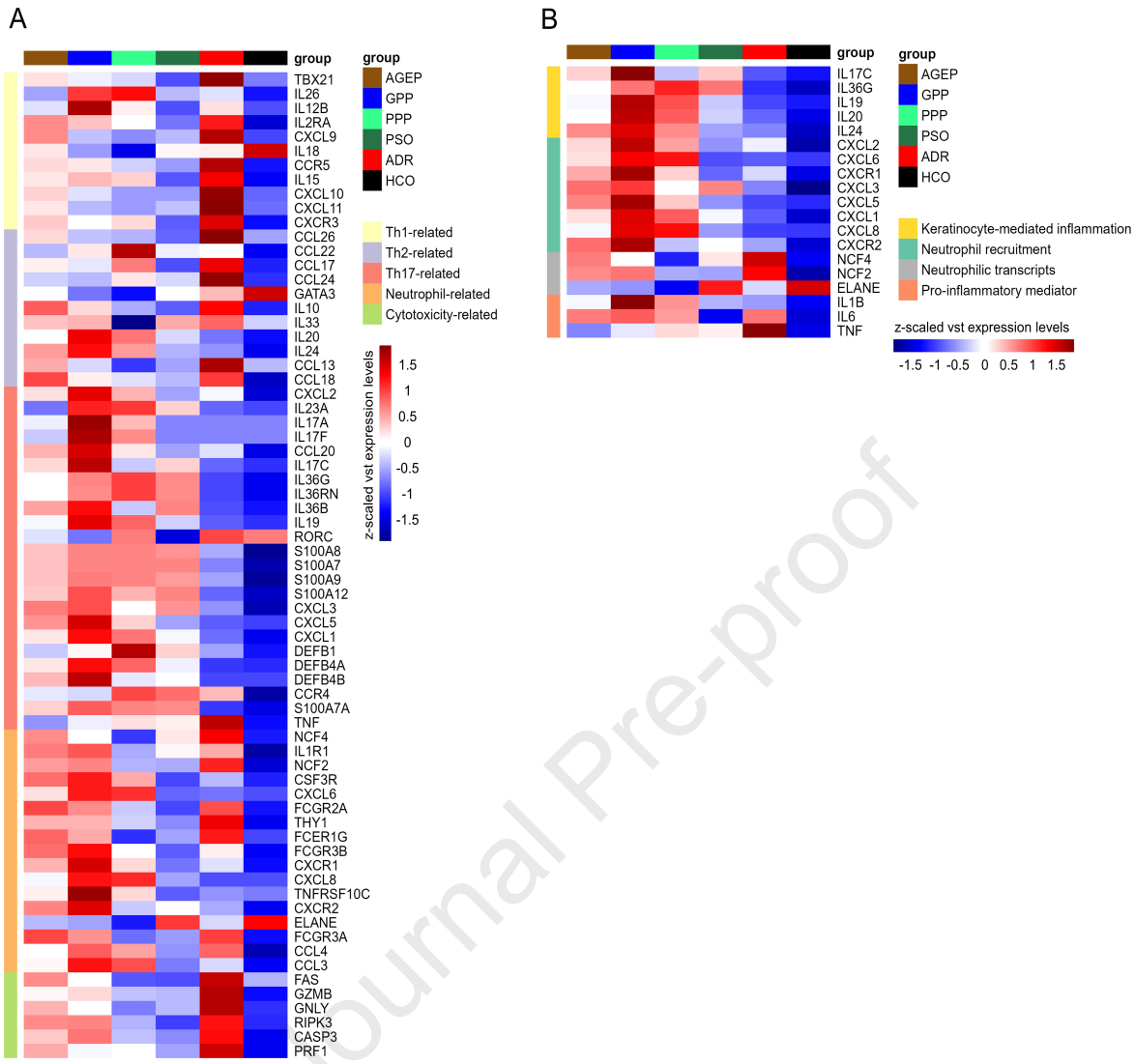
C



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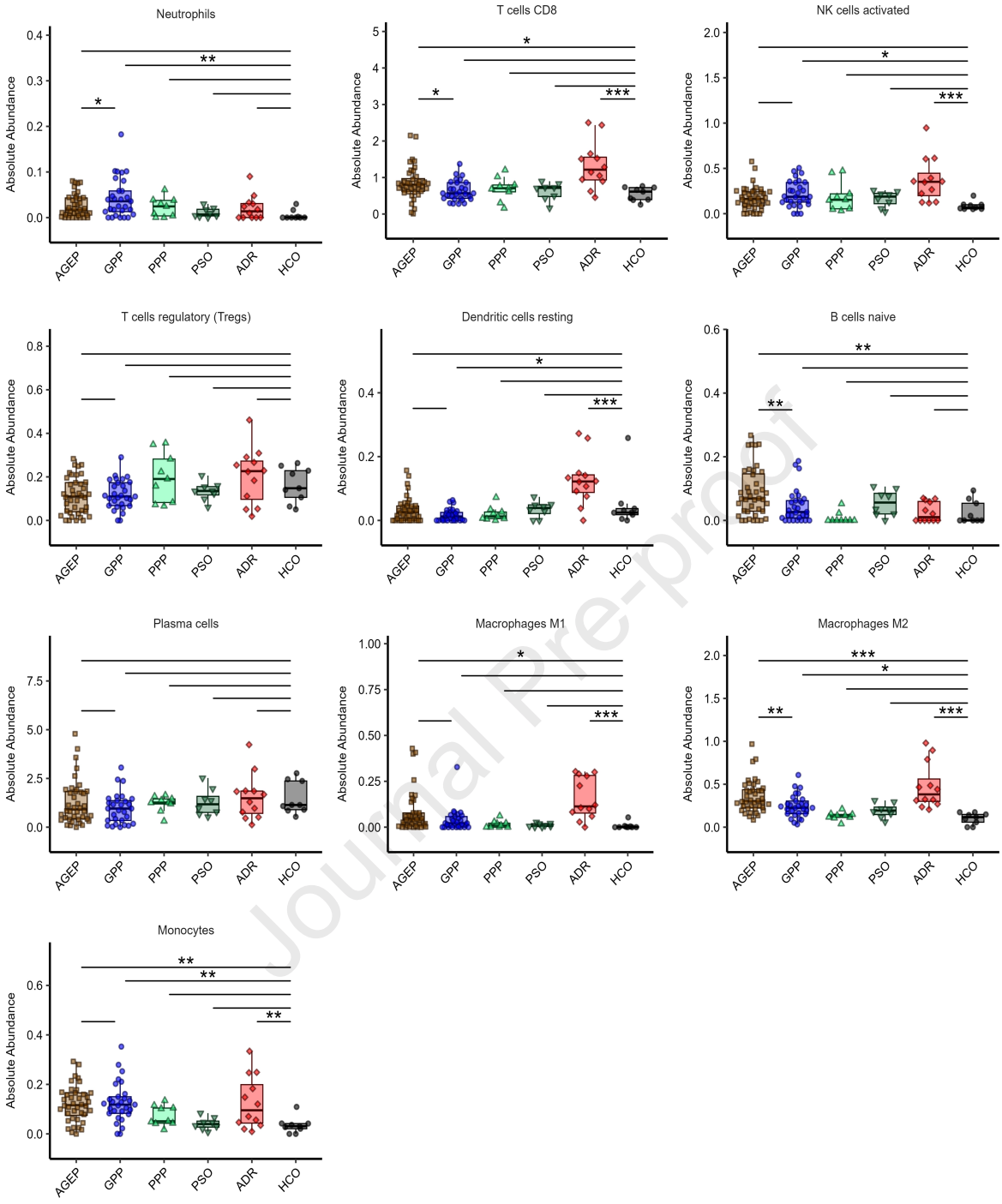
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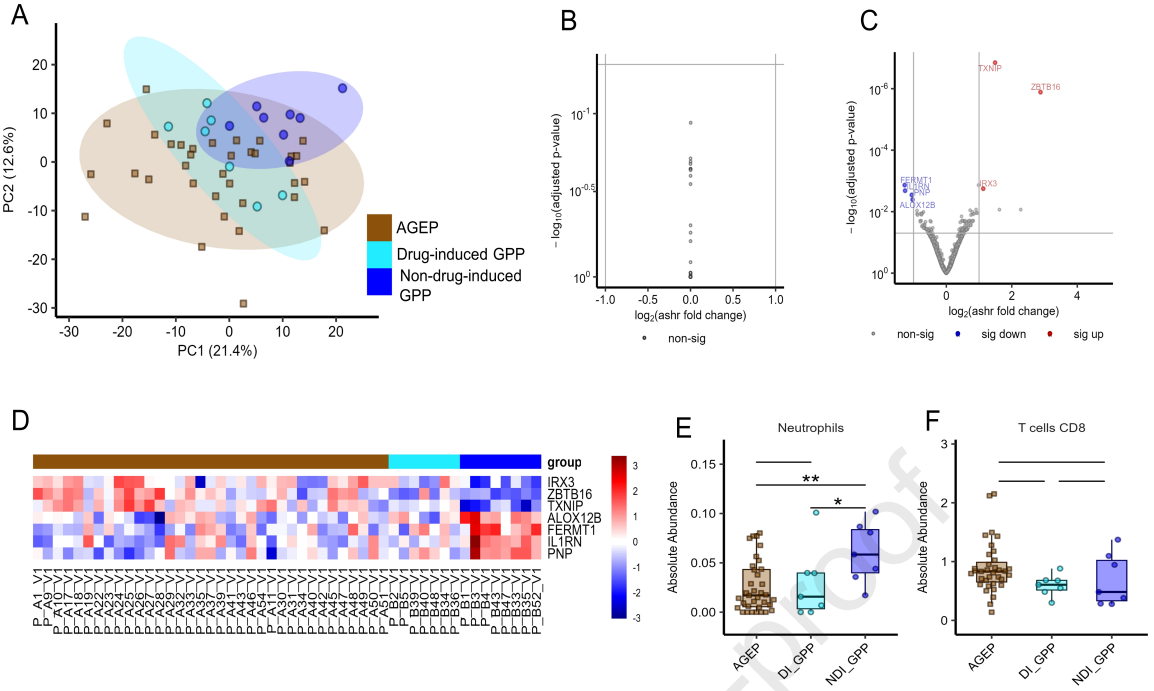


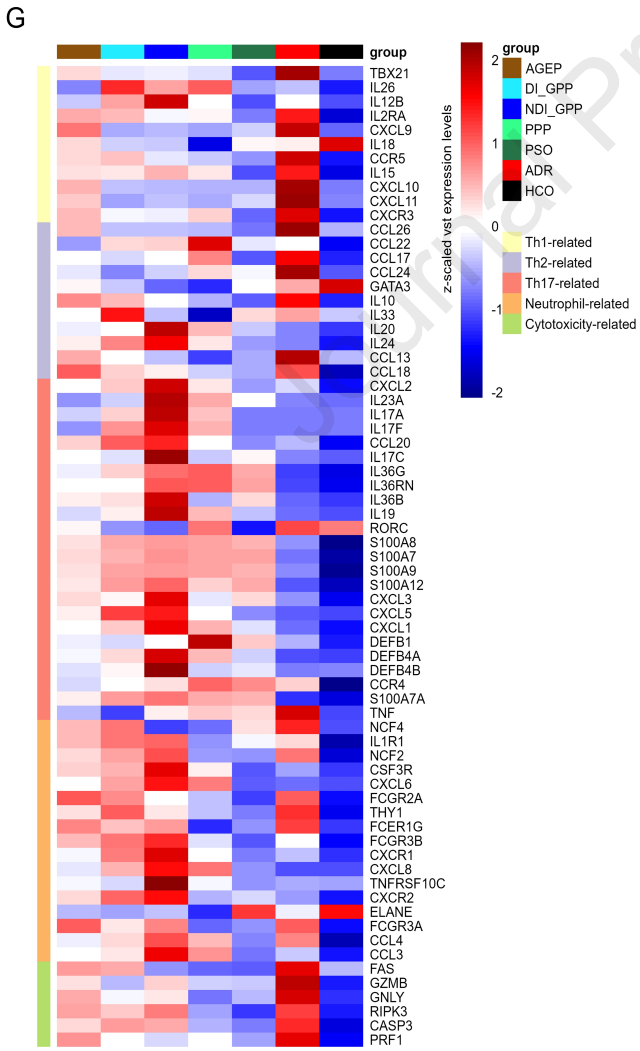
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