Jennifer Strunck: http://orcid.org/0000-0003-3042-9089
Pamela Cassidy: http://orcid.org/0000-0002-4310-9554
Teri M. Greiling: http://orcid.org/0000-0002-0028-8986

CONFLICT OF INTEREST

TMG has received research funding to study pityriasis rubra pilaris from Eli Lilly and Co for this study; has funding from Janssen Scientific Affairs to study unrelatd aspects of pityriasis rubra pilaris; and is an author for UpToDate on pityriasis rubra pilaris. The remaining authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: DH, RV, JS, TMG; Formal Analysis: DH, TR, MC, JS, TMG; Funding Acquisition: TMG; Investigation: RK, GK, JS, PC; Methodology: DH, TMG, RK; Project Administration: PC, TMG; Supervision: RK, PC, TMG; Visualization: DH, TR, TMG; Writing — Original Draft Preparation: DH, TR, TMG; Writing — Review and Editing: DH, TR, RV, MC, RK, GK, JS, PC, TMG.

***Dylan Haynes*[1], *Taylor Reiter*[2,3], *Rose Velasco*[1], *Matthew Chang*[1], *Rajan Kulkarni*[1], *Gail Kent*[1], *Jennifer Strunck*[1], *Pamela Cassidy*[1] and *Teri M. Greiling*[1,*]**

[1]*Department of Dermatology, Oregon Health & Science University, Portland, Oregon, USA;* [2]*Center for Health AI, University of Colorado School of Medicine, Aurora, Colorado, USA; and* [3]*Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, Colorado, USA*
[*]*Corresponding author e-mail: Greiling@ohsu.edu*

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2022.09.005

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Damaging Alleles Affecting Multiple *CARD14* Domains Are Associated with Palmoplantar Pustulosis

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TO THE EDITOR

Palmoplantar pustulosis (PPP) is a severe pustular eruption that affects the palms and/or soles, with detrimental effects on quality of life. The disease is notoriously difficult to treat because its immune and genetic determinants remain poorly defined (Twelves et al., 2019). Although mutations of the *IL36RN* and myeloperoxidase *MPO* genes have been convincingly associated with generalized pustular psoriasis, they are rarely found in patients with PPP (Haskamp et al., 2020; Twelves et al., 2019; Vergnano et al., 2020). Further candidate genes therefore need to be examined.

CARD14 encodes a keratinocyte scaffold protein that mediates NF-κB signaling downstream of TRAF2 and TRAF6. Activating *CARD14* mutations have been documented in a variety of inflammatory skin disorders, including familial psoriasis, erythrodermic psoriasis, generalized pustular psoriasis, pityriasis rubra pilaris, and *CARD14*-associated papulosquamous eruption

Abbreviation: PPP, palmoplantar pustulosis

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Table 1. Rare- and Low-Frequency *CARD14* Variants Detected in PPP Cases

Rs Number	Amino Acid Substitution	Minor Allele Frequency[1]	Pathogenicity Predictions					Occurrences
			CADD Score[2]	PROVEAN	MutationTaster	Spliceman	Consensus	
rs143747620	p.Lys78Asn	0.0004	25.0	Neutral	Polymorphism	—	Benign	1
—	p.Ile86Met	—	17.7	Neutral	Polymorphism	—	Benign	1
rs372403419	p.Arg182Cys	0.00009	22.7	Neutral	Disease causing	—	Deleterious	1
rs200790561	p.Glu197Lys	0.0007	27.3	Deleterious	Disease causing	—	Deleterious	1
rs375882704	p.Ala367Thr	0.00009	24.0	Neutral	Polymorphism	—	Benign	1
rs150536049	p.Ser378Arg	0.002	14.8	Deleterious	Polymorphism	—	Benign	1
rs780034490	p.Ser384Phe	0.000009	23.3	Deleterious	Polymorphism	—	Deleterious	2
rs200102454	p.Thr591Met	0.00008	24.3	Neutral	Disease causing	—	Deleterious	1
rs73429414	p.Arg597Trp	0.00007	25.8	Neutral	Disease causing	—	Deleterious	1
rs371910172	p.Arg610Cys	0.00003	24.7	Neutral	Disease causing	—	Deleterious	1
rs138833596	p.Val774Ile	0.0001	16.9	Neutral	Disease causing	—	Deleterious	1
rs2289541	p.Arg883His	0.0002	8.1	Neutral	Polymorphism	—	Benign	1
rs146678380	c.2569+4T>C	0.003	3.0	—	Disease causing	Deleterious	Deleterious	4
rs61751629	p.Glu422Lys	0.033	14.8	Neutral	Polymorphism	—	Benign	25
rs117918077	p.Arg682Trp	0.016	35.0	Deleterious	Disease causing	—	Deleterious	13

Low-frequency variants are reported in the two bottom rows. We reported the p.Arg182Cys and p.Thr591Met deleterious alleles in a previous study (Twelves et al., 2019).

Abbreviations: CADD, Combined Annotation Dependent Depletion; PPP, palmoplantar pustulosis.

[1]Frequency among non-Finnish Europeans, gnomAD 2.1.1.

[2]Variants with CADD scores > 15 are considered deleterious.

(Berki et al., 2015; Fuchs-Telem et al., 2012; Jordan et al., 2012b; Nieto-Benito et al., 2020; Signa et al., 2019). More recently, loss-of-function *CARD14* alleles have been observed in a small number of patients with severe atopic dermatitis, further extending the spectrum of *CARD14*-associated diseases (Peled et al., 2019).

In this study, we investigated the possibility that *CARD14* variants might also be associated with PPP. We examined 236 unrelated cases of European descent, recruited through United Kingdom dermatology departments participating in the APRICOT clinical trial (approved by the London Dulwich Research Ethics Committee; reference 16/LO/0436 [Cro et al., 2021]) or its sister research study PLUM (approved by the London Bridge Research Ethics Committee; reference 16/LO/2190) (Supplementary Table S1). PPP was diagnosed by dermatologists in line with the consensus criteria set by the European Rare And Severe Psoriasis Expert Network (Navarini et al., 2017). The study was undertaken in accordance with the declaration of Helsinki, and all participants granted their written informed consent.

CARD14 variants were identified by querying whole-exome sequence profiles generated on an Illumina HiSeq2000 instrument (n = 212) or by Sanger sequencing the gene coding region and exon/intron junctions (n = 24). Rare changes (minor allele frequency < 1%) were assessed using three independent algorithms (see Supplementary Materials and Methods), and those that were

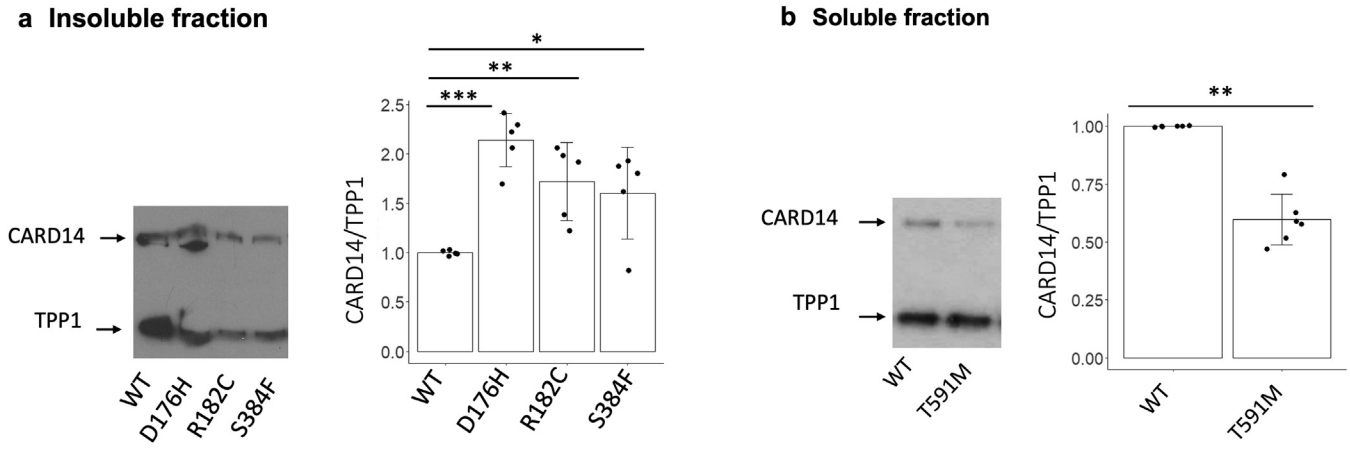

Figure 1. Western blot analysis of FLAG–CARD14 constructs cotransfected into HEK293 cells, alongside control FLAG–TPP1 plasmids. (**a**) Increased formation of insoluble aggregates is observed for p.Asp176His (positive control), p.Arg182Cys, and p.Ser384Phe proteins, (**b**) whereas reduced accumulation of mutant p.Thr591Met proteins is apparent in the soluble fraction. Representative images are shown on the left, with average densitometry readings (n ≥ 5 independent experiments) plotted on the right. *$P < 0.05$, **$P < 10^{-2}$, and ***$P < 10^{-3}$ with (**a**) ANOVA with Dunnett's posthoc test or (**b**) *t*-test. HEK293, human embryonic kidney 293; WT, wild-type.

classified as damaging by at least two predictors were considered potentially pathogenic. This approach identified eight deleterious variants, affecting 12 unrelated individuals (Table 1). Meanwhile, an analysis of 62,222 controls (non-Finnish European dataset) sequenced by the gnomAD consortium identified 1,123 rare alleles that met the same pathogenicity criteria. Fisher's exact test showed that the *CARD14* mutational burden was significantly different in the two groups (2.5 vs. 0.9%; $P = 1.5 \times 10^{-3}$; OR = 2.9, 95% confidence interval = 1.5–5.1), showing an association between rare *CARD14* alleles and PPP. Importantly, the frequency of rare and synonymous *CARD14* changes was comparable in cases and controls ($P > 0.05$), showing that there were no systematic differences between our patient population and the external control dataset.

We next examined low-frequency *CARD14* variants, identifying multiple occurrences of a known p.Arg682Trp substitution (Jordan et al., 2012a) (Table 1). This change was also more common in cases than in controls (2.7 vs. 1.6%; $P = 0.044$; OR = 1.7; 95% confidence interval = 1.0–3.0).

Although our dataset was not powered for subgroup analysis, we found that *CARD14* mutations were not restricted to a particular demographic (i.e., females or smokers) and were detectable regardless of plaque psoriasis affection status (Supplementary Table S2). Of note, this argues against the suggestion that PPP presenting with concurrent psoriasis might have a distinct genetic etiology (Murakami and Terui, 2020).

To better understand the significance of our association findings, we compared the location of the rare damaging changes detected in PPP cases with that of known *CARD14* mutations. We first carried out a systematic literature review, which identified 61 *CARD14* genetic studies (Supplementary Figure S1), reporting a total of 65 rare variants. We then assessed the deleterious potential of each change on the basis of their predicted pathogenicity, recurrence, and segregation (see Supplementary Materials and Methods). This identified 18 variants that were likely to be deleterious (Supplementary Table S3). Strikingly, all damaging missense alleles clustered to two specific gene regions (Supplementary Figure S2).

The gain-of-function mutations described in familial psoriasis, generalized pustular psoriasis, pityriasis rubra pilaris, and *CARD14*-associated papulosquamous eruption mapped between amino acids 117 and 197, affecting the CARD14 coiled–coil and the preceding linker region. Conversely, the recurrent loss-of-function allele documented in atopic dermatitis lies within the PDZ domain (residue 593).

Interestingly, the damaging missense changes detected in PPP cases were found in both mutation hot spots. Three variants (p.Arg182Cys, p.Glu197Lys, and p.Ser384Phe) localized to the coiled–coil and three to the PDZ domain (p.Thr591Met, p.Arg597Trp, p.Arg610Cys), with one substitution mapping to the C-terminal linker region (p.Val774Ile) (Supplementary Figure S2).

These data suggest that PPP is associated with both gain- and loss-of-function *CARD14* alleles. To further investigate this possibility, we overexpressed mutagenized cDNA constructs harboring representative coiled–coil (p.Arg182Cys, p.Ser384Phe) and PDZ (p.Thr591Met) variants. We found that the p.Arg182Cys and p.Ser384Phe alleles led to the formation of insoluble CARD14 aggregates (Figure 1a). Because these promote constitutive NF-κB activation (Berki et al., 2015), the two variants are very likely to have gain-of-function properties. Conversely, we observed that the p.Thr591Met substitution was associated with reduced protein accumulation (Figure 1b), indicating a loss-of-function effect.

Interestingly, the notion that variants with opposing effects can result in the same clinical phenotype is supported by the characterization of *CARD14* alleles associated with plaque psoriasis. This identified both gain- and loss-of-function changes, suggesting that CARD14 activity levels need to be finely balanced to maintain skin immune homeostasis (Jordan et al., 2012a). Importantly, this implies that CARD14 might be a problematic therapeutic target.

Although *CARD14* has been previously investigated in PPP, earlier studies were mostly restricted to the proximal coiled–coil domain and had in retrospect limited the potential to detect disease alleles (Berki et al., 2015; Mössner et al., 2017). Of note, evidence gathered in other inflammatory conditions (e.g., pityriasis rubra pilaris and *CARD14*-associated papulosquamous eruption) indicates that IL-12p40 blockade (ustekinumab) may be effective in individuals with *CARD14* mutations (Eytan et al., 2014; Nieto-Benito et al., 2020; Signa et al., 2019). In this context, our work suggests that whole-gene mutational screens could identify patients with *CARD14* disease alleles who may benefit from personalized ustekinumab treatment.

Data availability statement

All the patient allele frequency data are reported in the text and table of this manuscript. Control allele frequency data were retrieved from the gnomAD database.

ORCIDs

Athanasios Niaouris: http://orcid.org/0000-0002-8407-2014
Ariana Hernández-Cordero: http://orcid.org/0000-0002-4132-1949
Salma Haddad: http://orcid.org/0000-0002-8123-6853
Niina Karoliina Hassi: http://orcid.org/0000-0002-1548-1199
Natashia Benzian-Olsson: http://orcid.org/0000-0002-5287-7707
Carmen Bugarin Diz: http://orcid.org/0000-0001-6257-3734
A. David Burden: http://orcid.org/0000-0001-7395-9931
Hywel L. Cooper: http://orcid.org/0000-0001-8016-706X
Christopher E.M. Griffiths: http://orcid.org/0000-0001-5371-4427
Richard Parslew: http://orcid.org/0000-0002-9430-1394
Andrew E. Pink: http://orcid.org/0000-0001-5151-5539
Nick J. Reynolds: http://orcid.org/0000-0002-6484-825X
Shyamal Wahie: http://orcid.org/0000-0003-2798-8429
Richard B. Warren: http://orcid.org/0000-0002-2918-6481
Andrew Wright: http://orcid.org/0000-0002-6072-5148
Michael Simpson: http://orcid.org/0000-0002-8539-8753
Patrick Baum: http://orcid.org/0000-0002-1399-8610
Sudha Visvanathan: http://orcid.org/0000-0002-1170-9272
Jonathan N. Barker: http://orcid.org/0000-0002-9030-183X
Catherine H. Smith: http://orcid.org/0000-0001-9918-1144
Francesca Capon: http://orcid.org/0000-0003-2432-5793

AUTHOR CONTRIBUTIONS

Conceptualization: FC; Formal Analysis: AN, AHC, CBD, SH, NBO; Funding Acquisition: FC, CHS, PB, SV; Resources: ADB, HLC, CEMG, RP, AEP, NJR, MS, SW, RBW, AW, JNB, CHS; Investigation: AN, NKH; Supervision: FC; Writing - Original Draft Preparation: FC

CONFLICT OF INTEREST

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Disclaimer

The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, or the Department of Health and Social Care.

***Athanasios Niaouris*[1], *Ariana Hernández-Cordero*[1], *Salma Haddad*[1], *Niina Karoliina Hassi*[1], *Natashia Benzian-Olsson*[1], *Carmen Bugarin Diz*[1], *A. David Burden*[2], *Hywel L. Cooper*[3], *Christopher E.M. Griffiths*[4], *Richard Parslew*[5], *Andrew E. Pink*[6], *Nick J. Reynolds*[7,8], *Shyamal Wahie*[9,10], *Richard B. Warren*[11], *Andrew Wright*[12,13], *Michael Simpson*[1], *Patrick Baum*[14], *Sudha Visvanathan*[15], *Jonathan N. Barker*[6], *Catherine H. Smith*[6] *and Francesca Capon*[1,*] *The APRICOT and PLUM study team*

[1]Department of Medical & Molecular Genetics, School of Basic & Medical Biosciences, King's College London, London, United Kingdom; [2]Institute of Infection Immunity and Inflammation, University of Glasgow, Glasgow, United Kingdom; [3]Portsmouth Dermatology Unit, Portsmouth Hospitals Trust, Portsmouth, United Kingdom; [4]Dermatology Centre, NIHR Manchester Biomedical Research Centre, University of Manchester, Manchester, United Kingdom; [5]Department of Dermatology, NHS Foundation Trust, Royal Liverpool Hospitals, Liverpool, United Kingdom; [6]St John's Institute of Dermatology, School of Basic & Medical Biosciences, King's College London, London, United Kingdom; [7]Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom; [8]Department of Dermatology and NIHR Newcastle Biomedical Research Centre, Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom; [9]Department of Dermatology, University Hospital of North Durham, Durham, United Kingdom; [10]Department of Dermatology, Darlington Memorial Hospital, Darlington, United Kingdom; [11]The Dermatology Centre, Salford Royal NHS Foundation Trust, University of Manchester, Manchester Academic Health Science Centre, Manchester, United Kingdom; [12]Department of Dermatology, St Lukes Hospital, Bradford, United Kingdom; [13]Centre for Skin Science, University of Bradford, Bradford, United Kingdom; [14]Boehringer Ingelheim International, Biberach, Germany; and [15]Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut, USA

[*]Corresponding author e-mail: francesca.capon@kcl.ac.uk

SUPPLEMENTARY MATERIAL

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TFAM Deficiency–Mediated Mitochondrial Disorder Affects Langerhans Cell Maintenance and Function

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TO THE EDITOR

The skin is the outermost organ and confers biophysical and immunological protection from the external environment (Kabashima et al., 2019). Increased susceptibility to skin infections and rising incidence of cutaneous tumors observed in the elderly indicate that skin immunity decreased with age (Chambers and Vukmanovic-Stejic, 2020). Langerhans cells (LCs), characterized by high expression of Langerin (CD207) (Seré et al., 2012), are professional antigen-presenting dendritic cells that reside in the epidermis, which form the first immunological barrier and play a vital role in maintaining skin homeostasis (Doebel et al., 2017; Kaplan, 2017). The number and maturation of LCs are significantly reduced during the skin aging processes, which is closely related to aging-associated skin disorders (Pilkington et al., 2021; Xu et al., 2012a). However, the reasons underlying the change of LC characteristics with age are not well-known.

Mitochondria are dynamic biosynthetic and bioenergetic organelles and are the main powerhouse to regulate cell homeostasis and function (Angajala et al., 2018). It has long been appreciated that a decline in mitochondrial quality and activity were associated with normal aging and correlated with the development of a wide range of age-related diseases; for example, UV-induced skin aging, photoaged skin, and skin cancer are the direct consequence of mitochondrial dysfunction (Sreedhar et al., 2020; Sun et al., 2016). Interestingly, the age-associated loss of epidermal LCs in sun-exposed and covered skin was observed to have a slow response to UV irradiation, which might play a critical permissive role in the development of cutaneous carcinoma (Gilchrest et al., 1982; Thiers et al., 1984). However, it is unknown whether mitochondrial abnormality is involved in skin disorders at least in part by regulating LC homeostasis and function.

TFAM, a nuclear-encoded transcription factor, binds mtDNA to govern its replication, packaging, and transcription, whereas stabilized mtDNA is essential for cell fitness and function (Ekstrand et al., 2004; Picca and Lezza, 2015). A number of studies have shown that TFAM-mediated mitochondrial function is critical for the maintenance and function of immune cells, such as T cells, regulatory T cells, and macrophages, using conditional *Tfam* gene deletion mice (Baixauli et al., 2015; Fu et al., 2019; Gao et al., 2022), whereas homozygous-knockout embryos were not viable and died before embryonic day 10.5 (Larsson et al., 1998). In this study, we crossed $Tfam^{fl/fl}$ mice with $CD11c^{cre}$ mice to generate $CD11c^{Cre}Tfam^{fl/fl}$ conditional-knockout (cKO) mice to investigate the role of TFAM-mediated mitochondrial stability in LC homeostasis and function.

At the adult age (6–8 weeks), the deletion of *Tfam* on epidermal LCs from cKO mice was confirmed by RT-qPCR (Figure 1a, left), and *Tfam* expression of epidermal LCs was decreased with age (Figure 1a, right), suggesting that defective mitochondrial function of aging LCs might be related with decreased *Tfam* expression. As shown in Figure 1b, epidermal LCs almost disappeared in $CD11c^{Cre}Tfam^{fl/fl}$ adult mice (0.15 ± 0.02%) compared with that of wild-type littermates (1.94 ± 0.02%); the result was further confirmed by immunofluorescence staining of epidermis tissue sections (Figure 1c). Next, we sought to assess the kinetics of LC responses after *Tfam* deficiency and examined LC numbers at the juvenile stage (age 3–4 weeks postnatally). The data showed that *Tfam* expression in cKO mice was dramatically reduced compared with that of wild-type mice (Figure 1d); meanwhile, the frequency of LCs in $CD11c^{Cre}Tfam^{fl/fl}$ mice was significantly decreased compared with that of $Tfam^{fl/fl}$ littermates (Figure 1e), albeit to a lower extent than in the adult

Abbreviations: cKO, conditional-knockout; LC, Langerhans cell

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SUPPLEMENTARY NOTES

Membership of the APRICOT and PLUM study team

The members of the APRICOT and PLUM include the following: Thamir Abraham (Peterborough City Hospital, Peterborough, United Kingdom), Muhmad Ali (Worthing Hospital, Worthing, United Kingdom), Suzannah August (Poole Hospital NHS Foundation Trust, Poole, United Kingdom), David Baudry (Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom), Gabrielle Becher (NHS Greater Glasgow and Clyde, Glasgow, United Kingdom), Anthony Bewley (Whipps Cross Hospital, London, United Kingdom), Victoria Cornelius (Imperial College London, London, United Kingdom), Giles Dunnill (University Hospitals Bristol NHS Foundation Trust, Bristol, United Kingdom), Adam Ferguson (Royal Derby Hospital, Derby, United Kingdom), Sharizan Ghaffar (Ninewells Hospital and Medical School, Dundee, United Kingdom), John Ingram (University Hospital of Wales, Cardiff, United Kingdom), Svetlana Kavakleiva (Royal Lancaster Infirmary, Lancaster, United Kingdom), Susan Kelly (The Royal Shrewsbury Hospital, United Kingdom), Mohsen Khorshid (Basildon Hospital, Basildon, United Kingdom), Helen Lachmann (Royal Free Hospital, London, United Kingdom), Effie Ladoyanni (Russels Hall Hospital, Dudley, United Kingdom), Helen McAteer (The Psoriasis Association, Northampton, United Kingdom), John McKenna (Leicester Royal Infirmary, Leicester, United Kingdom), Freya Meynell (Guy's and St Thomas' NHS Foundation Trust), Nick Levell (Norfolk and Norwich University Hospital, Norwich, United Kingdom), Prakash Patel (Guy's and St Thomas' NHS Foundation Trust), Angela Pushparajah (Guy's and St Thomas' NHS Foundation Trust), Catriona Sinclair (Mid and South Essex NHS Foundation Trust, Southend-on-Sea, United Kingdom), Rachel Wachsmuth (Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom), Rosemary Wilson (Guy's and St Thomas' NHS Foundation Trust).

SUPPLEMENTARY MATERIALS AND METHODS

Whole-exome sequencing and Sanger sequencing

A total of 95 affected individuals were whole-exome sequenced as part of a previous study (Vergnano et al., 2020). The same reagents and computational pipeline were then used to generate variant profiles for a further 117 patients. Briefly, libraries were prepared with Agilent SureSelect Human All Exome kit and run on an Illumina HiSeq instrument (Illumina Inc, San Diego, CA). Reads were aligned to the hg19 genome using Novoalign (Novocraft Technologies, Petaling Jaya, Malaysia), and variants were called with SAMtools (Li et al., 2009) and annotated with ANNOVAR (Wang et al., 2010). A total of 24 additional palmoplantar pustulosis cases were screened by Sanger sequencing using the primers in Supplementary Table S4.

Pathogenicity predictions

The rare *CARD14* alleles detected in palmoplantar pustulosis cases and gnomAD controls were analyzed using the same approach. Briefly, the impact of missense variants was assessed with Combined Annotation Dependent Depletion (CADD) (Rentzsch et al., 2019), MutationTaster (Schwarz et al., 2010), and PROVEAN (Choi and Chan, 2015). Given that the latter program can only be used for the analysis of coding changes, splicing variants were assessed using CADD, MutationTaster, and Spliceman (Lim and Fairbrother, 2012). Missense and splice site variants that were classified as damaging by at least two predictors were considered potentially pathogenic. Frameshift and nonsense variants were automatically considered potentially pathogenic.

Systematic literature review

A systematic literature review was performed by interrogating the PubMed database with the terms (CARD14 or CARMA2) and (variants or mutations or GWAS or genome-wide linkage). The cut-off date was October 31, 2021. Duplicate articles, conference abstracts, reviews, irrelevant studies, and papers that were not written in English were removed. The rare variants (minor allele frequency < 1%) described in the remaining studies were classified as pathogenic if they were predicted to be damaging by at least two algorithms and met one of the following criteria: described in at least two case reports, segregating with inflammatory skin disease in pedigrees, inherited de novo.

In vitro mutagenesis

Mutant constructs were generated using the QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA), and primers were designed using Agilent's QuikChange Primer Design tool (https://www.agilent.com/store/primerDesignProgram.jsp) (Supplementary Table S5). All constructs were validated by sequencing the entire *CARD14* coding region, pCMV promoter, bovine growth hormone polyadenylation site, and the FLAG sequence.

Cell culture

Human embryonic kidney 293 cells were cultured in DMEM supplemented with 2 mM L-Glutamine, 50 U/ml of penicillin, and 50 μg/ml of streptomycin (all from Life Technologies, Carlsbad, CA) and 10% fetal calf serum (LabTech, Heathfield, United Kingdom). Lipofectamine 2000 (Life Technologies) was used to cotransfect cells with the FLAG–CARD14 constructs and FLAG–TPP1 (kindly provided by Tracey Mitchell, King's College London, London, United Kingdom). Pellets were harvested after 48 hours.

Western blotting

Cell pellets were incubated with nondenaturing lysis buffer (50 mM Tris-hydrogen chloride [pH 7.4], 50 mM sodium chloride, 10% glycerol, 5 mM EDTA, 1% NP-40) and then centrifuged. The supernatant containing the soluble protein fraction was frozen, and the pellet with the insoluble proteins was resuspended in denaturing cell extraction buffer (Thermo Fisher Scientific, Waltham, MA). The two fractions were analyzed by western blotting using a mouse anti-FLAG antibody (Sigma-Aldrich, St. Louis, MO) at 1:3,000 dilution. Autoradiography films were analyzed with ImageJ (National Institutes of Health, Bethesda, MD)

(Schneider et al., 2012) to measure FLAG−CARD14/FLAG−TPP1 ratios.

Statistics

Counts of rare and damaging *CARD14* alleles were compared in cases vs. controls using Fisher's exact test. Densitometry results were analyzed with a *t*-test or one-way ANOVA followed by Dunnett's post-test, as appropriate. $P < 0.05$ was deemed statistically significant.

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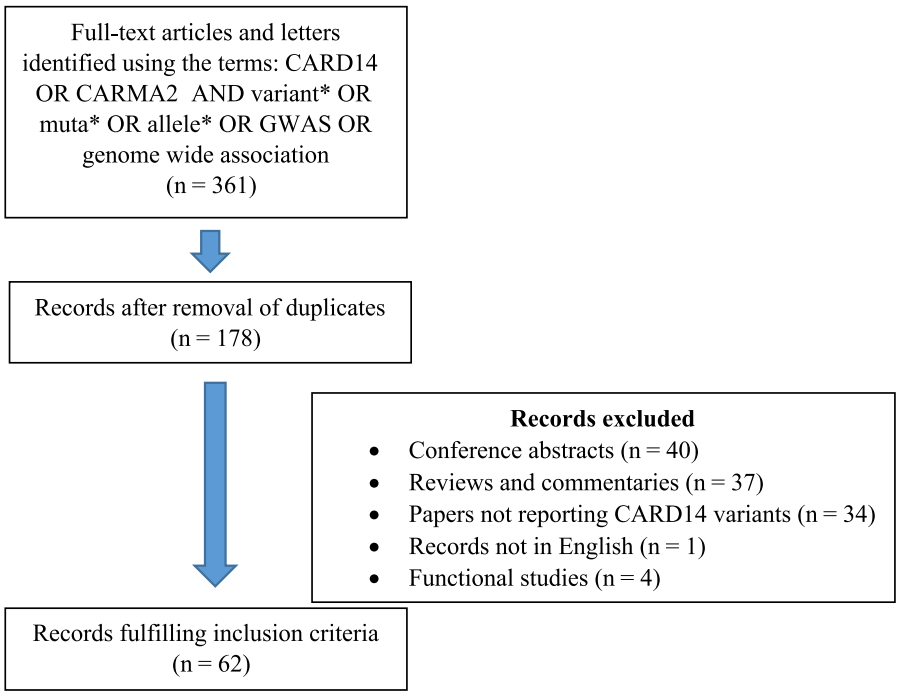
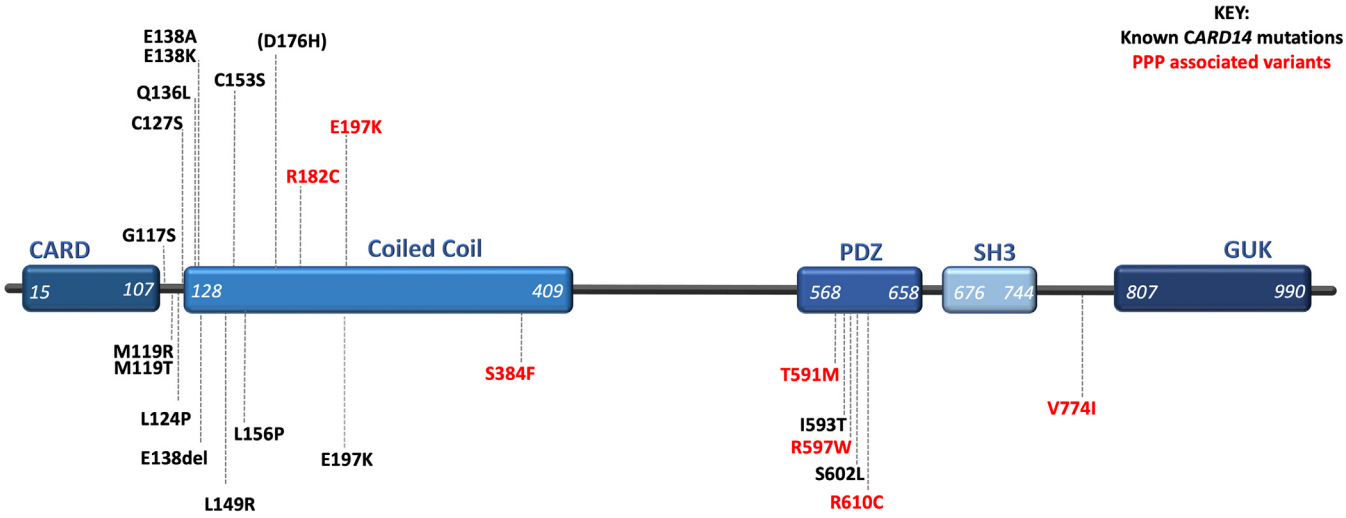


Supplementary Figure S1. Flowchart illustrating the steps taken during the literature review.



Supplementary Figure S2. Distribution of *CARD14* missense alleles associated with inflammatory skin disease. The diagram shows the localization of the *CARD14* mutations validated through the systematic literature review and the rare variants associated with PPP. The D176H allele is shown in brackets because its frequency exceeds 1% in Asian populations. GUK, guanylate kinase; PPP, palmoplantar pustulosis.

Supplementary Table S1. Patient Demographics

Demographic	Observations
Sex (%)	169 females (79.2%); 43 males (20.8%)
Median age of onset (IQR)	46 (36–55)
Median PPPASI (IQR)	9.6 (3.5–16.2)
Concurrent plaque psoriasis, n (%)	67 (31.6%)
Current/former smokers, n (%)	181 (76.4%)

Abbreviations: IQR, interquartile range; PPASI, palmoplantar pustulosis area and severity index.

Supplementary Table S2. Characteristics of the 12 Individuals Harboring *CARD14* Mutations

Demographic	Observations
Sex (%)	11 females (91.6%); 1 male (8.4%)
Median age of onset (IQR)	48 (35.5–56.5)
Median PPPASI (IQR)	10.3 (2.2–18)
Concurrent plaque psoriasis, n (%)	4 (33.3%)
Current/former smokers, n (%)	7 (58.3%)

Abbreviations: IQR, interquartile range; PPASI, palmoplantar pustulosis area and severity index.

Supplementary Table S3. Damaging *CARD14* Alleles Associated with Inflammatory Skin Disease

Change	Reference(s)
p.Gly117Ser	Ammar et al., 2016; Craiglow et al., 2018; Eskin-Schwartz et al., 2016; Jordan et al., 2012; Mössner et al., 2017; Takeichi et al., 2017b
c.349+1G>A	Fuchs-Telem et al., 2012; Takeichi et al., 2017a
c.349+5G>A	Jordan et al., 2012
p.Met119Arg	Craiglow et al., 2018; Lwin et al., 2018
p.Met119Thr	Craiglow et al., 2018; Frare et al., 2021
p.Leu124Pro	Craiglow et al., 2018; Eytan et al., 2014; Spoerri et al., 2018
p.Cys127Ser	Craiglow et al., 2018; Takeichi et al., 2017b
p.Gln136Leu	Takeichi et al., 2017b
p.Glu138Lys	Has et al., 2016
p.Glu138Ala	Jordan et al., 2012
p.Glu138del	Fuchs-Telem et al., 2012
p.Leu149Arg	Signa et al., 2019
p.Cys153Ser	Chiramel et al., 2020
p.Leu156Pro	Fuchs-Telem et al., 2012
p.Asp176His	Berki et al., 2015; Mössner et al., 2017; Sugiura et al., 2014; Takeichi et al., 2017b
p.Glu197Lys	Ammar et al., 2016
p.Ile593Thr	Peled et al., 2019
p.Ser602Leu	Ammar et al., 2016

Supplementary Table S4. *CARD14* Sequencing Primers

Target	Primer Name	Sequence (5′–3′)	Annealing (°C)
Exon 4	CARD14_Exon4F	ATGGCCACTGGAATGCTTC	63
	CARD14_Exon4R	CAGGACGAGAAGAGACCCC	
Exon 5	CARD14_Exon5F	ACCCAGCAGAACCCAGAAA	64
	CARD14_Exon5R	AAGGGGGAGTAGGGCAAAT	
Exon 6	CARD14_Exon6F	TGCTCACCTGCTCACCTAC	66
	CARD14_Exon6R	AAGGAGTTCCAGGGAGATGG	
Exon 7	CARD14_Exon7F	TTCAGTCTCGAGGCAGGAAG	63
	CARD14_Exon7R	AACCACCTGTCAGAAACCCC	
Exon 8	CARD14_Exon8F	AAGACTGCATCCGTCCACA	63
	CARD14_Exon8R	AATTATGTGAGCTCGGCGTG	
Exon 9	CARD14_Exon9F	AGAACTGTCTCCCTCCCTC	66
	CARD14_Exon9R	TGTGGACCGAGGAAAGAGAC	
Exon 10	CARD14_Exon10F	CACTGCACATGTGAACACGA	63
	CARD14_Exon10R	TCGCTCATCACAGTGACACT	
Exon 11	CARD14_Exon11F	CTGGAAGCTGACGAGAGGAA	63
	CARD14_Exon11R	CGTACCAACCTCTTCCCTGT	
Exons 12 + 13	CARD14_Exon12_13F	TGTGTCCTTCTTTCCCCTCC	66
	CARD14_Exon12_13R	TATCTGCCCTTTCCCTGGAG	
Exons 14 + 15	CARD14_Exon14_15F	AGATCTGTGAAGAAGGGGCT	66
	CARD14_Exon14_15R	TGAAGTCTGCCTGGGTCAC	
Exon 16 + 17	CARD14_Exon16_17F	TGCAGGCAGTGGTCCTAC	63
	CARD14_Exon16_17R	CGCCCACCCTCTATTGCT	
Exon 18	CARD14_Exon18F	AAAGCTCTGGAGACTGGCAT	63
	CARD14_Exon18R	TTTGAAGGGGTGCAGAGGAG	
Exon 19	CARD14_Exon19F	ACACACCTCAGGCTGTTCTC	63
	CARD14_Exon19R	CCCAGCCCCATGATTCTTGA	
Exon 20 + 21	CARD14_Exon20_21F	TGGAATTCTAGGTGCTGGGG	64
	CARD14_Exon20_21R	GGTCGGTCCCTGTACCTTTA	
Exon 22	CARD14_Exon22F	AAATTCAGCTCTGCCCAGCTCC	63
	CARD14_Exon22R	TCCCAAAGTTGTGCGGAAAC	
Exon 23	CARD14_Exon23F	TGTCATCACTACCCTAGCCA	63
	CARD14_Exon23R	GCCTCATGTGCCAAGGAG	

Supplementary Table S5. Mutagenesis Primers

Variant	Primers (5′–3′)
p.Arg182Cys	CACAGCCGCATGAAGTGTGAGGTTAGCGCAC
	GTGCGCTAACCTCACACTTCATGCGGCTGTG
p.Ser384Phe	CTGCGGAGGAAGTCCTTCTCCACCAGG
	CCTGGTGGAGAAGGACTTCCTCCGCAG
p.Thr591Met	GAAGATGCCCATGAGGTTCCCGCCGATGA
	TCATCGGCGGGAACCTCATGGGCATCTTC

The p.Asp176His mutagenesis primers were described by Berki et al. (2015).