CARD14 Missense Variant Underlying *CARD14*-Associated Papulosquamous Eruption with Beneficial Response to Secukinumab

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CARD14-associated papulosquamous eruption is an autosomal dominant genodermatosis characterized by early-onset, generalized erythematous patches and plaques with prominent scales, mostly with facial involvement. Heterozygous gain-of-function variants in the *CARD14* gene have been reported to be causative for this entity. The pathogenesis mainly involves the IL-23–IL-17 inflammatory circuit, yet the efficacy of anti–IL-17 treatment remained less examined. In this study, we report one previously unidentified variant underlying the *CARD14*-associated papulosquamous eruption and showed its gain-of-function property. Furthermore, we present the beneficial effect of anti–IL-17A treatment in our patient.

JID Innovations (2023);**3:**100174 doi:10.1016/j.xjidi.2022.100174

INTRODUCTION

Variants in the *CARD14* gene have been reported to cause psoriasis, pityriasis rubra pilaris, atopic dermatitis, and *CARD14*-associated papulosquamous eruption (CAPE) (Craiglow et al., 2018; Jordan et al., 2012; Peled et al., 2019; Takeichi et al., 2017). The term CAPE was first introduced by Craiglow et al. in 2018, which described a spectrum of diseases with overlapping clinical manifestations of psoriasis and pityriasis rubra pilaris. The patients typically present with generalized erythematous patches or plaques with marked scales. Other features of patients with CAPE include early age of onset, prominent facial involvement, and minimal response to conventional therapies for psoriasis (Craiglow et al., 2018). The ambiguity of clinical presentations often leads to misdiagnosis, and the treatment options are limited in these patients.

Heterozygous gain-of-function variants in *CARD14* gene have been reported to be causative for CAPE (Craiglow et al., 2018). CARD14 is a scaffolding protein that belongs to the CARMA protein family (Wang et al., 2018). The activation of the CARMA protein can lead to binding of BCL10 and MALT1, thus forming the CARD–BCL10–MALT1 complex. The CARD–BCL10–MALT1 complex has been identified as a signaling hub and plays a central role in the activation of different cell types such as T-cells or keratinocytes. The downstream effects of CARMA activation include the activation of NF-kB and MAPK signaling pathway as well as the promotion of cell proliferation and the proinflammatory process (Ruland and Hartjes, 2019).

We and others have previously shown that *Card14* variant in mice caused enhanced response to IL-17A in keratinocytes through self-aggregation (Howes et al., 2016; Wang et al., 2018), thus leading to psoriasis-like phenotype. Under pathological conditions, CARD14 activation results from the downstream effects of IL17A receptor signaling and facilitates the formation of the IL-23–IL-17 inflammatory circuit in both psoriasis and CAPE pathogenesis. Anti–IL-23 treatment has been successful in alleviating the phenotype of CAPE, yet the efficacy of anti–IL-17 treatment was less examined (Craiglow et al., 2018).

In this study, we present one case of CAPE with previously unreported variant in *CARD14* and further show the beneficial response to anti–IL-17A treatment in this patient.

RESULTS

Clinical and pathological characteristics of the patient

A boy of Chinese Han ethnicity aged 5 years was born to unaffected, nonconsanguineous parents. Erythematous, erosive, pruritic patches appeared on his face, elbows, and knees in the first year of his life. The lesions progressed into erythroderma, with marked scaling and severe itching (Figure 1a). Contractural scarring was noted on lips, eyelids, and popliteal fossa, leading to ectropion and impaired walking (Figure 1b). He also suffered from malnutrition and recurrent skin infection. The patient denied any allergy history. No elevation of eosinophils or total IgE in the blood was detected. The patient was treated with oral retinoids and topical agents, including corticosteroids and emollient but with minimal response. Histological examination revealed orthohyperkeratosis, parakeratosis, and mild spongiosis with prominent infiltration of lymphocytes and an increased number of eosinophils in the

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Abbreviation: CAPE, CARD14-associated papulosquamous eruption

Received 26 June 2022; revised 9 November 2022; accepted 11 November 2022; accepted manuscript published online XXX; corrected proof published online XXX

Cite this article as: JID Innovations 2023;3:100174

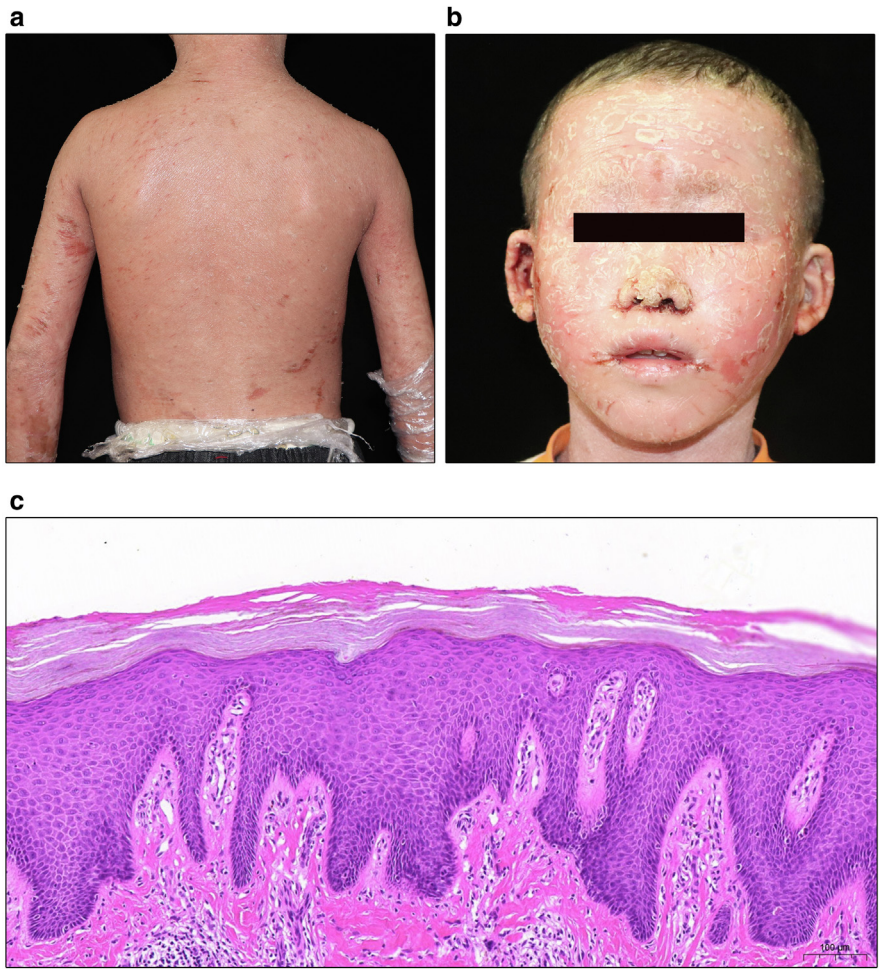

Figure 1. Clinical manifestation and treatment response of our patient. (**a, b**) Photograph of the patient. (**c**) Lesional skin biopsy of the patient showing orthohyperkeratosis, parakeratosis, spongiosis, and increased infiltration of lymphocytes and eosinophils. Bar = 100 μm. Written informed consent for the publication of the patient's images has been obtained from the patient's parents.

dermis, whereas the stratum granulosum is intact (Figure 1c). Staining for proliferation markers and immune cells indicated an increased expression of Ki-67, keratin 10, and loricrin in the epidermis and CD3, Ly6g, CD11c, and F4/80 in the dermis, which exhibited a psoriasis-like pattern (Figure 2).

Identification of one heterozygous variant in *CARD14*

After obtaining written informed consent from the parents and approval from the Clinical Research Ethics Committee of Peking University First Hospital (Beijing, China), we performed whole-exome sequencing of the peripheral blood sample from the patient. A heterozygous missense variant (c.353T>G, p.Leu118Arg) in *CARD14* was identified and confirmed by Sanger sequencing (Figure 3a). This variant was not detected in his parents and was absent from public databases. Leu118 was highly conserved across species.

Therapeutic response to secukinumab in our patient

On the basis of clinical features and the *CARD14* variant, the patient was diagnosed with CAPE. The patient was started with a subcutaneous injection of secukinumab at a dose of 150 mg (6 mg/kg) per week for 5 weeks, followed by monthly administration at 150 mg. A marked resolution was observed after 2 weeks of treatment, leaving mild scaling on the face and hyperkeratotic patches on the extremities (Figure 3b and c). Occasional relapses on the nape and extremities were reported. PASI90 response has been reached and sustained at a 40-week follow-up (Figure 4).

Luciferase reporter assay showed gain-of-function property of the variant

CARD14 is a scaffolding protein, which belongs to the CARMA protein family (Jiang and Lin, 2012). Variants such as Glu138Ala have been reported in patients with CAPE, which have been proven to enhance the activation of NF-kB signaling in keratinocytes in response to IL-17A stimulation (Wang et al., 2018). To confirm the downstream effect of the Leu118Arg variant in our patient, NF-kB–dependent luciferase reporter was coexpressed with CARD14 Leu118Arg and Glu138Ala mutants. The Leu118Arg variant showed a comparable constitutively activated NF-kB signal with Glu138Ala (Figure 5a), indicating a gain-of-function property.

The Leu118Arg variant induced skin inflammation through IL-23–IL-17 axis

It has been shown that in a *Card14*-mutated mouse model, enhanced signaling of NF-kB can induce a skin inflammatory circuit through the IL-23–IL-17 axis (Zhang et al., 2021). We next examined the cytokine expression profile in our patient. RNA was extracted from biopsy samples of lesional skin before treatment. qPCR was utilized to determine the

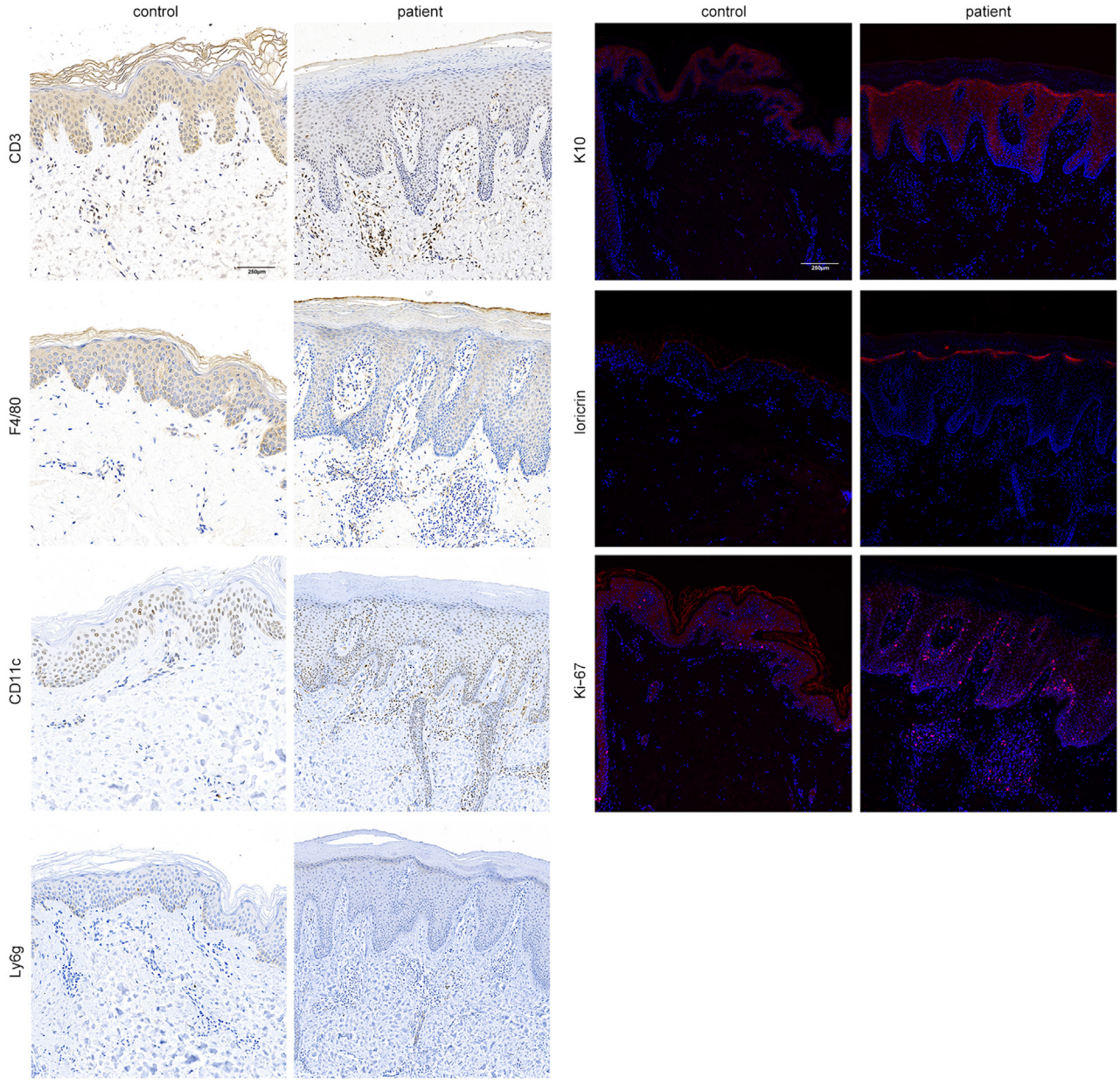

Figure 2. Immunohistochemical and immunofluorescent staining for proliferation markers and immune cells. Representative immunohistochemical sections of CD3, F4/80, CD11c, and Ly6G (left panel) and immunofluorescent sections of K10, loricrin, and Ki-67 (right panel) in the skin of healthy control and our patient. Bars = 250 μm. K10, keratin 10.

expression level of proinflammatory cytokines and IL-17A–targeted genes. Comparable with that in a mouse model, the expression of IL-17A, IL-17F, IL-22, and IL-23A displayed a significant increase as well as other IL-17A–targeted genes, including *S100A8* and *S100A9,* and chemokines such as *CCL20* and *CXCL1* (Figure 6). Our results indicated that the CARD14 Leu118Arg variant in patients with CAPE induced skin inflammation through the IL-23–IL-17 axis.

The Leu118Arg variant can cause CARD14 activation through self-aggregation

The inactive CARD14 displayed a diffused pattern of distribution in the cytoplasm, whereas the activated form aggregated near the nuclear (Wang et al., 2018). The inactive state of CARD14 is kept through intramolecular interaction between coiled–coil domain and linker domain, which can be disrupted through variants such as Glu138Ala in coiled–coil domain, leading to CARD14 self-aggregation and NF-kB activation (Wang et al., 2018). To test whether the variant in our patient can cause CARD14 self-aggregation and autoactivation, we transfected wild-type and mutants of CARD14, including Glu138Ala and Leu118Arg, into 293T cells. We observed that the Leu118Arg and Glu138Ala mutants displayed similar patterns of perinuclear aggregation without stimulation (Figure 5b) compared with the diffused pattern in the wild-type group, which suggest that the Leu118Arg variant can cause CARD14 activation through self-aggregation.

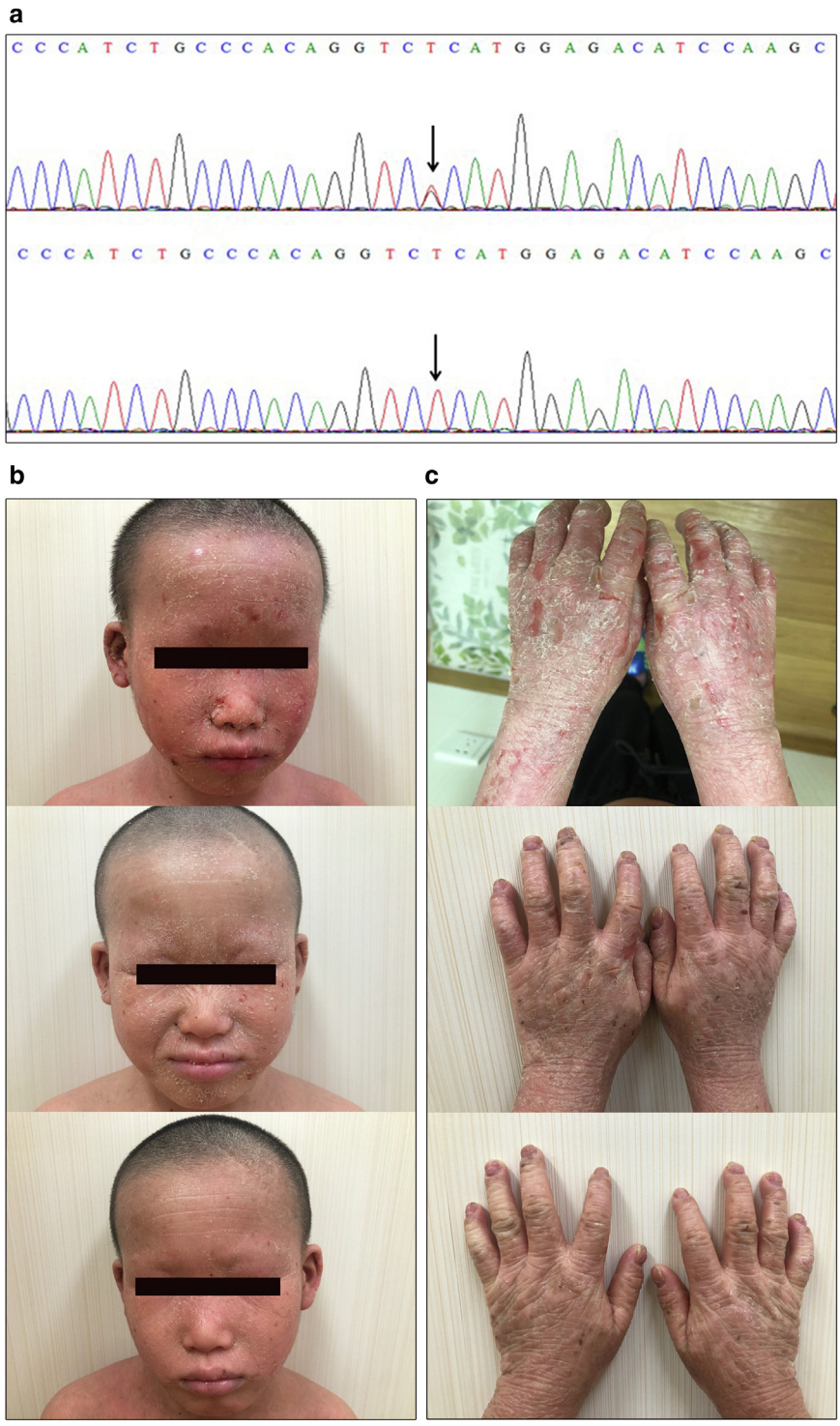

Figure 3. Variant identification and therapeutic response of the patient during initiation of secukinumab. (**a**) Sanger screening of the patient (upper panel) indicated heterozygous missense variant (c.353T>G, p.Leu118Arg) in *CARD14*, compared with that in the control (lower panel). (**b, c**) Photographs of skin lesions on (**b**) face and (**c**) dorsum of hands from our patient before (upper panel) and after (middle panel) the first and second (lower panel) injection of secukinumab. Written informed consent for the publication of the patient's images has been obtained from the patient's parents.

DISCUSSION

Combined with data from a mouse model and qPCR results of human samples, the skin and systemic inflammation in CAPE are mediated through the IL-23–IL-17 axis, similar to that of psoriasis vulgaris (Nestle et al., 2009). However, the inflammatory circuit of CAPE is driven by the intrinsic activation of CARD14 in keratinocytes, making it recalcitrant in response to conventional psoriasis medication (Zhang et al., 2021).

Both psoriasis vulgaris and pityriasis rubra pilaris have been reported to be associated with *CARD14* variants (Takeichi et al., 2017). CARD14 lies in the center of the activation of CARD–BCL10–MALT1 complex in keratinocytes. One important output of CARD–BCL10–MALT1 signalosome is the activation of the proteolytic activity of MALT1. Substrates of MALT1 mainly include proteins associated with inhibition of the NF-κB signaling pathway, such as A20 and CYLD (Ruland and Hartjes, 2019). The self-aggregation of the mutated

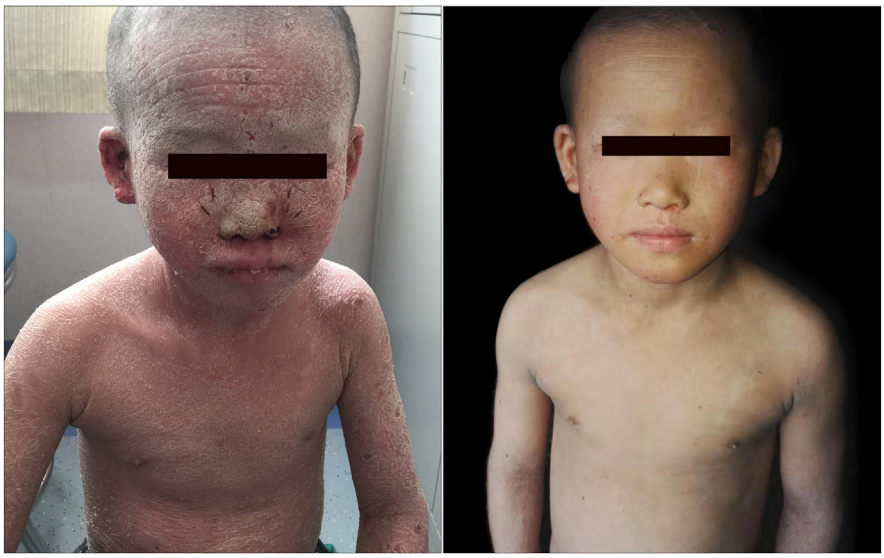

Figure 4. Clinical outcome of the patient. After a 40-week follow-up (right panel), the patient has achieved a PASI90 response, with occasional relapses on the nape and extremities, compared with that before treatment (left panel). Written informed consent for the publication of the patient's images has been obtained from the patient's parents.

CARD14 could enhance the cleavage of A20 and CYLD, thus reinforcing the NF-κB signaling. In addition to A20 and CYLD, there might be other substrates of MALT1 that can be associated with the varied phenotypes of patients with *CARD14* variants, which merits further exploration.

Biologic agent targeting IL-23 has been successful in treating both psoriasis and CAPE by blocking the crucial step of IL-23–IL-17 axis (Craiglow et al., 2018; Nieto-Benito et al., 2020; Zhang et al., 2021). On the basis of the pathogenesis of CAPE, a beneficial response to IL-17A inhibitor can be expected (Craiglow et al., 2018; Zhang et al., 2021). Thus far, only two cases harboring *CARD14* Leu156Pro variant were reported to be treated with ixekizumab, with a partial and near complete response, respectively (Klein et al., 2022; Manils et al., 2020; Zhang et al., 2021). Our case suggested anti–IL-17A treatment to be effective in CAPE. However, because of the intrinsic activation of mutated CARD14, higher or more frequent dosing might be required to achieve satisfactory improvement. The dosing of previously treated patients was not described in detail. Different dosing might lead to inconsistent treatment responses in patients receiving anti–IL-17A agents. Optimal dosing of IL-17A inhibitors in treating CAPE warrants further investigations, especially in pediatric patients.

In conclusion, we reported one case of CAPE with a missense variant in *CARD14* and provided further evidence that *CARD14* variants could cause NF-kB activation and subsequent inflammation through IL-23–IL17 axis, thus leading to psoriasis-like phenotype in humans. For patients with CAPE, secukinumab can be considered a therapeutic option.

MATERIALS AND METHODS

Patient and treatment

The Clinical Research Ethics Committee of Peking University First Hospital approved this study. Written informed consent was obtained from the patient's parents. A washout period of 4 weeks for previous treatment was conducted. The study subject received secukinumab (150 mg equals 6 mg/kg, subcutaneously) at weeks 0, 1, 2, 3, and 4 and then every 4 weeks thereafter until week 40.

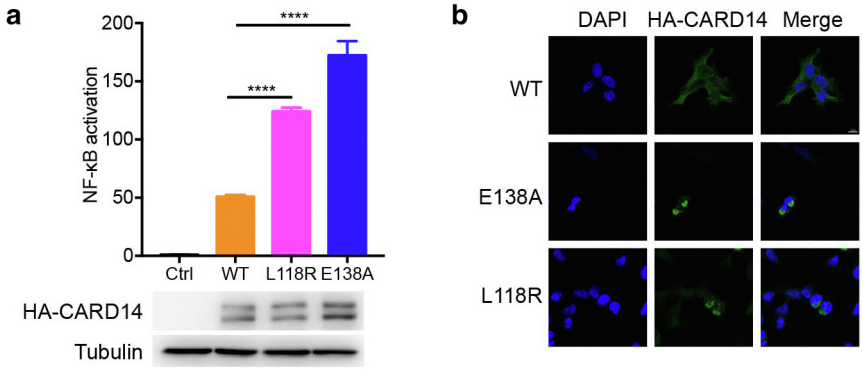

Figure 5. The *CARD14* Leu118Arg variant cause NF-κB activation through self-aggregation. (**a**) The 293T cells were transfected with NF-kB luciferase reporter plasmid and indicated HA-tagged CARMA2 wild type or variants and analyzed at 12 hours. (**b**) Representative confocal micrographs of 293T cells transiently transfected with indicated *CARMA2* variants stained for HA (CARMA2, green) and DAPI (nuclei, blue). Bars = 10 μm. (**a**) Data are representative of three repeated experiments. Data are presented as mean ± SEM. *P*-values were determined by two-tailed Student's *t*-test. ****$P < 0.001$. Ctrl, control; HA, hemagglutinin; WT, wild type.

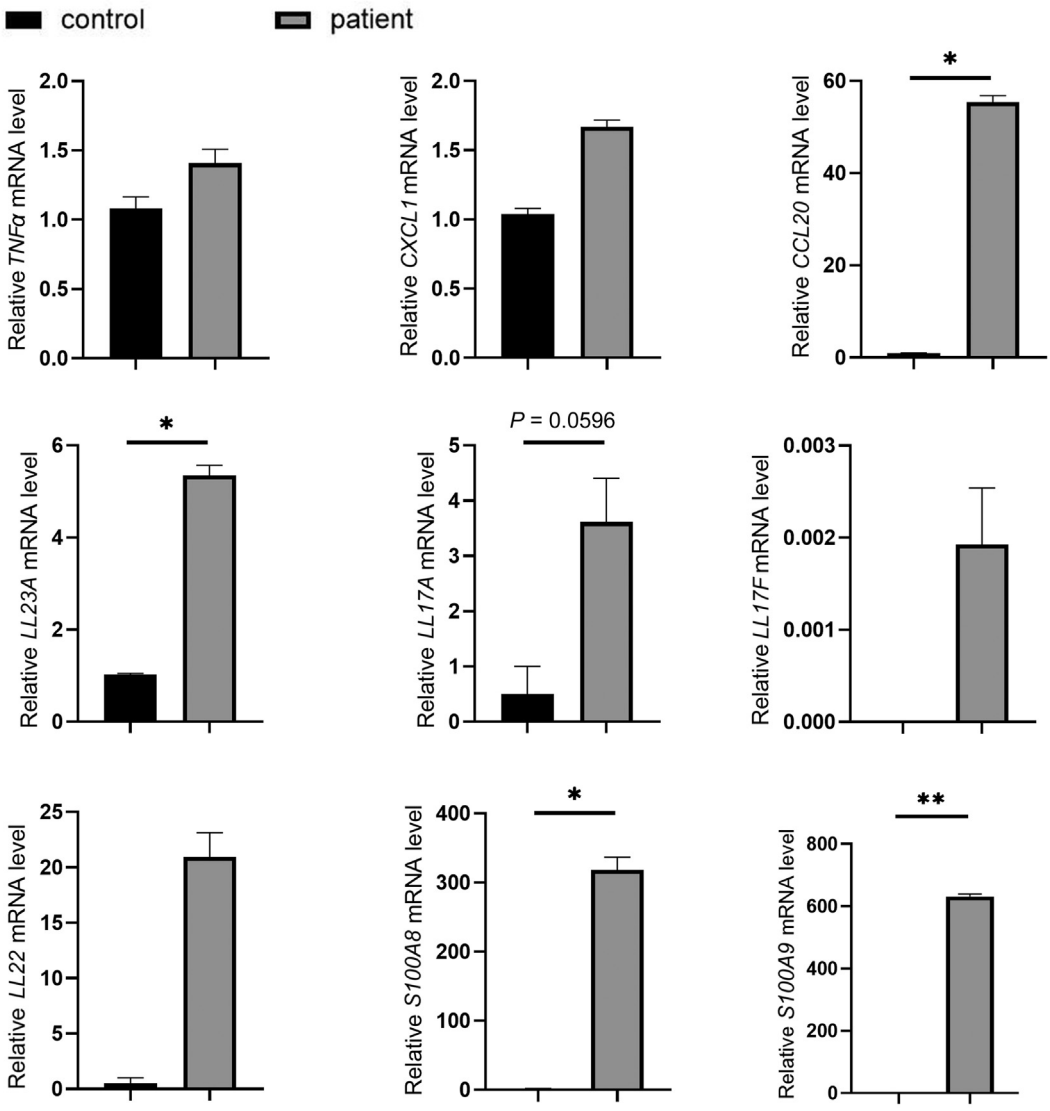

Figure 6. The Leu118Arg variant induced skin inflammation through IL-23–IL-17 axis. qPCR analysis of mRNA encoding *IL23*/*IL17A* axis cytokines among total mRNA in the skin of healthy control (matched by age and sex) and our patient. Results (calculated by the $2^{-\delta\delta Ct}$ method) are normalized to internal control gene *GAPDH*. Data are representative of two repeated experiments (repeat of the same tissue). Data are presented as mean ± SEM. *P*-values were determined by two-tailed Student's *t*-test. $^{*}P < 0.05$ and $^{**}P < 0.01$.

Pretreatment skin punch biopsy was obtained from lesions on the forearm. PASI assessments were performed at weeks 0, 4, and 40. Age- and sex-matched normal control skin sample was obtained from surgical waste.

Histopathology, immunohistochemistry, and immunofluorescence

For histopathology analysis, the skin biopsy sample was fixed with 4% formaldehyde overnight, followed by dehydration, and embedded in paraffin. The 5-μm tissue sections were stained with H&E according to standard procedures. Stained sections were scanned using an Olympus microscope (IX73, Olympus, Tokyo, Japan). For immunohistochemistry and immunofluorescence, 4% formaldehyde-fixed paraffin-embedded skin sections were deparaffinized, and antibodies were retrieved with sodium citrate and then stained with Ki-67 (Abcam, Cambridge, United Kingdom), keratin 10 (Abcam), loricrin (Abcam), CD45 (Ceville, Wuhan, China), CD11c (Ceville), F4/80 (Ceville), and Ly6G (Ceville) according to standard procedures. The images were captured with an Olympus microscope (IX73).

RNA isolation and quantitative RT-PCR

Snap-frozen skin tissues were ground in liquid nitrogen and dissolved directly in TRIzol (Invitrogen, Carlsbad, CA). Total RNA was extracted, and reverse transcription was performed using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. An ABI 7500 Real-Time PCR system (Applied Biosystems, Waltham, MA) and Power SYBR Green PCR Master Mix (Genestar, Waltham, MA) were used for qPCR. Results were normalized to *GAPDH*, and quantification was carried out using the 2-DDCt method. Melting curves were confirmed to ensure the amplification of a single product.

Luciferase reporter assays

Luciferase assays were performed as described previously (Afonina et al., 2016). Briefly, Human embryonic kidney 293T cells were cotransfected with 200 ng of different *CARMA2* variants and 100 ng

of the luciferase (firefly) reporter plasmid pGL3 containing NF-kB promoter together with 0.5 ng of EF1a-promoter–dependent Renilla luciferase reporter into a 24-well plate. After 24-hour transfection, cells were collected and lysed. Firefly and Renilla luciferase activities were measured with Dual-Luciferase Reporter System (Promega, Madison, WI), and Renilla luciferase activity was used to normalize for transfection efficiency.

Immunocytofluorescence staining

Cells were fixed with 4% paraformaldehyde, permeabilized with 0.25% Triton X-100, and blocked with 10% horse serum. Antibodies to hemagglutinin (Cell Signaling Technology, Danvers, MA or Abmart, Shanghai, China) and flag (Abmart) were used as primary antibodies. Alexa Fluor 488–conjugated donkey antimouse IgG (Thermo Fisher Scientific, Eugene, OR) and Alexa Fluor 546–conjugated goat anti-Rabbit IgG (Thermo Fisher Scientific) were used as secondary antibodies. DAPI (Beyotime Biotech, Suzhou, China) was used as a counterstain to label cell nuclei. Fluorescence was detected using Nikon A1.

Statistical analysis

All the statistical analysis was performed using Prism 6 (GraphPad Software, San Diego, CA). Statistical significance was evaluated by Student's *t*-test. Asterisk coding is indicated in figure legends as $*P < 0.05$, $**P < 0.01$, $***P < 0.005$, and $****P < 0.001$. Statistical parameters, including the number of biological replicates and repeat experiments, data dispersion, and precision measures (mean and SEM), are reported in figure legends.

Data availability statement

No large datasets were generated or analyzed during this study. Minimal datasets necessary to interpret and/or replicate data in this paper are available upon request to the corresponding author.

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AUTHOR CONTRIBUTIONS

Conceptualization: ZL, XL; Formal Analysis: SD, SZ, CW; Funding Acquisition: ZL, XL; Investigation: SD, SD, CW; Resources: ZL, XL; Supervision: ZL, XL; Writing - Original Draft Preparation: SD, SZ; Writing - Review and Editing: ZL, XL

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This study was funded by the National Natural Science Foundation of China (grant numbers 81872515, 82073423, and 82003327). We thank the support from the Post-doctoral Foundation of Tsinghua-Peking Joint Center for Life Sciences, Beijing, China.

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