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LETTER TO EDITOR

Autosomal Recessive Inflammatory Skin Disease Caused by a Novel Biallelic Loss-of-Function Variant in CARD11

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Abbreviations

BENTA	B cell expansion with NF-κB and T cell anergy
CADINS	CARD11-associated atopy with dominant interference of NF-κB signaling
CARD11	Caspase recruitment domain family member 11
CBM	CARD11-BCL10-MALT1
CID	Combined immunodeficiency
FTT	Failure to thrive
GFP	Green fluorescent protein
HSCT	Hematopoietic stem cell transplantation
IVIG	Intravenous immunoglobulin
LOF	Loss-of-function
MFI	Mean fluorescence intensity
NF-κB	Nuclear factor kappa B
OS	Omenn syndrome
PBMC	Peripheral blood mononuclear cell
PMA	Phorbol-12 myristate 13-acetate
WBC	White blood cell
WES	Whole exome sequencing

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To the Editor

Caspase recruitment domain family member 11 (CARD11) is a critical scaffold protein that together with B cell CLL/lymphoma 10 and MALT1 paracaspase forms the CBM complex, which links lymphocyte antigen receptor engagement with the activation of nuclear factor kappa B (NF-κB), c-Jun N-terminal kinase, and mechanistic target of rapamycin complex 1 [1]. Here, we describe the workup of an unusual patient with erythroderma, lymphadenopathy, and a polyclonal T cell receptor (TCR) repertoire who was discovered to carry a novel germline homozygous loss-of-function (LOF) variant in *CARD11* (p.Trp1125Ser). Human CARD11 deficiency is usually associated with homozygous premature stop variants and profound combined immunodeficiency (CID) [1]. To our knowledge, this is the first identified case of a germline homozygous missense LOF *CARD11* variant, manifesting in an autosomal recessive inflammatory/atopic skin disease without CID.

A 4-month-old Hispanic boy born to healthy first cousin parents presented to the emergency department with fevers, diffuse lymphadenopathy, as well as extensive exfoliative erythroderma not responsive to topical steroids (Fig. 1A, B). The patient had a normal newborn screen (TREC 376 copies/microliter) and family history was otherwise unremarkable, including a healthy older brother. The patient's lymphadenopathy and dermatitis developed at 1.5 months of age and progressively worsened. Although initial presentation was reminiscent of Omenn syndrome (OS), there was no evidence of failure to thrive (FTT), chronic diarrhea, or frequent infections aside from one episode of *Staphylococcus aureus* skin superinfection responsive to cephalexin.

Laboratory investigations revealed marked T cell lymphocytosis with mild eosinophilia, hypogammaglobulinemia, and mildly elevated IgE (Table 1). Atopy screening was negative for antigen-specific IgE to egg, milk, or peanut, and his ImmunoCAP Rapid test for respiratory allergens was also negative. He had a higher proportion of memory T cells (69% $CD45RO^+$) compared to naïve T cells (30% $CD45RA^+$). B cell phenotyping revealed normal naïve and

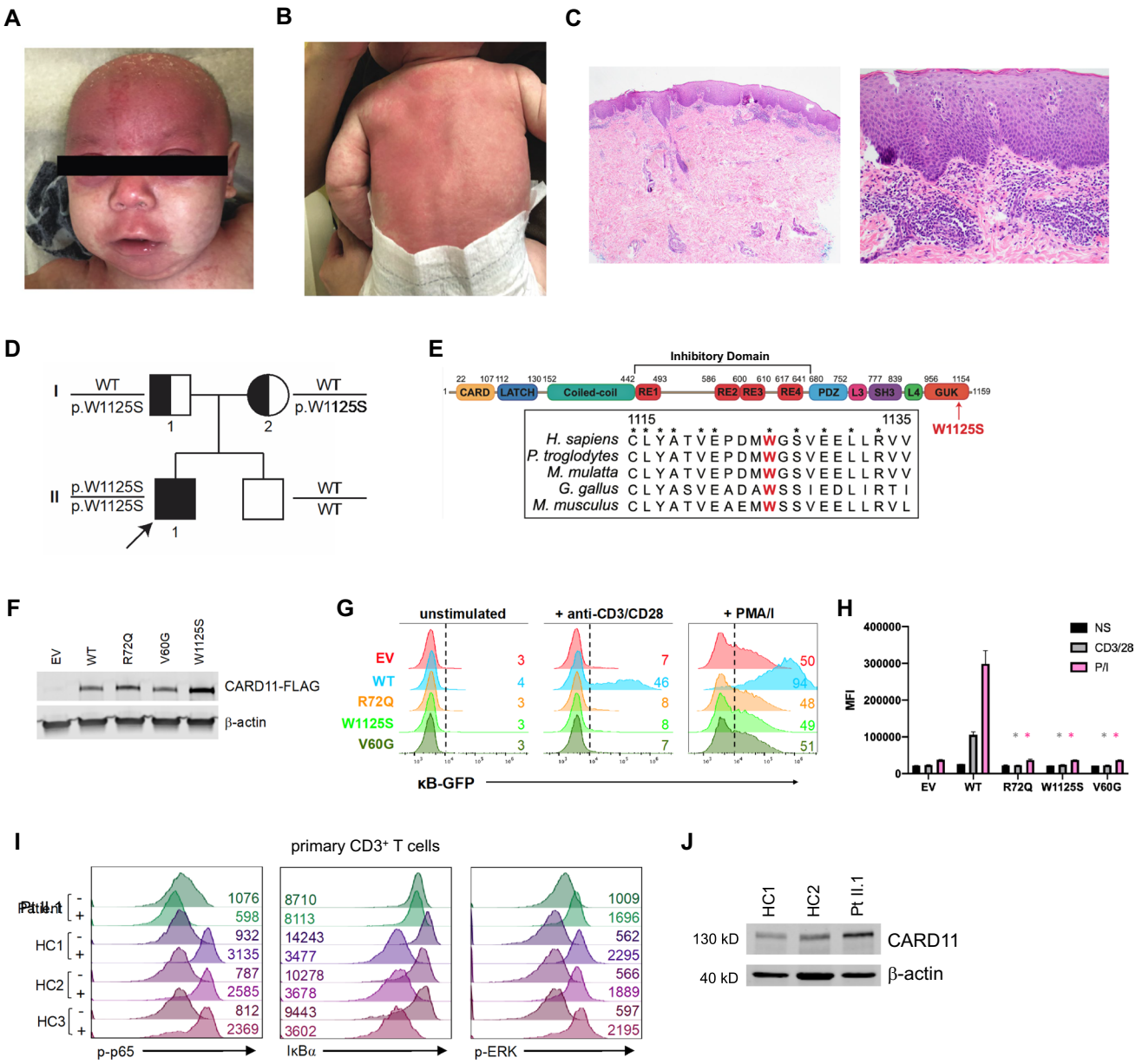

Fig. 1 Clinical phenotype of the patient. **A**, **B** Total body exfoliative erythroderma in the patient at age 3 months. **C** Hematoxylin and eosin immunohistochemical staining of the patient's skin biopsy, showing spongiotic dermatitis with mild superficial lymphocytic inflammation in the dermis (left image: 4×, right image: 20×). **D** Family pedigree of the patient. Squares = males, circles = females, half-filled shapes = heterozygous carriers, filled shapes = homozygous affected, black arrow = proband. **E** Schematic representation of the CARD11 protein and the location of the p.Trp1125Ser variant. The region was aligned to sequences of model organisms. Asterisks = conservation. **F**–**H** CARD11-deficient Jurkat T leukemia cells (JPM50.6) carrying a canonical NF-κB-GFP reporter were transfected with empty vector (EV), wild-type (WT), p.Arg72Gln, p.Val60Gly, or p.Trp1125Ser FLAG-tagged CARD11 constructs. **F** CARD11 expression in transfected cell lysates was detected by immunoblotting using an anti-FLAG antibody (clone M2); β-actin served as a loading control. **G**, **H** Relative NF-κB activation was assessed by stimulating transfected cells with anti-CD3/CD28 (1 µg/ml each) or PMA (20 ng/ml) + ionomycin (1 µg/ml) (PMA/I) for 24 h and measuring GFP expression by flow cytometry. ($N = 3$). Asterisks denote statistically significant differences from WT ($p < 0.01$). **I** PBMC from patient II.1 and 3 healthy controls were thawed, rested overnight, and stimulated for 20 min with PMA/I. Phospho-p65, IκBα, and phospho-ERK were quantified in gated $CD3^+$ T cells by intracellular flow cytometry ($N = 2$). **J** CARD11 expression in PBMC lysates was detected by immunoblotting using an anti-CARD11 antibody (clone 1D12), β-actin served as a loading control

Table 1 Key laboratory features for patient pre- and post-HSCT

Immunologic parameter	4 months of age	5 months of age	8 months of age^{++}
White blood cell count (cells/μL)	41 (5.5–18.5 × 10^3)**	61 (5.5–18.5 × 10^3)	83 (6–17.5 × 10^3)
WBC differential	67% lymphocytes, 26% neutrophils, 1% bands, 3.5% eosinophils	66% lymphocytes, 27% neutrophils, 2% eosinophils	77% lymphocytes, 15% neutrophils, 4% monocytes
$CD3^+$ T cells (cells/mm^3)	20,827 (3505–5009)	24,180 (5395–7211)	54,481 (3409–4575)
$CD4^+$ T cells (cells/mm^3)	16,199 (2780–3908)	22,246 (3505–5009)	38,423 (2630–3499)
$CD8^+$ T cells (cells/mm^3)	4628 (351–2479)	6287 (351–2479)	17,778 (351–2479)
IgA (mg/dL)	< 6 (6–47)	< 6 (6–47)	< 6 (8–89)
IgG (mg/dL)	77 (166–547)	281 (166–547)$^+$	840 (156–829)$^+$
IgM (mg/dL)	36 (18–98)	13 (18–98)	13 (27–132)
IgE (mg/dL)	94 (< 13)		
$CD56^+$ NK cells (cells/μL)	694 (13–441)	242 (13–441)	1147 (13–441)
$CD19^+$ B cells (cells/μL)	694 (14–816)	967 (14–816)	1147 (14–816)
$CD20^+$ B cells (cells/μL)	787 (66–529)	958 (66–529)	1090 (66–529)
Non-Switched Memory B cells (%) ($CD19^+CD27^+IgD^+IgM^+$)		9% (3–7%)	
Class-Switched Memory B cells (%) ($CD19^+CD27^+IgD^-IgM^-$)		< 1% (2–7%)	
Absolute CD45RA (cells/μL)		8222 (800–5900)	
Absolute CD45RO (cells/μL)		19,083 (100–950)	

*WBC and T/B/NK cell subsets were performed on different blood samples

**Numbers in parentheses represent reference ranges

$^+$Drawn on IVIG supplementation

$^{++}$After HSCT

total memory B cell numbers but impaired B cell class switching. Mitogen proliferation to phytohemagglutinin, concanavalin A, pokeweed mitogen, and anti-CD3/CD28 were comparable to controls—a highly atypical phenotype for OS. There was no evidence of maternal engraftment.

Epstein-Barr virus and cytomegalovirus viral loads were undetectable. Computed tomography scanning confirmed diffuse lymphadenopathy involving the head, neck, and inguinal regions with mild hepatomegaly. Bone marrow biopsy was negative for malignancy. Skin biopsy demonstrated chronic spongiotic dermatitis with parakeratosis and moderately dense superficial perivascular and interstitial predominantly lymphocytic infiltrate (98% T cells with CD4:CD8 ratio of 3.26:1) with a few neutrophils and eosinophils (Fig. 1C). There was no evidence of vasculitis, infection, or malignancy in the skin. On admission, he was found to have *Enterococcus faecium* bacteremia and was treated with antibiotics along with 1 dose of intravenous immunoglobulin (IVIG).

Due to high suspicion of an immune defect, he was subsequently started on prophylactic trimethoprim-sulfamethoxazole, acyclovir and fluconazole, as well as 1 mg/kg/day of oral prednisolone. While on prednisolone, he experienced improvement in his skin to near normal and a significant reduction in his lymphadenopathy, though leukocytosis persisted (white blood cell [WBC] ~ 60,000). He later developed fever, rhinitis, and fatigue and was found to be infected with Influenzae B (treated 10 days with oseltamivir) and at 8 months his WBC rose to 83,000 despite daily prednisolone. He continued to suffer from rhinitis symptoms and a respiratory panel demonstrated influenza clearance, though he was unable to clear a new coronavirus infection (non-SARS-CoV-2).

Trio whole exome sequencing identified a homozygous variant of uncertain clinical significance affecting an evolutionarily conserved codon in *CARD11* (c.3374G > C;p.Trp1125Ser), which was heterozygous in both parents (Fig. 1D, E). This variant is absent from population databases and was predicted to be pathogenic by a variety of in silico pathogenicity prediction tools, including CADD (score = 32), MutationTaster (1, disease_causing), SIFT (0.0, damaging), PROVEAN (8.53, damaging), M-CAP (0.056, damaging), and Polyphen-2 (1.0, probably_damaging).

The patient underwent a hematopoietic stem cell transplant (HSCT) from his brother who was an 8/10 HLA match and not a carrier of the p.Trp1125Ser (W1125S) *CARD11* variant. Prior to transplant, he underwent lymphodepletion with alemtuzumab and was then conditioned with anti-thymocyte globulin, busulfan, thiotepa, fludarabine, and rituximab. The patient is now 8 months status-post-HSCT and has had excellent engraftment results. He has not had any issues with infections, FTT, chronic diarrhea, nor lymphadenopathy since

HSCT. His skin is also much improved with only mild transient dermatitis that is responsive to topical therapies. He was able to wean off of IVIG 120 days post-transplant.

To assess whether the W1125S CARD11 variant affected CARD11 protein function, we transfected a W1125S variant CARD11 plasmid into CARD11-deficient JPM50.6 Jurkat T leukemia cells and compared it to wild-type (WT) and other recently described LOF variants [2–4] after stimulation with both anti-CD3/CD28 and PMA + ionomycin (P/I). Similar to previously described missense LOF variants, the W1125S variant did not affect CARD11 protein expression but failed to support NF-κB activation after stimulation (Fig. 1F–H).

In order to investigate whether patient cells had impaired NF-κB activation, we stimulated peripheral blood mononuclear cells (PBMC) with PMA/ionomycin and measured the phosphorylation of p65, degradation of IκBα and phosphorylation of ERK. While ERK activation was intact, NF-κB pathway induction was completely abrogated in patient T cells versus healthy controls despite comparable CARD11 protein expression (Fig. 1I, J), consistent with a homozygous missense LOF variant. Collectively, these data indicate that the W1125S CARD11 variant is pathogenic as it leads to severely impaired NF-κB activation.

Interestingly, there is a single report that previously linked a germline *CARD11* variant to OS [5]. This patient possessed a p.Cys150* CARD11 variant, which was unable to support NF-κB signaling nor IL-2 production. Further studies revealed a somatic second site p.Cys150Leu variant present in ~ 50% of marrow cells, which rescued CARD11 protein expression and NF-κB signaling. It was thought that the partial reconstitution of CARD11 function conferred a survival advantage to revertant T cells, which allowed them to become activated and proliferate in response to chronic viral infections. These virus-specific T cell clones, unchecked by regulatory T cells, were then able to infiltrate organs and cause an OS phenotype. To investigate whether a similar mechanism was in play in our patient, we conducted further whole genome sequencing (WGS) and deep RNA sequencing on patient T cells. We found no evidence of somatic reversion, with the p.Trp1125Ser variant detected at 100% allele frequency (Supplemental Fig. 1). Furthermore, clinical genome annotation analysis revealed no other variants of potential clinical significance (Supplemental Table 1), highlighting p.Trp1125Ser as the likely sole pathogenic mutation. Finally, TCR Vβ repertoire analyses revealed no evidence of oligoclonality in the patient's CD4 and CD8 T cells (Supplemental Fig. 2), which is inconsistent with classic OS. Since cell/biopsy samples collected prior to HSCT were limited, we were unable to do further work to investigate whether tissue T cell oligoclonality was present. It also remains puzzling why mitogen proliferation was largely intact in our initial tests, given the profound NF-κB defect we noted (Fig. 1I).

Here, we report a novel homozygous LOF variant in *CARD11* (p.Trp1125Ser) associated with an autosomal recessive inborn error of immunity featuring dermatitis and lymphadenopathy without severe, recurrent, or opportunistic infections. Germline *CARD11* variants are associated with 3 major phenotypes depending on the nature of the mutations present: profound CID, B cell expansion with NF-κB, and T cell anergy (BENTA) and CARD11-associated atopy with dominant interference of NF-κB signaling (CADINS) [1]. Our patient's phenotype, albeit only observable for less than a year, was perhaps more severe than CADINS, though lacked evidence for CID. Nevertheless, this case provides confirmation that *CARD11* variants should be considered in patients who initially present with early-onset severe exfoliative erythroderma, diffuse lymphadenopathy, and lymphocytosis regardless of other comorbidities. Importantly, disease pathogenesis appears distinct from OS, CADINS, or pure CARD11 deficiency—we noted no evidence of circulating T cell oligoclonality, antigen-specific atopy, or cutaneous viral or opportunistic respiratory infections. Nevertheless, functional lymphocyte testing should be pursued whenever possible for similar patients with a homozygous CARD11 VUS. If this reveals impaired NF-κB activation, patients should be started on *Pneumocystis jirovecii* pneumonia prophylaxis and immunoglobulin replacement therapy. Our case establishes a distinct, autosomal recessive primary immune regulatory disorder (PIRD) caused by homozygous CARD11 LOF mutations without features of CID, further expanding the phenotypic variety of "CARD11-opathies".

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10875-023-01440-8.

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Data Availability All data and material will be made freely accessible upon request.

Code Availability Not applicable.

Declarations

Ethics Approval Research study protocols were approved by institutional review and ethics boards and performed in accordance with the ethical standards of the 1964 Declaration of Helsinki.

Consent to Participate The patient/their parents and healthy donors provided written informed consent to participate in the study.

Consent for Publication All study participants have consented to having their data published.

Conflict of Interest The authors declare no conflict of interest.

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