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LETTER TO EDITOR

A Novel *CARMIL2* Mutation Resulting in Combined Immunodeficiency Manifesting with Dermatitis, Fungal, and Viral Skin Infections As Well as Selective Antibody Deficiency

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So far, more than 350 monogenic inborn errors of immunity have been reported, resulting in a wide range of clinical conditions, including increased susceptibility to severe infections, autoimmunity, and malignancy [1, 2].

In order to identify the genetic basis of 100 patients diagnosed with common variable immunodeficiency (CVID) or combined immunodeficiency (CID), we used HaloPlex enrichment technology, a targeted, next generation sequencing (NGS)-based method. Targeted NGS enables a high throughput, low cost, and quick turnaround time due to the limited number of sequenced genes. Our HaloPlex panel included a selected number of PID-related genes, including genes involved in immune responses to viral infections (Table S1) [3]. Here we identified a novel germline homozygous splice-acceptor site mutation in the invariant AG sequence of the intron 6 of the *CARMIL2* gene g.1587G>A [c.795-1 G>A] in three CID patients born to consanguineous parents, which segregated with their phenotype (Fig. 1a–c). Patients' parents and siblings were found heterozygous carriers by Sanger sequencing. This nucleotide substitution was predicted to be disease causing by Mutation Taster. Sequencing of the *CARMIL2* cDNA, produced by reverse transcription of total RNA from patients' PBMCs, revealed skipping of the second nucleotide in exon 7 (Fig. 1d, e), which causes a reading frame shift with a subsequent premature stop codon (M227*). CARMIL2 expression was absent in PHA-blasts, PBMCs, and naïve $CD4^+$ T cells of all three patients but not of wild type controls and heterozygous mutation carriers (Fig. 1f, g). Similar to previously reported CARMIL2-deficient patients, all here presented patients suffered from viral skin infections such as molluscum contagiosum and verruca vulgaris as well as eczematous dermatitis (Fig. 1a). One patient (P. II) suffered from recurrent respiratory tract infections since early childhood, which associated with selective antibody deficiency with normal immunoglobulins (SADNI). This patient displayed inadequate responses to vaccinations against diphtheria, tetanus, and pneumococcal infections (Table S2) and was therefore treated with immunoglobulin replacement therapy. Clinical and immunological characteristics of all here presented patients are described in detail in the Supplementary Notes and summarized in Table S2. Regarding the previously reported 21 CARMIL2-deficient patients, 9 patients presented hypogammaglobulinemia or SADNI, and 2 of them benefited from immunoglobulin replacement [3–6]. Clinical heterogeneity among patients with CARMIL2 deficiency, and especially among those harboring the same deleterious mutation, such as the coexistence of antibody deficiency in some patients or marked differences in age of disease onset, suggests the pathogenic relevance of additional genetic and/or epigenetic modifying factors.

Overall, our patients present similar immunologic features to the previously described patients (Table S2) [3–6]. Similar

Faranaz Atschekzei and Roland Jacobs contributed equally to this work.

Electronic supplementary material, please consider that we corrected the reference range of recent thymic emigrant (RTE) T helper cells (% T cells) from (64-42) to (6.4-42) for P.I in S2 table. See attached file S2. The online version of this article (https://doi.org/10.1007/s10875-019-00628-1) contains supplementary material, which is available to authorized users.

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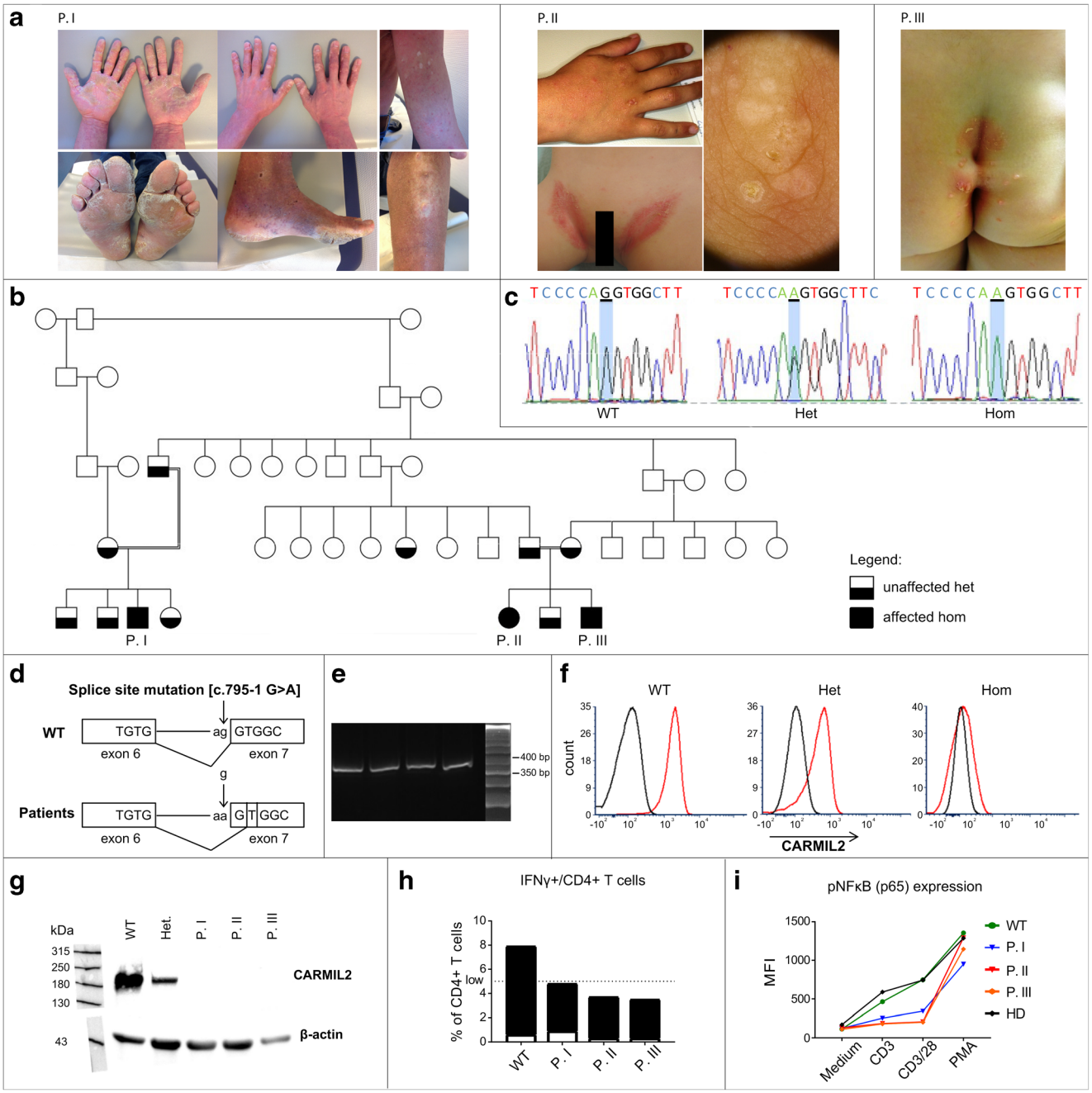

Fig. 1 **a** Viral skin infections and eczematous dermatitis in patients with CARMIL2 deficiency. P.I: index case P. I with disseminated warts (verruca vulgaris) on the hands/feet and molluscum contagiosum on the arm and leg. P.II: multiple verrucous papules on the dorsal hand of patient P.II at the age of 7 years; recurrent severe cutaneous candida infection of her inguinal region; dermoscopic image of hyperkeratotic papules (age of 4 years). P.III: perianal/gluteal mollusca contagiosa (age of 3 years). **b** Family pedigree indicating the segregation of the variant g.1587G>A (c.795-1 G>A) from the parents (heterozygous) and siblings (heterozygous) to the affected patients (homozygous). **c** Electropherograms of wild type (WT), heterozygous state (Het), and homozygous state (Hom) for the variant g.1587G>A. **d** Schematic representation of splicing event and skipping of the second nucleotide in exon 7. **e** CARMIL2 cDNA amplicon spanning exon 5 to 9 separated in 2% agarose gel electrophoresis. **f** Representative histograms of CARMIL2 expression (red) and isotype controls (black) in a normal individual (WT) and individuals with heterozygous (Het) and homozygous (Hom) mutations, respectively, are shown. **g** CARMIL2 immunoblot of WT, patients (P.I, P.II, P.III), heterozygous member of family and β-actin loading control. **h** IFN-γ production was intracellularly determined by flow cytometry gating on $CD4^+$ T cells after overnight incubation with (black bars) or without (white bars in front) PMA/ionomycin. **i** PBMC of the patients (P.I, II, and III), a healthy donor (HD), and a non-affected relative of the patients (wildtype, WT) were activated with CD3, CD3 in combination with CD28, and PMA/ionomycin or cultured in media as negative control for 15 min. Cells were then intracellularly stained for phosphorylated NFκB p65 and analyzed by flow cytometry

to Sorte et al. [3], we found alterations of the $CD4^+$ T cell compartment in all three patients. Absolute numbers of $CD4^+$ T cells and the proportion of $CD4^+$ T naïve T cells were increased, while percentages of $CD4^+$ T memory and $CD4^+$ T follicular-like T cells were reduced. We also found increased proportions of naïve B cells and decreased class-switched B cells and ascertained that $CD8^+$ T cell subsets were not affected. Furthermore, the phosphorylation of NFκB subunit p65 in $CD4^+$ T cells was abolished indicating the contribution of CARMIL2 in activation of NF-κB signaling (Fig. 1i).

Treg and Th17 cell counts were reduced in P.II and P.III, as previously described for other CARMIL2 deficient patients [3], but not in P.I. Furthermore, in line with the study published by Sorte et al. [3], we identified decreased percentages of IFN-γ producing NK cells in patients P.II and P.III. $CD4^+$ T and $CD8^+$ T cells from these two patients displayed reduced IFN-γ production as well (Fig. 1h). P. I however, presented normal IFN-γ production by both NK and T cells.

CARMIL2 protein is required for coupling CD28 to CARMA-1 and PKC-θ, an important step in CD28-mediated co-stimulation [7] and NF-κB signaling, and is therefore indispensable for T cell activation. Sorte et al. and Wang et al. have shown that the role of CARMIL2 in CD28-mediated co-stimulation is important for $CD4^+$ T cell but not $CD8^+$ T cell activation [3, 6]. Here, we could show that patients' PBMCs exhibited normal proliferation in response to mitogens, anti-CD3 alone, and the combination of anti-CD3/28. However, employing enriched $CD4^+$ T cells in a proliferation assay revealed the inability of CD28 to synergize with CD3 stimulation in CARMIL2-deficient patients, which is in line with the above mentioned previous findings.

In conclusion, we report on three patients with a recently described combined immunodeficiency disorder, CARMIL2-deficiency, bearing a novel homozygous mutation on splice-acceptor site region on *CARMIL2*-gene. These cases underline the role of CARMIL2 in immunity and suggest that *CARMIL2* mutations should be considered in patients presenting disseminated and/or persistent warts or other virus-related skin conditions. Furthermore, differences in clinical and immunological phenotypes among our patients highlight the variable clinical presentations of CARMIL2-deficiency, which cannot be explained by an additional rare variant of another PID-related gene, included in current panel.

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Compliance with Ethical Standards

Informed consent was obtained from patients and patients' family members, respectively, according to ethical and legal guidelines (ethics vote number 5582).

Conflict of Interest The authors declare that they have no conflict of interest.

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