Clinical Immunology 208 (2019) 108228

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Letter to the Editor

Profound immunodeficiency with severe skin disease explained by concomitant novel *CARMIL2* and *PLEC1* loss-of-function mutations

ARTICLE INFO

Keywords:
Immunodeficiency
Epidermolysis bullosa
CARMIL2
Plectin
Whole exome sequencing
RLTPR

ABSTRACT

This study reports a patient with severe skin disease in the context of profound immunodeficiency explained by two concomitant genetic diseases caused by two novel homozygous loss-of-function mutations in *PLEC1* and *CARMIL2*. The work provides additional information on the clinical and immunological manifestations of CARMIL2 deficiency and highlights the particular diagnostic and therapeutic challenge represented by the concomitant presence of two rare monogenic disorders.

To the Editor,

CARMIL2 (Capping protein, Arp2/3 and myosin-I linker protein 2; or RLTPR) is a multidomain cytosolic protein, fundamental for cell migration and T cell signaling [1,2]. A primary immunodeficiency due to biallelic loss-of-function *CARMIL2* mutations has been recently reported in 30 patients [2–8] (Supplementary Table 1). The clinical presentation appears heterogeneous but mainly characterized by eczematous skin disease, recurrent respiratory infections and susceptibility to Epstein-Barr Virus (EBV)$^+$ smooth muscle tumors (SMT) (Supplementary Table 1). Interestingly, in the first two reports [2,3], the causing mutations were found by analyzing families with well-defined but different presentations, namely the unusual phenotype of EBV$^+$SMT [2] and mycobacterial and staphylococcal diseases in the context of pulmonary and cutaneous allergy [3]. However, immunological studies revealed a homogeneous picture with high naïve CD4 T cells, absent regulatory T cells (Treg) and low or absent T cell proliferation upon CD3/CD28 stimulation.

Here, we report the case of a patient with recurrent infections and persistent EBV viremia, harboring a novel loss-of-function *CARMIL2* mutation. The patient presented also a severe skin disease, which is not rare in profound combined immunodeficiencies. However, in this patient it was not caused by CARMIL2 deficiency but by a second recessive genetic disorder, epidermolysis bullosa (EB).

The patient is a 4-year-old boy born to consanguineous Syrian parents (Supplementary Fig. 1A) referred to our center at the age of 2. The boy developed respiratory insufficiency and suffered from an episode of cardiac arrest immediately after birth. He recovered and was discharged two months later. Since birth, he had severe blistering and partially itching, eczematous skin lesions and dystrophic nails, not suggestive for *Candida* infection (Fig. 1A). The skin lesions were associated with a chronic, generalized lymphadenopathy without hepatosplenomegaly. The patient presented also with non-infectious chronic diarrhea and failure to thrive. He suffered from recurrent respiratory infections and developed sepsis due to *Klebsiella* infection. Moreover, three second-degree cousins had presented with skin blistering at birth and an older brother had died at the age of 6 due to suspected immunodeficiency and an unclear abdominal tumor (Supplementary Fig. 1A). Because of severe early-onset infections, severe skin condition, lymphadenopathy and family history, an Omenn-like syndrome was considered as possible diagnosis. Further immunological evaluation revealed hypogammaglobulinemia and elevated leukocyte and lymphocyte counts, but normal T- and B-cell distribution, including naïve and memory subsets (Supplementary Table 2). Severe microcytic anemia was interpreted as a result of the gastrointestinal iron malabsorption. The patient had Cytomegalovirus and EBV viremia but no clinical signs of related infection (Supplementary Table 2). He was treated with intravenous IgG, leading to improvement of the eczema and recurrent infections.

Upon admission, immunofluorescence mapping from a skin biopsy showed alterations compatible with EB simplex with plectin deficiency (Fig. 1B). However, given the recurrent and persistent infections – including skin infections with fever, not typical for EB – as well as the family history, an additional immunodeficiency seemed likely. Therefore, whole exome sequencing (WES) of patient and parents was performed, resulting in two possibly damaging novel homozygous mutations. One of these, in *PLEC1* (c.7468C > T; p.Gln2490*), causing a premature stop codon (Supplementary Fig. 1B), genetically confirmed the EB diagnosis and explained the blistering skin condition. Plectin is a member of the plakin protein family and is crucial for hemidesmosomes formation and the interaction of intermediate filaments with the plasma membrane. Biallelic mutations in *PLEC1* have been associated with EB simplex with muscular dystrophy [9]. The muscle disease limits long-term prognosis, but may present at variable age, ranging from 0 to 35 years. Our patient presents no signs of muscular dystrophy so far.

The second novel homozygous mutation was found in *CARMIL2*

Abbreviations: CARMIL2, Capping protein, Arp2/3 and myosin-I linker protein 2; EB, epidermolysis bullosa; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IκB, inhibitor of kappa B; PHA, phytohemagglutinin; PKC-θ, Protein kinase C, theta; RLTPR, RGD Motif, Leucine Rich Repeats, Tropomodulin Domain And Proline-Rich Containing; SMT, Smooth muscle tumors; WES, Whole exome sequencing

https://doi.org/10.1016/j.clim.2019.06.004
Received 27 March 2019; Received in revised form 25 April 2019; Accepted 9 June 2019
Available online 10 June 2019

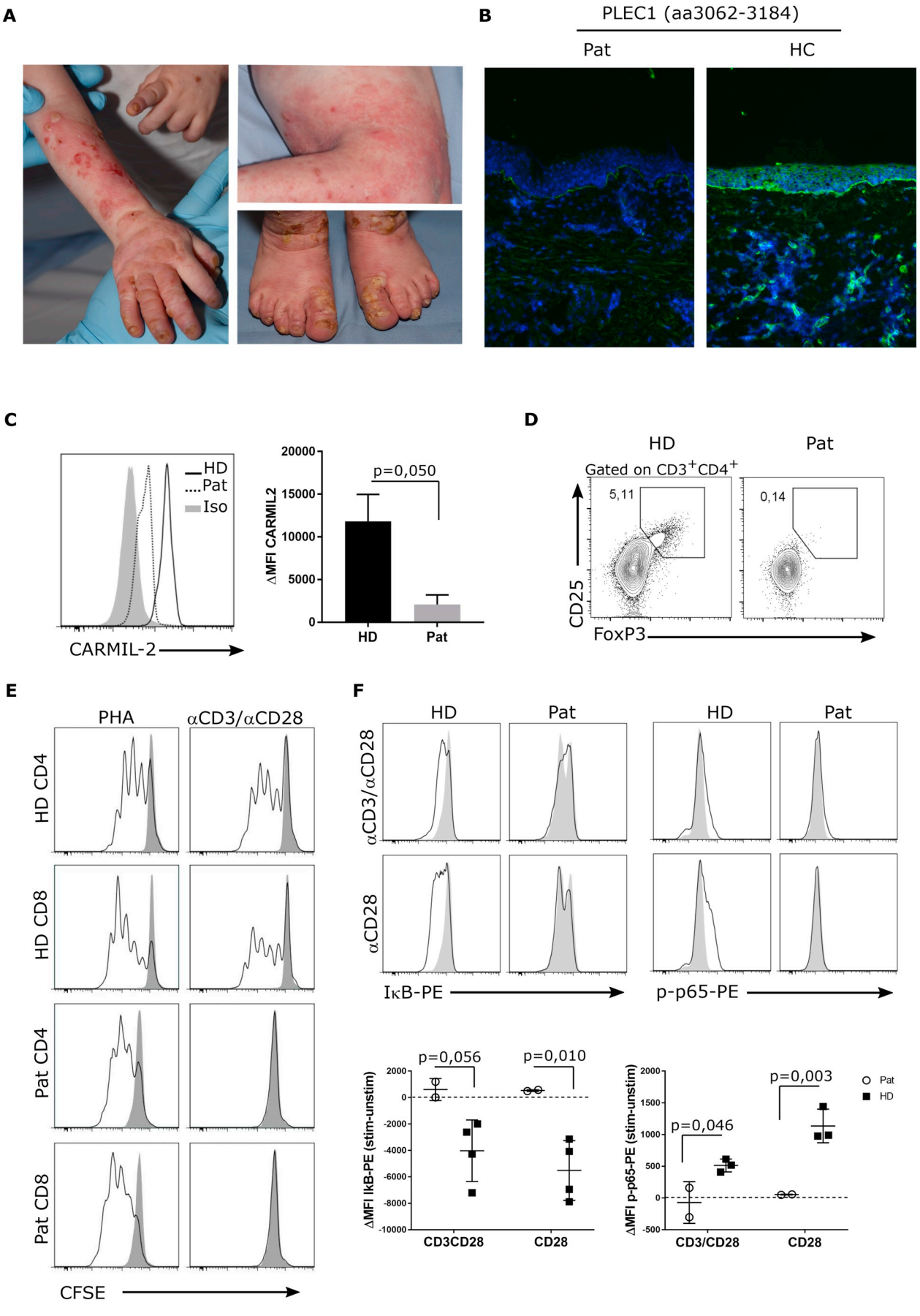

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Fig. 1. Immunodeficiency and epidermolysis bullosa explained by homozygous *PLEC1 and CARMIL2* mutations. A. Clinical features of the patient's skin lesions: right lower arm showing both eczematous patches and serous blisters (3 years and 4 months of age, left); eczematous lesions on lower back and abdomen, hip and upper leg (4 years and 2 months of age, upper right); nail dystrophy and hyperkeratotic plaques on both feet and multiple crusts and few serous blisters on the lower legs (4 years and 2 months of age, lower right). B. Immunofluorescence analysis of plectin on skin biopsy of the patient (Pat) and a healthy donor (HD). C. Representative histogram showing CARMIL2 protein expression (left) and ΔMFI CARMIL2 ($MFI_{CARMIL2}$-$MFI_{isotype}$)(right). Values represent mean ± SD from two independent experiments using PBMC from four different healthy donors and two different patient's samples. D. Representative contour plots showing %$CD25^+FOXP3^+$Treg from $CD3^+CD4^+$ T cells. E. Representative histograms showing proliferation of T cells 96 h after stimulation. F. Representative histograms (up) and ΔMFI (down) of IκB and phopsphorylated-p65 expression upon stimulation, the dotted line indicates ΔMFI = 0 (no change after stimulation compared to medium). Values represent mean ± SD from two independent experiments using cells from four healthy donors (filled squares) and two different patient's samples (open circles). Unpaired *t*-test was used in C; two-way ANOVA with Sidak multiple comparison test was used in F.

(c.1071 + 1G > T). This intronic mutation is located at an essential splice-site and leads to aberrant splicing (Supplementary Fig. 1C) with reduction of protein expression (Fig. 1C). The segregation analysis of the family was consistent with an autosomal recessive trait with complete penetrance, since the unaffected parents and siblings were heterozygous or wild-type. CARMIL2 is fundamental for the regulation of actin polymerization at the barbed end of actin filaments [10]. Moreover, it is essential for CD28 co-signaling and signal transduction to its effector protein PKC-θ [1]. *Rltpr*-mutant mice abrogate the connection of CD28 with PKC-θ, thus impairing antigen-induced CD4 and CD8 T cell activation and Treg development [1]. In line with previous reports, our patient presented markedly reduced Treg (defined as $CD4^+CD25^+FOXP3^+$) (Fig. 1D) and a reduced CD28-dependent T cell proliferation, while showing an adequate response to PHA (Fig. 1E). This impaired CD28 signaling was further confirmed by flow cytometry analysis of canonical NF-κB pathway in CD3 T cells, which showed reduced phosphorylation of p65 and impaired IκB degradation upon CD28 stimulation (Fig. 1F). However, unlike the majority of the previously published patients, a high fraction of naïve CD4 T cells was not observed (Supplementary Table 2). Thus, normal or low naïve T cells, especially in young individuals, should not be taken as a simple parameter to exclude CARMIL2 deficiency in the differential diagnosis.

In summary, we describe a clinical phenotype resulting from two independent genetic diseases caused by novel loss-of-function mutations in the *PLEC1* and *CARMIL2* genes, found by WES. Although severe skin disease can be part of various profound immunodeficiencies, additional independent genetic diseases have to be considered, in particular in families with high degree of consanguinity. Both mutations lead to severe clinical phenotypes and thus to management challenges. The reported risk of developing EBV^+SMT in *CARMIL2* deficient patients speaks for the evaluation of a hematopoietic stem cell transplantation as a possible cure for this immunodeficiency [11]. This treatment has also been performed in few patients with other forms of EB with mixed results [12] and some cases of severe drug-related cutaneous toxicity. In the particular case of EB simplex with plectin deficiency, the progressive muscle disease would not be affected by this therapy. For this reason, our patient is not currently listed for this treatment. However, during the last checkup, two suspicious lesions were found in the liver. They were completely resected and histological evaluation revealed EBV + SMT. Since resection was complete, no chemotherapy was given, and a close follow-up is planned. Furthermore, SMT tissue is being evaluated for upregulated pathways that could be targeted with a specific therapy, as an alternative to conventional chemotherapy.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2019.06.004.

Ethics approval

All subjects gave written informed consent in accordance with the Declaration of Helsinki. The immunological studies were performed according to the CCI Biomaterial Bank (IRB approval No. 282/11).

Funding

This work was supported by funds of the German Federal Ministry of Education and Research (BMBF 01EO1303) and the *E*-RARE Euro-CID program. MH and AR are supported by the Berta-Ottenstein-Programme for Clinician Scientists, Faculty of Medicine, University of Freiburg.

Author's contribution

MEM, CS, MH and SE are the treating physicians in the immunology department. AR, FC and CH are the treating physicians in the dermatology department. They provided clinical information and biological material. MEM was a major contributor in the design of the study, interpreted the data and wrote the manuscript. CNC designed, performed and analyzed experiments concerning CARMIL2 deficiency and wrote the manuscript. SE identified the patient, designed the study, discussed results and reviewed the paper. AR, FC and CH performed experiments and studies concerning the skin condition and PLEC1 deficiency. All of the authors edited the manuscript.

Declaration of Competing Interest

The authors declare no competing financial interests.

Acknowledgments

The authors thank the technicians of the Advance Diagnostic Unit and the Biobank of the Center for Chronic Immunodeficiency Freiburg for excellent technical assistance. The authors also highly appreciate the support by our nurse Henrike Ritterbusch. This work was supported by funds of the German Federal Ministry of Education and Research (BMBF 01EO1303) and the *E*-RARE Euro-CID program. MH and AR are supported by the Berta-Ottenstein-Programme for Clinician Scientists, Faculty of Medicine, University of Freiburg.

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