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Authors

Kolukisa, Burcu
Baser, Dilek
Akcam, Bengu
et al.

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Evolution and Long-Term Outcomes of Combined Immunodeficiency Due to CARMIL2 Deficiency

Burcu Kolukisa, MD[1,2,3], **Dilek Baser, MSc**[1,2,3], **Bengu Akcam, MSc**[1,2,3], **Jeffrey Danielson, MSc**[4,5], **Sevgi Bilgic Eltan, MD**[1,2,3], **Yesim Haliloglu, MSc**[6], **Asena Pinar Sefer, MD**[1,2,3], **Royale Babayeva, MD**[1,2,3], **Gamze Akgun, BS**[1,2,3], **Louis-Marie Charbonnier, PhD**[7], **Klaus Schmitz-Abe, PhD**[8], **Yasemin Kendir Demirkol, MD**[9], **Yu Zhang, PhD**[4,5], **Claudia Gonzaga-Jauregui, PhD**[10], **Raul Jimenez Heredia, MSc**[11,12], **Nurhan Kasap, MD**[1,2,3], **Ayca Kiykim, MD**[13], **Esra Ozek Yucel, MD**[14], **Veysel Gok, MD**[15], **Ekrem Unal, MD**[15], **Aysenur Pac Kisaarslan, MD**[16], **Serdar Nepesov, MD**[17], **Gokhan Baysoy, MD**[18], **Zerrin Onal, MD**[19], **Gozde Yesil, MD**[20], **Tulin Tiraje Celkan, MD**[21], **Haluk Cokugras, MD**[13], **Yildiz Camcioglu, MD**[13], **Ahmet Eken, PhD**[6], **Kaan Boztug, MD**[11,12], **Bernice Lo, PhD**[22,23], **Elif Karakoc-Aydiner, MD**[1,2,3], **Helen C. Su, MD, PhD**[4,5], **Ahmet Ozen, MD**[1,2,3], **Talal A. Chatila, MD**[7], **Safa Baris, MD**[1,2,3]

[1]Marmara University, Faculty of Medicine, Pediatric Allergy and Immunology, Istanbul, Turkey

[2]Istanbul Jeffrey Modell Diagnostic and Research Center for Primary Immunodeficiencies, Istanbul, Turkey

[3]The Isil Berat Barlan Center for Translational Medicine

[4]Human Immunological Diseases Section, Laboratory of Clinical Immunology and Microbiology, NIAID, NIH, Bethesda, MD, USA

[5]Clinical Genomics Program, NIAID, NIH, Bethesda, MD, USA

[6]Erciyes University School of Medicine, Department of Medical Biology, Kayseri, Turkey

[7]Boston Children's Hospital and Department of Pediatrics, Harvard Medical School, Division of Immunology, Boston, MA, USA

[8]Boston Children's Hospital, Division of Immunology and Newborn Medicine, Harvard Medical School, Boston, MA, USA

[9]Genomic Laboratory (GLAB), Umraniye Teaching and Research Hospital, University of Health Sciences, Istanbul, Turkey

Corresponding author: Safa Baris, MD, Marmara University, Division of Pediatric Allergy/Immunology, Fevzi Çakmak Mah. No: 41, Pendik/Istanbul/Turkey. Phone: +90 216 625 78 11; Fax: +90 216 432 06 95, safabaris@hotmail.com.

Author Contributions: S.B. conceptualized and supervised the study. D.B., B.A., G.A., J.D., Y.H., L.M.C., K.S.A., Y.K.D., Y.Z., C.G.J., R.J.H., A.E., G.Y. and B.L. performed the experiments. B.K., S.B.E., A.P.S., R.B., N.K., A.K, E.O.Y., V.G., E.U., A.P.K, S.N., G.B., Z.O., T.T.C., H.C., Y.C., E.K.A., A.O. and S.B. provided patient care, collected samples and clinical data. B.K. and S.B. wrote the paper. K.B., H.C.S. and T.A.C. edited the manuscript. All authors reviewed and approved the final version of the manuscript.

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[10]Regeneron Genetics Center, Tarrytown, NY, USA

[11]Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, Vienna, Austria

[12]St. Anna Children's Cancer Research Institute (CCRI), Vienna, Austria

[13]Istanbul University-Cerrahpasa, Faculty of Medicine, Pediatric Allergy and Immunology, Istanbul, Turkey

[14]Istanbul University, Istanbul Faculty of Medicine, Pediatric Allergy and Immunology, Istanbul, Turkey

[15]Erciyes University School of Medicine, Pediatric Hematology and Oncology, Kayseri, Turkey

[16]Erciyes University School of Medicine, Pediatric Rheumatology, Kayseri, Turkey

[17]Medipol University Medical Faculty, Department of Pediatric Allergy and Immunology, Istanbul, Turkey

[18]Medipol University Medical Faculty, Department of Pediatric Gastroenterology, Istanbul, Turkey

[19]Istanbul University, Istanbul Faculty of Medicine, Department of Pediatric Gastroenterology, Hepatology and Nutrition, Istanbul, Turkey

[20]Istanbul University, Istanbul Faculty of Medicine, Department of Medical Genetics, Istanbul, Turkey

[21]Istanbul University-Cerrahpasa, Faculty of Medicine, Division of Pediatric Hematology and Oncology, Istanbul, Turkey

[22]Sidra Medicine, Research Branch, Division of Translational Medicine, Doha, Qatar

[23]College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, Qatar.

Abstract

Background: Biallelic loss-of-function mutations in *CARMIL2* cause combined immunodeficiency associated with dermatitis, inflammatory bowel disease (IBD), and EBV-related smooth muscle tumors. Clinical and immunological characterizations of the disease with long-term follow-up and treatment options have not been previously reported in large cohorts. We sought to determine the clinical and immunological features of CARMIL2 deficiency and long-term efficacy of treatment in controlling different disease manifestations.

Methods: The presenting phenotypes, long-term outcomes, and treatment responses were evaluated prospectively in 15 CARMIL2-deficient patients, including 13 novel cases. Lymphocyte subpopulations, protein expression, regulatory T (Treg), and circulating T follicular helper (cT_{FH}) cells were analyzed. Three-dimensional (3D) migration assay was performed to determine T-cell shape.

Results: Mean age at disease onset was 38±23 months. Main clinical features were skin manifestations (n=14, 93%), failure to thrive (n=10, 67%), recurrent infections (n=10, 67%), allergic symptoms (n=8, 53%), chronic diarrhea (n=4, 27%), and EBV-related leiomyoma (n=2, 13%). Patients had reduced proportions of memory $CD4^+$ T cells, Treg, and cT_{FH} cells. Memory B and NK cells were also decreased. CARMIL2-deficient T-cells exhibited reduced T-cell

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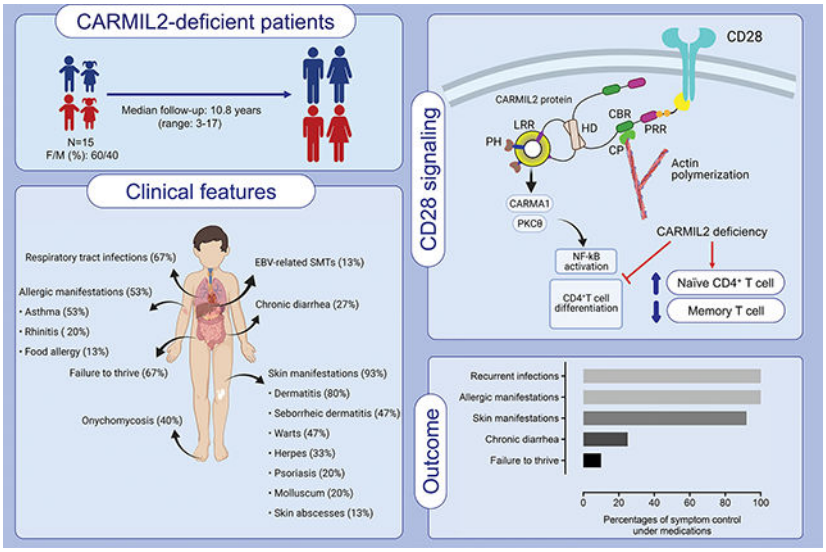
proliferation and cytokine production following CD28 co-stimulation and normal morphology when migrating in a high-density 3D collagen gel matrix. Skin manifestations ranged from atopic and seborrheic dermatitis to psoriasiform rash. IBD was the most severe clinical manifestation, leading to growth retardation, requiring multiple interventional treatments. All patients were alive with a median follow-up of 10.8 years (range: 3–17 years).

Conclusion: This cohort provides clinical and immunological features and long-term follow-up of different manifestations of CARMIL2 deficiency.

Graphical Abstract



Keywords

CARMIL2; CD28 co-signaling; combined immune deficiency; inflammatory bowel disease; recurrent infections; RLTPR; long-term follow-up

Introduction

The capping protein regulator and myosin 1 linker 2 (CARMIL2) deficiency is an autosomal recessive inborn error of immunity (IEI) leading to combined T, B, and NK cell defects[1,2]. CARMIL2, also known as RGD motif, leucine-rich repeats, tropomodulin domain and proline-rich containing protein (RLTPR), is a member of the CARMIL family. This family consists of three paralogous genes (*CARMIL1, CARMIL2, CARMIL3*), producing multidomain proteins with high sequence homology. They contain an N-terminal pleckstrin homology (PH) domain, a leucine-rich repeat (LRR) domain, a homodimerization domain (HD), and a C-terminal domain including a capping protein binding region (CBR) and a proline-rich region (PRR). While all CARMILs have a capacity to bind to the capping proteins and regulate actin assembly, each protein also has a unique cellular function[3]. CARMIL1 activates the Trio-Rac1 pathway to enhance Arp2/3-mediated actin polymerization[4,5], whereas CARMIL2 binds to cellular membranes via vimentin, and activates T cells by ligating CD28 and CARMA1 to mediate NF-κB signaling[6,7]. Mice

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expressing mutated *Carmil2* gene are not able to conduct CD28-mediated activation of its effector protein kinase C theta (PKCθ), abrogating effector memory $CD4^+$ T and regulatory T cells (Treg) development[7,8]. CARMIL2 is also necessary for invadopodia formation, cell polarity, lamellipodial assembly, membrane ruffling, macropinocytosis, and cell migration[3]. The CARMIL3 is expressed mainly in the brain and spinal cord, and identified as oncofetal gene, however, recently it was demonstated as essential regulator of the proinflammatory cytokines in macrophages[3,9].

The human *CARMIL2* gene was originally identified by Matsuzaka et al. and shown to be downregulated in affected skin cells of psoriasis vulgaris patients[10]. The CARMIL2 protein is expressed in the cytoplasm, especially in the skin, lymphoid tissue and gastrointestinal system, and was demonstrated to play a role in wound healing[3]. So far, fewer than 40 cases of CARMIL2 deficiency have been reported worldwide. Patients with CARMIL2 deficiency present with a broad range of symptoms, including cutaneous and respiratory allergies mainly characterized by eczematous lesions, early onset inflammatory bowel disease (EO-IBD), recurrent bacterial, fungal, and viral infections, mucocutaneous candidiasis, skin abscesses, mycobacterial infections, EBV-related smooth muscle tumors (SMT), and growth retardation[1,2,11–20]. Immunologically, affected individuals have elevated naive $CD4^+$ T cells and decreased proportions of circulating T follicular helper cells (cT_{FH}), mucosal-associated invariant T-cell, T_H1, T_H17, and regulatory $Foxp3^+$ T cells concomitant with impaired T-cell activation, proliferation, and cytokine responses due to the defective CD28 engagement[1,2]. Defective memory B-cell formation with poor specific antibody responses was also described in some patients, although this cellular defect is not as prominent as those observed in T cells[1,2,13,14]. Decreased NKG2D expression in $CD8^+$ T and NK cells delineates the role of CARMIL2 in normal cytotoxic function[2]. Heretofore, the majority of patients were reported under investigational research studies without long-term follow-up. Therefore, further detailed studies are needed to better characterize the clinical and immunological features of CARMIL2 deficiency.

In this study, we report clinical and immunological findings of 15 patients with biallelic loss-of-function *CARMIL2* mutations. Our well-defined cohort exhibits the broad clinical phenotypes of patients with long-term follow-up and provides detailed immunological assessments that can facilitate early diagnosis of the disease.

Materials and Methods

This multicenter study involved 15 patients with CARMIL2 deficiency (13 novel and two reported). Genetic diagnosis was made by next-generation (targeted, whole genome, or exome) sequencing, confirmed by Sanger sequencing[21,22]. Some patients were sequenced in collaboration with the Regeneron Genetics Center. Details are provided in the Supplementary File. Clinical and demographic features of the patients were retrieved from their medical records. The local ethics committee approved the study protocol of Marmara University and written informed consent was obtained from all patients and their parents. For 3D migration assays, control subjects were enrolled on a National Institute of Healthy-approved protocol after providing written informed consent.

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Clinical and laboratory evaluations

A questionnaire, including demographic and clinical data (age at onset of symptoms, age at diagnosis, family history, immunodeficiency, past infections, allergic manifestations, systems involved, treatments) was filled for every patient. The pathological examination with Immunohistochemistry analysis of the SMT was recruited. Updated follow-up for the previously reported patients (P11[1] and P12[12]) was provided.

Flow cytometric analysis and 3D migration assay

Peripheral lymphocyte subset analyses, intracellular protein and cytokine staining and proliferation assays were performed by flow cytometry as described previously[23–25]. 3D migration assays using a high-density collagen gel matrix were performed as described previously[26,27]. The details are provided in the Supplementary File.

Statistical analysis

Comparison between patient and control groups were carried out with Mann-Whitney U test, and One-way ANOVA with Tukey's posttest analysis, as indicated. Chi-square test was used to compare categorical values. Differences in mean values were considered significant at a p value <0.05. Statistical analysis was done using GraphPad Prism 8 (GraphPad Software Inc, San Diego, Calif).

Results

Demographic and clinical features

Fifteen patients from nine families with the mean age of 14±3.3 years were enrolled. Of 15 patients, 60% (n=9) were female, 40% (n=6) were male. The mean age at disease onset was 38±23 months (range: 1–120). Demographic features and clinical presentations of the patients are presented in Table 1 and Fig. 1A. All families had parental consanguinity except for Family (F) 6 (Fig. S1). One patient (P5) originated from Azerbaijan, while 14 were from Turkey.

The cardinal symptoms at disease onset were skin manifestation (n=14, 93%), failure to thrive (FTT) (n=10, 67%), recurrent infections (n=10, 67%), allergic symptoms (n=8, 53%), chronic diarrhea (n=4, 27%), and malignancy (n=3, 20%). By the end of the study, all patients were alive with a median follow-up of 10.8 years (range: 3–17 years).

Spectrum of Infections

Respiratory tract infections—The most common infections in CARMIL2-deficient patients were sinopulmonary infections. Mean age at onset for respiratory tract infections was 3.5±1.9 years. Recurrent upper respiratory tract infections (URTI), including otitis media and sinusitis, were seen in 67% of patients (P1, P3-P8, P11, P12, P15).

Seven patients (P3-P7, P9, P10) had a history of bronchiolitis during infancy (47%), and three (20%, P3, P6, P7) had a history of recurrent bronchopneumonia requiring hospital admission that resulted in bronchiectasis in P3 and P7. P6 had pneumonia at age nine years, which required intubation and admission to the intensive care unit. P11 was hospitalized for

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lung *Mycobacterium tuberculosis* infection at 10 years of age. P7 had recurrent croup during ages two to five, with only one proven infection of Parainfluenza virus type 4.

Skin manifestations—Twelve out of fifteen (80%) patients (P1, P3-P8, P10, P12-P15) had either active or past history of eczema (Fig. 1B). Mean age at onset for eczema was 2.7±2.1 years. P1 had mild eczema as a child, which is mainly resolved except for mild dryness as an adult. P3 and P4 have seborrheic dermatitis-like lesions predominantly on the scalp, face, and flexural areas (Fig. 1C). P13 and P14 had early-onset eczematous lesions, both initiated at one month old, predominating around the umbilical, inguinal area, neck, and scalp, which resolved in most regions after the age of 10 years.

HPV was the most common cutaneous infection (n=7, 47%, P3, P4, P6, P7, P11, P14, P15). The mean age at onset for warts was 10.2±3.8 years. Some patients had very persistent and widespread warts (P3 and P4, Fig. 1D), while others had only a few and self-limiting warts (P6, P7, P11, P14, P15). P7 had a few cutaneous warts on the toes, but an upper airway endoscopy also revealed warts on the patient's vocal cords (Fig. 1E). Another common viral skin infection was molluscum contagiosum (n=3, 20%, Table 1). Five patients (33%) had recurrent herpes infections, none severe; mainly herpes labialis or localized on trunk, concurrent with eczema.

Cutaneous candidiasis was seen in 40% of patients. P7, P12, and P15 had onychomycosis (Fig. 1F), while P5, P6, and P13 had persistent inguinal and genital Candida rash.

P8 has eczematous plaques on malar region, nose dorsum, ears, arms and on the back of his neck. Skin biopsy revealed psoriasis vulgaris (Fig. 1G and H). P12 suffers from early-onset psoriasis and psoriatic arthritis.

P6 and P7 suffered from skin abscesses on the trunk and umbilical area. P2 experienced leukocytoclastic vasculitis (Fig. 2I) without detectable autoantibodies.

Systemic viral infections—Three patients (20%, P3, P9, P10) had low-level EBV viremia, while two patients (13%, P3, P13) had CMV viremia. P3 had hepatomegaly and splenomegaly with no other symptoms, and has been monitored for viral load without antiviral treatment. P9, P10, P13 were asymptomatic, but P9 and P10 had EBV-related leiomyoma located to the adrenal glands at eight and three years, respectively. The pathological examination of the resected tumor showed bundle of spindle cells positive for actin, desmin, caldesmon, and Epstein–Barr virus-encoded small RNAs (EBER) staining, confirming the smooth muscle origin. Varicella infection was documented in P4 and P11.

Allergic manifestations

Asthma, allergic rhinitis and food allergies—Eight patients (P3-P7, P9, P10, P15) have diagnosed asthma, but inhalant allergen sensitivity could not be detected via skin prick tests (SPT) or specific IgE except for only one patient (P6) with house dust mite reactivity (Table 1). P3, P6, P7, and P15 were shown to have reversibility on spirometry and were treated with inhaled corticosteroids. P3, P7, and P15 (20% of patients) had rhinitis, with chronic nasal congestion and rhinorrhea, which was sensitive to intranasal corticosteroids.

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Food allergies were observed in two patients (13%, P6, P9). P9 was diagnosed with eosinophilic enteropathy.

Gastrointestinal manifestations—Chronic diarrhea with or without blood was a common symptom for four patients (27%, P1, P2, P5, P12), who were diagnosed with IBD. The mean age of symptom onset for these patients was 4.6±3.0 years. P1 and P2 were diagnosed with atypical Crohn's disease. P1 had chronic diarrhea since age eight years and had an intra-abdominal mass that was surgically excised. Biopsy showed a neuroendocrine tumor of the appendix with negative EBER staining. P2 was also diagnosed with EO-IBD at 10 years old, requiring colectomy. P5 has EO-IBD since birth and bloody diarrhea since two years of age. Colonoscopy revealed multiple ulcers in the colon, starting from the ileum, and the biopsies showed increased lymphocytes, focal active duodenitis, lymphoid hyperplasia in the ileum, and active colitis. P12 presented with abdominal pain, chronic diarrhea at five years of age, and also had recurrent aphthous stomatitis and perianal disease. She had an operation for duodenal stenosis at the age of six years. P9 had an episode of bloody diarrhea around 3 months old, and colonoscopy revealed eosinophilic enteropathy with negative SPT and serum specific IgE tests for food. Diarrhea was unresponsive to cow's milk elimination diet, but symptoms eventually diminished by around age one year.

Genetic analysis and genotype/phenotype assessments—There were seven distinct biallelic variants from nine families in the *CARMIL2* gene. The pathogenic variants and affected domains are shown in Fig. 2A. The consanguinity rate was high in our cohort (except P11). Most of the mutations were missense, while one was a splice site mutation (F1) and the other three mutations were frameshift (F2, F7 and F9). Mutations in F1-F5, F8 and F9 were novel and not reported previously (Table 1 and Fig. 2A). Mutations in F6 and F7 were reported previously[1,12]. Notably, in our cohort the R385T mutation was found in three distinct families (F4, F5, F8) who came from same region, which is consistent with a founder mutation. All the described mutations were deleterious as they led to undetectable or reduced protein expression (P5) in $CD4^+$ and $CD8^+$ T cells, as measured by flow cytometry (Fig. 2B). The current and previously reported mutations were located mostly in the LRR and HD domains, followed by the PH domain[1,2,11–20], and only one mutation in the CBR domain[28], while mutations in PRR domain have not been identified. Despite undetectable CARMIL2 expression (regardless of mutation location), the clinical presentation varied in severity even between members of the same family. Furthermore, the clinical and immunological comparisons between mutation types did not reveal any differences (Table S1). Therefore, we concluded that there was no strong genotype-phenotype relationship that governed the manifestations of CARMIL2 deficiency in our patient cohort.

Laboratory assessment of CARMIL2 deficiency—Detailed immunological evaluations of the patient cohort are presented in Table 2. During the time of evaluation, eosinophilia was detected in seven patients (47%). Serum IgG immunoglobulin levels before immunoglobulin replacement were normal in nine (60%), low in five (33%), and high in one (6.6%) patient. Serum IgA levels were normal in most patients (n=12, 80%), and elevated in three (20%). Serum IgM levels were normal in nine (60%), elevated in four (27%) and low in two (13%) patients. IgE was slightly elevated in three (20%) patients.

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All tested patients displayed some poor protein vaccine responses, whereas IgM responses against polysaccharide antigens (isohemagglutinins) were normal. Broad autoantibody screening was also carried out (Table S2). While five of 12 and four of 12 were positive for anti-thyroid peroxidase and anti-thyroglobulin antibodies, respectively, all had normal thyroid function tests. P2 had a positive anti-PR3 antibody result without pertinent clinical symptoms.

Extensive flow cytometric analysis was performed in all patients (Table 2 and Fig. 3A). All patients had normal or slightly elevated $CD3^+$ T-cell counts, but T-cell subsets revealed markedly high percentages of naive $CD4^+$ T cells in 12 (80%) patients accompanied by low memory $CD4^+$ T cells in most compared to the healthy controls ($p<0.0001$, $p<0.0001$, respectively). Defective maturation was less prominent in $CD8^+$ T-cell subtypes, which revealed a decrease in the central memory and effector memory $CD8^+$ T-cell compartment compared to age-matched healthy controls ($p<0.0001$ and $p<0.05$, respectively). To evaluate the CD28-costimulation defect in patients, we performed lymphocyte proliferation and intracellular cytokine staining for IL-2, IL-17, and IL-22 (P2, P3, P7, P12). While the CD3 and PMA/ionomycin-induced proliferation and cytokine responses of patients were comparable with healthy controls, anti-CD3/CD28 stimulation revealed blunted expansion and cytokine responses (Fig. S2 and S3). Similarly, B cell counts were low in only one patient (P2), but nine (60%) had low percentages of class-switched memory B cells concomitant with elevated naïve B cells. Notably, we observed low NK cells in 13 (87%) CARMIL2-deficient patients, with the predominance of reduced CD56 subtype (Table 2).

Since CARMIL2 protein is expressed in Treg and T cells' subtypes[1], we enumerated cT_{FH} ($CD4^+$ $PD1^+$ $CXCR5^+$) and Treg frequencies in CARMIL2-deficient patients. Significant reduction in cT_{FH} and $CD4^+$ $CD25^+$ $FOXP3^+$ Treg cells were observed in all tested patients compared to healthy controls ($p<0.0001$, $p<0.0001$, respectively, Fig. 3B and C). Furthermore, we also evaluated the induction of CTLA4 at baseline and after anti-CD2, anti-CD3 and anti-CD28 stimulation, which resulted in reduced levels compared to healthy controls ($p<0.0001$, $p<0.0001$, respectively, Fig. 3D).

CARMIL2 and DOCK8 proteins are involved in the actin polymerization pathway, and both diseases can share overlapping clinical phenotypes[7,29]. Therefore, we performed 3D migration assays to compare the cellular morphology in both diseases, using an ~3-fold higher concentration of collagen than previously tested for CARMIL2-deficient cells. While DOCK8-deficient T lymphocytes showed abnormal elongation and defective migration[26], CARMIL2-deficient T cells exhibited normal phenotype, resembling healthy controls (Fig. 4A and B).

Medications and disease course

During the follow-up, skin manifestations (n=14) were the most common feature of disease and were frequently accompanied by FTT and recurrent infections (n=10) and rarely by chronic diarrhea (n=4) and tumor (n=3) (Fig. 5A and B). The cumulative incidences for the development of dermatitis, warts, RTI, SMT and IBD over time are presented in Fig. S4. Because of recurrent infections and dysgammaglobulinemia with poor antibody responses, five patients (33%, P3, P4, P6, P7, P15) received prophylactic antibiotics and seven patients

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(47%, P3, P6, P7, P9–11, P15) were commenced on immunoglobulin replacement therapy (IgRT) with one receiving via subcutaneous and six via intravenous routes. P3 was on azithromycin prophylaxis, and chronic rhinosinusitis and productive cough benefited from the therapy. P4 was initiated on trimethoprim-sulfamethoxazole, while P6, P7, P9–11, and P15 were on trimethoprim-sulfamethoxazole and fluconazole prophylaxis. Generally, the combined therapies including IgRT and prophylaxis were effective in reducing the frequency of infections. Detailed therapy options are provided in the Supplementary File of patients' descriptions.

P1, P2, P5, and P12 received various systemic immunosuppressive therapies for IBD. P1 and P2 received mono or a combination of immunosuppressants, including mesalamine, prednisolone, azathioprine, adalimumab, vedolizumab, infliximab, and sirolimus. Due to unresponsiveness to immunosuppressants, both underwent colectomy, which resolved their symptoms. P5 is receiving mesalamine and prednisolone with partial response.

In general, skin manifestations were alleviated during the follow-up and controlled only by topical therapies in the majority of patients (Fig. 5C). P12 received steroids and methotrexate for IBD, psoriasis, and psoriatic arthritis. P13 and P14 are doing well without medication and only have mild eczema and FTT.

P9 received hematopoietic stem cell transplantation (HSCT) from full matched unrelated donor due to the SMT and is doing well with 99% donor chimerism on 90th day posttransplantation. The patient's skin lesions and abdominal pain were resolved and there was no progression in the adrenal masses.

Discussion

In this report, we demonstrated the clinical manifestations and laboratory results of 15 patients with seven different *CARMIL2* mutations. The disease was recently defined and most studies in the literature do not include long-term follow-up and different therapeutic options. Most prominent symptoms were various skin manifestations (atopic and seborrheic dermatitis, psoriasiform rash), increased frequency of infections (including EBV, CMV, Varicella infections) and chronic diarrhea. Patients also had allergic manifestations such as rhinitis and asthma without prominent allergen sensitivity, and two siblings developed EBV-related SMTs. Based on the long-term evaluation, the majority of patients benefited from the provided drug therapies, while refractory colitis usually required multistep therapy including surgery.

Early-onset moderate to severe eczema and seborrheic dermatitis concurrent with superficial bacterial or viral skin infections are widely reported in CARMIL2 deficiency (Table 3)[1,2,11,13]. Furthermore, urticaria, lupus-like erythema, psoriasis, photosensitivity, and skin abscesses were also observed in other cohorts, demonstrating the importance of CARMIL2 for normal skin maintenance[10,16]. Interestingly, some overlapping features resembling DOCK8 deficiency have been described in CARMIL2-deficient patients. Although both proteins have a shared biochemical interactome[7], CARMIL2 deficiency differs as it presents with less common symptoms for food allergies, anaphylaxis, severe viral infections,

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and autoimmunity compared to DOCK8 deficiency[30,31]. Furthermore, skin lesions and accompanied infections were not as severe as in DOCK8 deficiency and seem to progress with a slower tempo, responding well to topical treatments[11,32]. Previously, it was shown that CARMIL2-deficient T cells exhibited normal levels of F-actin but disorganized microtubule network and F-actin at the leading edge of migrating cells[2]. However, we found that CARMIL2-deficient T cells failed to manifest abnormal morphology when cells migrated under more stringent 3D conditions; these results are consistent with a milder cellular defect in dense tissues than in DOCK8 deficiency, which could contribute to the less severe clinical course of skin infections in CARMIL2 deficiency. In CARMIL2 deficiency, eczema can be bolstered via a T_H2 cell bias[1]. This skewing phenotype is reminiscent of other IEI disorders with mutations in *DOCK8*, *WAS*, *PGM3*, hypomorphic *CARD11*, Omenn's syndrome, and transforming growth factor-ß receptor associated disorders[29,33–36]. The majority of these diseases also have Tregopathies, as does CARMIL2 deficiency, contributing to the eczema phenotype. However, the elevated IgE and eosinophilia are not observed to be as high in CARMIL2 deficiency as hyper IgE syndrome. The cellular phenotype and T_H17 defect is more profound in DOCK8 deficiency as there is almost undetectable IL-17 in those patients and the molecule plays a central role in lymphocyte activation[29], while in CARMIL2 deficiency these defects can be compensated by other stimuli directed to CD3 and PKCΘ[2,7,8].

Another important clinical phenotype is FTT and IBD, which was previously described in 19 patients in the literature (Table 3). Our cohort includes 27% and 67% of patients with chronic diarrhea and FTT, respectively. It was shown in previous reports that CARMIL2 protein is expressed in colonic epithelial cells[12,18]; therefore, akin to the skin findings, epithelial CARMIL2 deficiency could potentially initiate and drive exaggerated immune responses, thus subverting the integrity of mucosa, and ending with colitis. On the other hand, FTT seems to be generally linked to chronic diarrhea and gastrointestinal disease[12]. Only a few patients were reported to have growth hormone insufficiency[18,19], as in our P4.

In our cohort, we detected RTI, HPV-related verrucae, molluscum contagiosum, skin abscesses, EBV and CMV viremia, mycobacterial infection, and EBV-related leiomyoma. Notably, severe infections or mortality due to these infections are less common than other CIDs, which usually require HSCT[37]. EBV-related SMTs were previously reported in CARMIL2 deficiency involving different areas such as liver, GI tract, brain, spleen, and lung[2,17,19–21]. Interestingly, in those patients EBV-induced B-cell lymphoproliferation was not observed as is usually expected in IEIs[38,39] and the plausible mechanisms related to the specific tumor susceptibility are not fully explored[2]. In our cohort, P9 and P10 had EBV-related leiomyoma located to the adrenal glands. Recently, two CARMIL2-deficient siblings with extensive EBV-related SMTs were reported and one of them received sirolimus and radiotherapy for the tumor. However, treatment failures led to the decision to transplant; one patient subsequently died of *Pneumocystis jirovecii* pneumonia and progression of pulmonary tumors, while the other recovered after transplantation[19]. Similarly, another study reported four patients who had multiple tumor site involvement without response to chemotherapy, which led to patients' demise[2]. It seems that the most fatal complication of the disease is EBV-induced SMTs, which probably requires transplantation for recovery, as experienced in other reported IEI disorders like GATA2 haploinsufficiency[40,41].

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All tested patients in our cohort had low Treg and cT_{FH} cells, impaired proliferation in response to anti-CD28, and low production of IL-2, IL-17, and IL-21 cytokines after CD28 crosslinking. Importantly, the underlying defect was reported to be partially rescued by IL-2 supplementation[2,15,19]. Compared to other CIDs with defective regulatory T-cell functions, CARMIL2-deficient patients exhibit much less autoimmune phenomenon[1,2]. While reduced conventional $FOXP3^+$ Treg cells can potentially pave a way for exaggerated autoimmunity in CARMIL2 deficiency, it can be speculated that insufficient CD28-driven engagement precludes the activation and development of memory T cells, which normally mediate the effectors of autoimmunity[42–44]. Furthermore, diminished cT_{FH} cells in those patients can prevent production of autoantibodies due to the inappropriate B-cell co-stimulation especially via ICOS-ICOSL and IL-21/IL-21R, as shown previously in mouse models [45,46]. Interestingly, our data also demonstrated decreased CTLA4 expression in CARMIL2-deficient Treg cells, resembling the Treg cell-specific conditional *Cd28* knockout mice characterized by decreased CTLA4, PD1, and CCR7 expressions on Tregs[47].

Treatment options in this disease are immunoglobulin replacement therapy and antimicrobial prophylaxis for recurrent infections. Eight out of 15 patients in our cohort benefited from IgRT and prophylactic therapies. Immunosuppressants are utilized for skin manifestations and inflammatory bowel disease. Topical corticosteroids and calcineurin inhibitors are effective in managing eczema and psoriatic lesions, and sun protection helped in cases with photosensitive dermatitis[16]. More commonly used options are mesalazine, sulfasalazine, steroids, azathioprine, and infliximab for IBD. Abatacept was received by one patient[12], and provided partial response, which can be promising, since lower CTLA4 expression was found in our tested patients. Colectomy is an option in the IBD patients who are refractory to immunosuppressants, and resulted in symptomatic control in our patients (P1, P2)[11,12,18]. Sirolimus was used in a patient with EBV+SMT, but discontinued after 5 days because of sepsis due to *Mycobacterium chelonae,* so the effect of the drug could not be evaluated[19].

The presented large cohort provides a long-term follow-up evaluation of CARMIL2-deficient patients with different clinical phenotype categories: CIDs, skin manifestations, and EO-IBD. Overall, patients have low mortality compared to other CIDs. Infections and allergic manifestations like eczema, asthma, and rhinitis are manageable and stable over the years, whereas gastrointestinal manifestations are more severe, leading to severe growth failure, anemia, and overall low quality of life, and requiring interventional treatments (corticosteroids, biologic agents, colectomy). Monitoring patients for SMTs and providing earlier therapies might be life-saving. Studies regarding indications and outcomes of HSCT are required in CARMIL2 deficiency. Finally, patients with persistent symptoms especially for skin manifestations, chronic diarrhea, and EBV-related smooth muscle tumors preferentially should be evaluated for the CARMIL2 deficiency.

Supplementary Material

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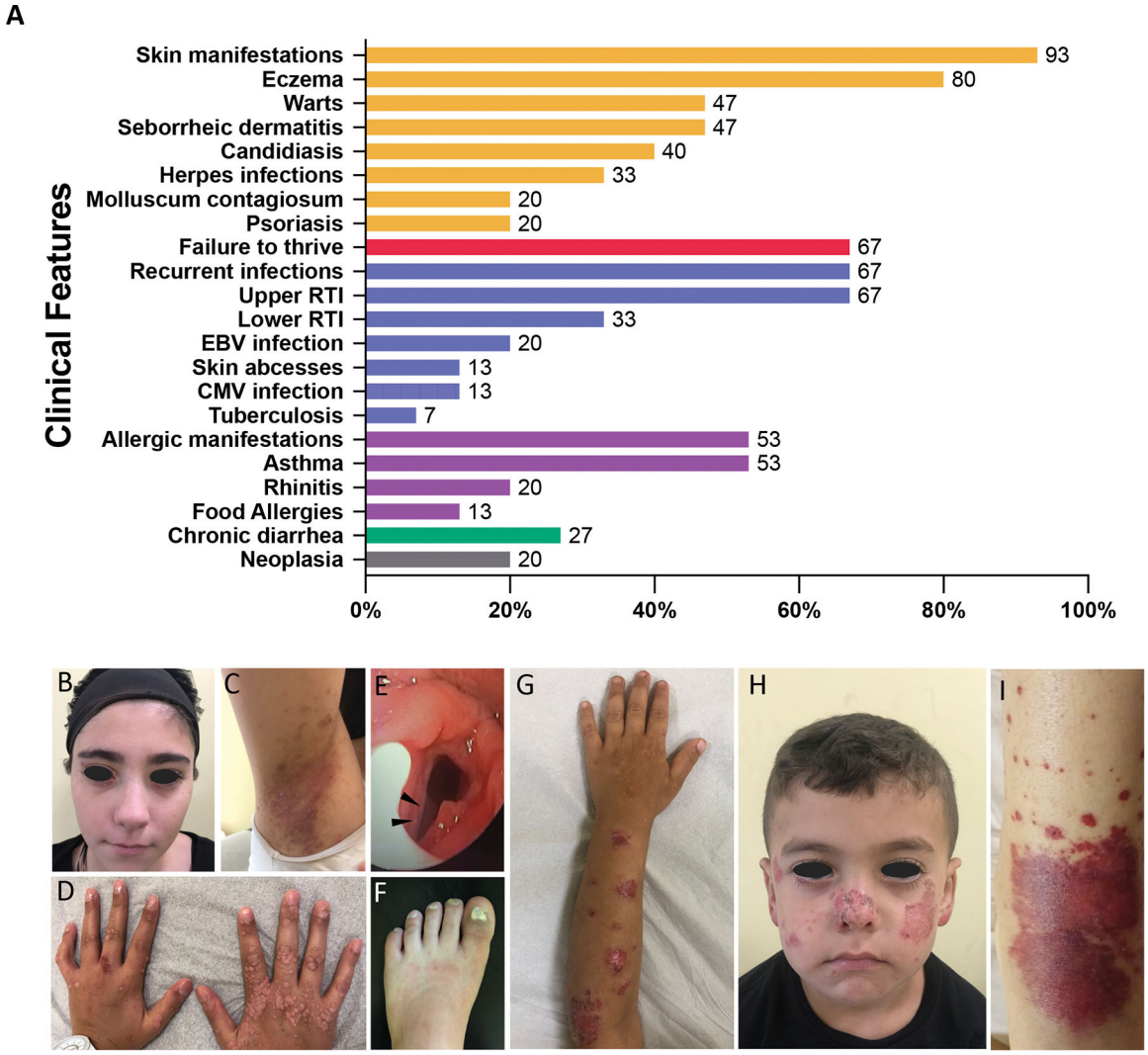

Figure 1. CARMIL2-deficient patients' clinical phenotypes.

(**A**) Clinical features of CARMIL2-deficient patients. The bars are depicted as percentages. Disease symptom categories are indicated with different colors. Orange bars, skin manifestations; red bar, failure to thrive; blue bars, infections; purple bars, allergic manifestations; green bar, chronic diarrhea; grey bar, neoplasia. **(B-I)** Representative pictures of patients' skin phenotypes: warts around eyes and molluscum contagiosum on cheek of P6 (**B**), seborrheic dermatitis on axillary region and multiple warts on dorsum of hands of P3 (**C** and **D**), warts around vocal cords seen in upper airway endoscopy of P7, indicated with arrows (**E**), onychomycosis on toes of P15 (**F**), psoriatic dermatitis on face and lower arm, and molluscum contagiosum on chin of P8 (**G** and **H**), vasculitic rash and edema on leg of P2 (**I**). RTI, Respiratory tract infections; EBV, Epstein-Barr virus; CMV, Cytomegalovirus.

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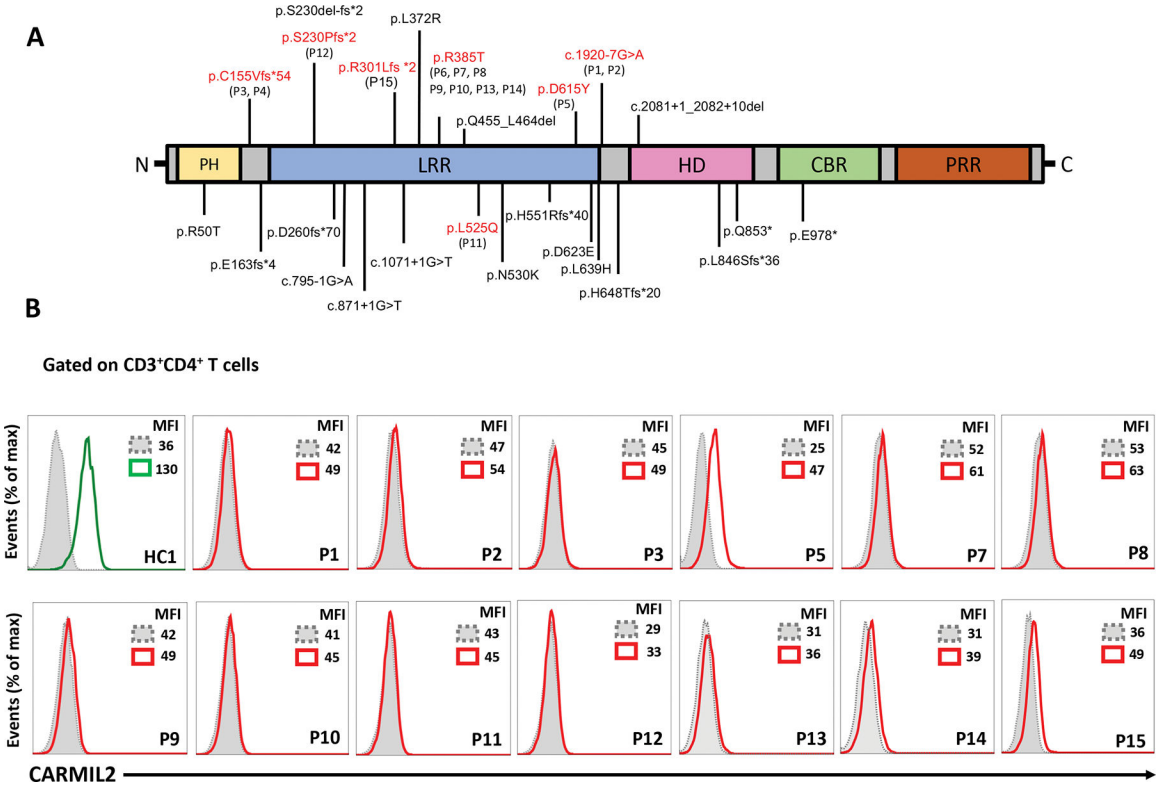

Figure 2. Mutations in CARMIL2 deficiency distributed throughout the gene and undetectable CARMIL2 protein expression in patients compared to healthy controls.

(**A**) Schematic diagram of CARMIL2 protein domains, with locations of previously known mutations (Black) and mutations observed in this cohort (Red). Asterisk (*) indicates a premature stop codon. The depicted domains are pleckstrin homology domain (PH), leucine-rich repeat (LRR), homodimerization domain (HD), capping protein binding region (CBR), and proline-rich regions (PRR). **(B)** Flow cytometric analysis of CARMIL2 protein expression in mutated patients and healthy controls. Mean fluorescence intensity (MFI) values are indicated with the related colors (Grey: Isotype, Green and Red: CARMIL2 protein). HC: Healthy control, P: Patient.

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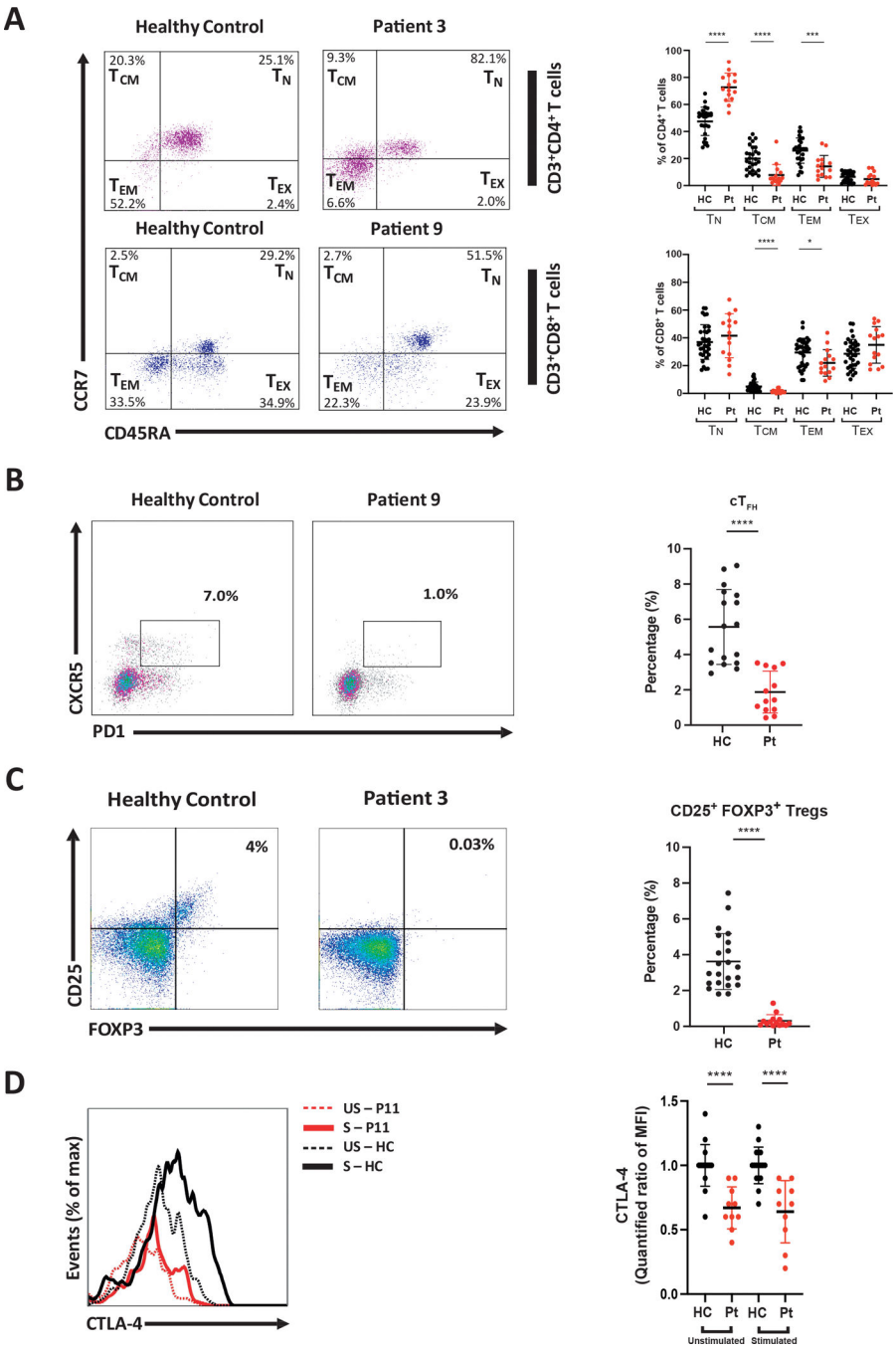

Figure 3. Defective differentiation of T cells in CARMIL2-deficient patients.

(A) Representative plot of T-cell subtypes of patients (P3 and P9) and age-matched healthy controls. Plots show $CD4^+$ and $CD8^+$T cells that are naïve (T_N, $CCR7^+$ $CD45RA^+$), central memory (T_{CM}, $CCR7^+$ $CD45RA^-$), effector memory (T_{EM}, $CCR7^-$ $CD45RA^-$), and exhausted (T_{EX}, $CCR7^-$ $CD45RA^+$). Corresponding percentages are indicated in each quadrant, and the summary of CCR7/CD45RA staining data from CARMIL2-deficient patients (n=15), along with age-matched healthy controls (n=32), is shown on the right. **(B)** Flow cytometric analysis of CXCR5 and PD1 expression in $CD4^+$ T cells in P9 and healthy

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control with the summary of cT_{FH} cell percentages for patients (n=13) compared to healthy controls (n=17). **(C)** Representative plots showing unstimulated $CD4^+$ $CD25^+$ $FOXP3^+$ Treg cells in P3 with the summary of Treg cell percentages data for (n=14) and healthy controls (n=23). **(D)** Quantification of CTLA4 expression in $CD45RO^+$ memory $CD4^+$ $FOXP3^+$ Treg cells of patients (n=10) and healthy controls (n=17) in unstimulated and 16 hours-stimulated (anti-CD2, anti-CD3 and anti-CD28) conditions. HC: Healthy control, P: Patient. Asterisks indicate significance levels (*p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001, Mann-Whitney U Test).

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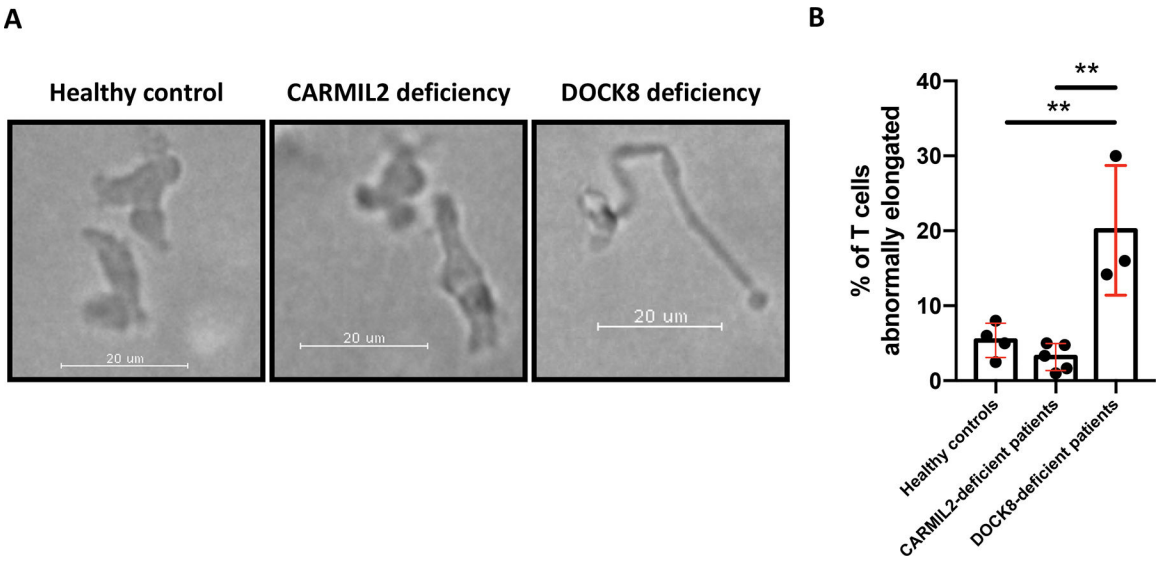

Figure 4. CARMIL2-deficient T cells showed normal morphology in a high-density 3D collagen gel matrix compared to DOCK8 deficient T cells.

A) Live cell imaging of T cells from CARMIL2-deficient patient (middle panel), DOCK8-deficient patient (right panel) and a healthy control (left panel). **B)** Percent of T cells abnormally elongated in 3D migration assay. Each symbol represents a different individual, from CARMIL2-deficient patients (n=5), DOCK8-deficient patients (n=3), and healthy controls (n=4). For each patient, the value shown was averaged from two or three experiments. Mean ± SD are indicated. **$p < 0.01$, by ordinary One-way ANOVA with Tukey's posttest analysis.

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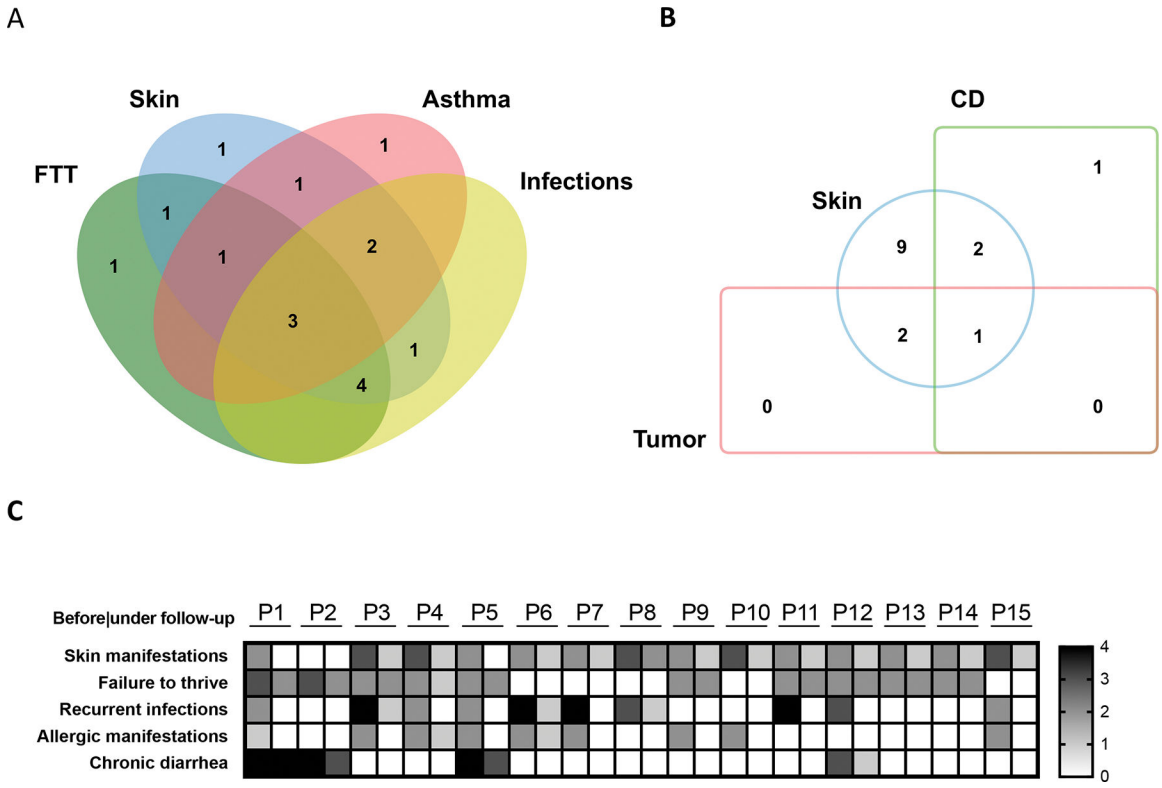

Figure 5. Main clinical features are controlled in CARMIL2 deficiency during follow-up.

A) Conventional Venn-diagram showing the frequent overlap phenotypes with skin manifestations. **B)** Edwards demonstrating the rare overlap phenotypes with skin manifestations. **C)** The clinical symptoms were graded from 0 (none), 1 (mild symptoms or not required medication), 2 (moderate symptoms or required intermittent medication), 3 (severe symptoms required frequent medication), to 4 (hospitalization to control symptoms) before and under follow-up, and are demonstrated as a heatmap per patient. FTT, failure to thrive.

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Table 1

The demographic, clinical and mutation data of CARMIL2-deficient patients

Patients	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
Family	F1		F2		F3	F4			F5		F6	F7	F8		F9
Current age (yrs)	25	22	22	11	7	16	10	4	9	5	23	15	15	11	16
Age at onset of symptoms (mo)	96	120	108	48	1	4	3	36	3	3	120	2	1	1	24
Mutation *	c.1920-7G>A	c.1920-7G>A	c.463delT p.C155Vfs*54	c.463delT p.C155Vfs*54	c.1843G>T p.D615Y	c.1154G>C p.R385T	c.1154G>C p.R385T	c.1154G>C p.R385T	c.1154G>C p.R385T	c.1154G>C p.R385T	c.1574T>A p.L525Q	c.688_689delAG p.S230Pfs*2	c.1154G>C p.R385T	c.1154G>C p.R385T	c.902delG p.R301 Lfs*6
Mutation	Novel	Novel	Novel	Novel	Novel	Novel	Novel	Novel	Novel	Novel	Reported **	Reported ***	Novel	Novel	Novel
Recurrent infections	+	–	+	+	+	+	+	+	–	–	+	+	–	–	+
Upper RTI	+	–	+	+	+	+	+	+	–	–	+	+	–	–	+
Lower RTI	–	–	+	+	–	+	+	–	–	–	+	–	–	–	–
Candidiasis	–	–	–	–	Diaper area	Vulvovaginal	Onychomycosis	–	–	–	–	Onychomycosis	Diaper area	–	Onychomycosis
EBV infection	–	–	+	–	–	–	–	–	+	+	–	–	–	–	–
CMV infection	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–
Skin abscess	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–
Tuberculosis	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
Chronic diarrhea	+	+	–	–	+	–	–	–	–	–	–	+	–	–	–
Other gastrointestinal findings	Hepatomegaly, intra-abdominal abscess	Congenital asplenia	Hepatosplenomegaly	Dysphagia	Hepatomegaly	–	–	–	Eosinophilic enteropathy	–	Hepatosplenomegaly, antral and duodenal ulcer	Aphthous stomatitis, esophagitis, duodenal stenosis	Dysphagia, perforation in esophagus during endoscopy	Intussusception at 11 mo	–
Failure to thrive	+	+	+	+	+	–	–	–	+	–	+	+	+	+	–
Skin manifestations	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+
Eczema	+	–	+	+	+	+	+	+	–	+	–	+	+	+	+
Psoriasis	–	–	–	–	–	–	–	+	–	–	+	+	–	–	–
Seborrheic dermatitis	–	–	+	+	–	–	–	–	+	+	+	–	+	+	–
Herpes infections	–	–	–	+	–	+	+	–	–	–	+	+	–	–	–
Molluscum contagiosum	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–

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Patients	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
Family	**F1**		**F2**		**F3**	**F4**			**F5**		**F6**	**F7**	**F8**		**F9**
Warts	–	–	+	+	–	+	+	–	–	–	+	–	–	+	+
Allergic Manifestations	–	–	+	+	+	+	+	–	+	+	–	–	–	–	+
Asthma	–	–	+	+	+	+	+	–	+	+	–	–	–	–	+
Rhinitis	–	–	+	–	–	+	–	–	–	–	–	–	–	–	+
Food Allergies	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–
Allergic sensitization	None	None	None	None	None	House dust mite, tomato, cashew	None	None	None	None	None	None	None	None	None
Lung Involvement	–	–	Bronchiectasis	–	–	Subplevral nodule with ground glass opacity in left lung	Bronchiectasis, ground glass opacities left lung	–	–	–	–	–	–	–	–
Tumor	Neuroendocrine neoplasia in appendix	–	–	–	–	–	–	–	Bilateral adrenal EBV-related leiomyoma	Bilateral adrenal EBV-related leiomyoma	–	–	–	–	–
Other findings	Epilepsy, secondary amyloidosis and chronic renal failure, rod-cone dystrophy	Leukocytoclastic vasculitis	Severe iron deficiency anemia	Growth hormone deficiency	Severe iron deficiency anemia	Gliosis in brain (right putamen)	–	–	–	–	Osgood Schlatter disease of left knee	Psoriatic arthritis	–	–	Cerebral vasculopathy

Abbreviations: RTI: Respiratory tract infection.

*Mutation annotation was performed according to the canonical transcript (NM_001013838.3).

**Magg et al. (12)

***Wang et al. (1).

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Table 2

The Immunological evaluation of CARMIL2-deficient patients

Parameters (age at evaluation)	P1 (21 yrs)	P2 (18 yrs)	P3 (20 yrs)	P4 (11yrs)	P5 (5 yrs)	P6 (14 yrs)	P7 (10 yrs)	P8 (4 yrs)	P9 (9 yrs)	P10 (5 yrs)	P11 (23 yrs)	P12 (15 yrs)	P13 (15 yrs)	P14 (11 yrs)	P15 (16 yrs)	Reference values (3–4 yrs)*	Reference values (5–6 yrs)*	Reference values (9–13 yrs)*	Reference values (14–20 yrs)*
ANC/mm^3	6300	4600	5400	3800	4600	6500	5700	4900	5700	4200	2800	4580	3700	3200	4700	1500–8500	1500–8500	1500–8500	1800–8000
ALC/mm^3	4800	1500	2300	2500	3700	3800	4400	7300	5700	6200	3200	2330	5200	3100	3500	2000–8000	1500–7000	1500–6500	1200–5200
Eo/mm^3	**1600**	400	200	80	**780**	**1600**	210	**2160**	**4200**	**3400**	**500**	**850**	330	220	460	0–500	0–500	0–500	0–500
Serum immunoglobulins																			
IgG (mg/dl)	**693**	1200	**2405**	**719**	1450	1102	**613**	**564**	1370	1011	948	1270	1391	1090	**566**	640–2810	746–1804	835–2694	913–1884
IgM (mg/dl)	**481**	245	**352**	**27**	124	172	87	62	182	124	93	**330**	**418**	223	**74**	52–297	78–261	47–484	88–322
IgA (mg/dl)	261	252	**798**	72	237	120	112	47	**474**	149	217	**401**	344	254	192	44–244	57–282	62–433	139–378
IgE (IU/ml)	9.6	71.6	1.0	<1.0	17	33.5	1.1	23	**228**	**158**	34	36.9	**120**	30	7	<100	<100	<100	<100
Specific antibody titers																			
Anti-Hbs (IU/L)	**0.48**	**0.08**	**0.38**	**2**	**1.3**	**0**	**0.38**	**0**	**0.21**	**4.39**	**0.14**	n.d.	**0.46**	**0.3**	**2**	>10	>10	>10	>10
Anti-Measles (IU/L)	n.d.	n.d.	**0.71**	**0.20**	n.d.	**0.43**	**5.87**	**0.21**	n.d.	n.d.	**0**	n.d.	**5.77**	**2.29**	**3.8**	>11	>11	>11	>11
Anti-Mumps (AI/mL)	n.d.	n.d.	19.2	n.d.	n.d.	n.d.	40.2	**0.67**	n.d.	n.d.	**<0.5**	n.d.	17	**5.1**	**1.5**	>11	>11	>11	>11
Anti-Rubella (IU/mL)	n.d.	n.d.	>400	35.4	n.d.	n.d.	>400	>400	n.d.	n.d.	>350	n.d.	257	387	33	≥15	≥15	≥15	≥15
Isohemagglutinin level	n.d.	n.d.	1/256 (Anti-A)	1/16 (Anti-A)	n.d.	n.d.	1/16 (Anti-B)	1/8 (Anti-A)	n.d.	n.d.	n.d.	n.d.	1/128 (Anti-B)	1/16 (Anti-B)	1/16 (Anti-B)	≥1/8	≥1/8	≥1/8	>1/8
Lymphocyte subsets (/mm^3)																			
CD3$^+$T cells	**3912**	1224	1803	1720	**3315**	3081	3146	**5956**	**4987**	**5239**	2473	2080	**4196**	2219	2450	1500–3870	1420–3120	1070–2270	1090–2600
CD3$^+$4$^+$T cells	**2352**	930	1012	733	**2160**	1470	**1860**	**3737**	**2844**	**3292**	1238	813	**1892**	815	**1599**	880–2360	540–1840	640–1290	560–1450
CD3$^+$8$^+$T cells	**1296**	**252**	621	887	1073	**1356**	**1346**	**2080**	**1744**	**1481**	**1091**	**1057**	**1445**	**945**	700	410–1280	470–1200	380–880	320–960
CD19$^+$B cells	446	**133**	338	**675**	317	399	**1126**	1073	558	830	544	165	**852**	**722**	**987**	310–1130	260–970	170–630	140–560
CD16$^+$56$^+$NK cells	283	**49**	**62**	**90**	**11**	**148**	**48**	175	**22**	**43**	**108**	**51**	**67**	**96**	**130**	150–810	180–510	170–530	150–700
CD56$^+$ cells %	n.d.	n.d.	**11.2**	**6.0**	**0.9**	n.d.	**1.7**	**3.6**	**4.1**	**4.0**	**6.3**	**7.2**	**5.1**	7.5	**6.5**	7–15.8	5.4–12.9	12.3–19.4	12.1–19.6
T cell subsets (%)																			
CD4$^+$CD45RA$^+$CCR7$^+$	**58.8**	53.5	**82.2**	**63.2**	**74.6**	**70.5**	**80.3**	**91.6**	**77.1**	**85.8**	**79.9**	**59.2**	51.0	59.4	**69**	46–72	35–69	25–63	31–57
CD4$^+$CD45RA$^-$CCR7$^+$	**32.5**	**38.5**	9.7	**3.6**	**6.9**	16.2	**3.3**	**1.8**	**5.7**	5.2	**5.3**	**4.6**	11.9	**2.6**	**5.4**	11–20	9–25	11–25	10–27

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Parameters (age at evaluation)	P1 (21 yrs)	P2 (18 yrs)	P3 (20 yrs)	P4 (11yrs)	P5 (5 yrs)	P6 (14 yrs)	P7 (10 yrs)	P8 (4 yrs)	P9 (9 yrs)	P10 (5 yrs)	P11 (23 yrs)	P12 (15 yrs)	P13 (15 yrs)	P14 (11 yrs)	P15 (16 yrs)	Reference values (3–4 yrs)*	Reference values (5–6 yrs)*	Reference values (9–13 yrs)*	Reference values (14–20 yrs)*
$CD4^+CD45RA^-CCR7^-$	**8.1**	**7.5**	**6.2**	**31.1**	10.7	12.8	12.5	**4.3**	14.7	**7.5**	**11.4**	29.6	25.3	30.8	18.7	9–20	10–30	12–30	12–44
$CD4^+CD45RA^+CCR7^-$	**0.5**	**0.5**	**1.8**	**1.9**	7.8	**0.6**	3.9	**2.2**	**2.6**	**1.5**	**3.4**	6.5	11.7	7.2	6.6	3–14	4–24	4–21	4–12
$CD8^+CD45RA^+CCR7^+$	36.7	59.8	43.7	**13.8**	52.4	39.9	28.5	**51.2**	50.6	**60.1**	57.3	19.9	**5.6**	**7.4**	48.5	5–25	22–58	22–58	18–61
$CD8^+CD45RA^-CCR7^+$	**2**	**1.9**	4.7	**0.9**	**1.5**	4.2	**0.3**	**0.03**	**1.5**	**0.9**	**0.8**	**1.5**	**2.6**	**0.4**	**1.3**	2–13	4–11	2–15	3–12
$CD8^+CD45RA^-CCR7^-$	15.3	**7.3**	**11.1**	43.6	18	27	33.1	**9.1**	**21.8**	18	24.6	36.6	25.7	32.8	31	14–49	14–59	24–58	12–44
$CD8^+CD45RA^+CCR7^-$	**45.9**	**31**	**40.9**	**41.5**	28	**28.9**	38.1	**39.7**	26.1	20.9	17.2	**41.9**	**65.9**	**59.4**	19.2	5–25	6–30	7–26	5–20
$CD4^+CD45RA^+CD31^+$	59	41.9	50.1	50.7	**40.5**	61.3	**71.1**	68.7	58.0	70.1	52.8	**33.1**	**36.1**	49.1	55	55–70	52–71	43–67	37–62
B cell subsets (%)																			
$CD19^+CD27^-IgD^+$	84.9	67.6	**63**	**91.7**	**87**	82.1	**91**	73.2	**89.8**	**87.8**	**89.9**	82.1	84.3	**88.4**	**88.3**	63–86	65–86	64–84	65–88
$CD19^+CD27^+IgD^+$	**3.5**	3.9	**3.3**	7.2	**2.4**	**3.2**	**0.8**	**17.4**	7.0	**3.1**	**1.4**	**12.9**	7.1	8.4	**0.6**	4–13	5–16	4–14	4–12
$CD19^+CD27^+IgD^-$	8.4	**22.1**	5.4	**0.2**	**3**	11	**1.02**	3.9	**0.4**	**1.7**	**1.9**	**2.6**	4.8	**1.6**	**1.7**	4–15	4–16	6–16	4–16
$CD21^{low}CD38^{low}$	3.9	**15.3**	3.4	**1.0**	**0.9**	**7.9**	**8.6**	**1.8**	**1.4**	**0.78**	**2.6**	3.7	**1.6**	**0.9**	**1.0**	2–6	3–10	2–7	2–6

Abbreviations: ALC: Absolute lymphocyte number, ANC: Absolute neutrophil number, Eo: Eosinophil number, $CD4^+$ naïve T cells ($CD4^+CD45RA^+CCR7^+$), $CD8^+$ naive T cells ($CD8^+CD45RA^+CCR7^+$), recent thymic emigrants ($CD4^+CD45RA^+CD31^+$), central memory $CD4^+$T cells ($CD4^+CD45RA^-CCR7^+$), effector memory $CD4^+$T cells ($CD4^+CD45RA^-CCR7$), terminally differentiated effector memory $CD4^+$T cells ($CD4^+CD45RA^+CCR7^-$), central memory $CD8^+$T cells ($CD8^+CD45RA^-CCR7^+$), effector memory $CD8^+$T cells ($CD8^+CD45RA^-CCR7^-$), terminally differentiated effector memory $CD8^+$T cells ($CD8^+CD45RA^+CCR7^-$), naive mature B cells ($CD19^+CD27^-IgD^+$), non-switched memory B cells ($CD19^+CD27^+IgD^+$), switched memory B cells ($CD19^+CD27^+IgD^-$). Abnormal values are shown as bold.

*Normal ranges were adopted from published normal reference [48].

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Table 3.

Comparison of clinical and immunological findings of our cohort of CARMIL2-deficient patients to those previously reported in the literature.

Publications	Wang et al. (1)	Schober et al. (2)	Sorte et al. (11)	Magg et al. (12)	Alazami et al. (13)	Atschekzei et al. (14)	Shamriz et al. (15)	Shayegan et al. (16)	Kim et al. (17)	Kurolap et al. (18)	Yonkof et al. (19)	Maccari et al. (20)	Bosa et al. (28)	This Study
Number of Patients (n)	6	4	4	5	7	3	4	1	1	1	2	1	4	15
Dermatitis (n, %)	6 (100)	2 (50)	4 (100)	3 (60)	6 (86)	3 (100)	1 (25)	1 (100)	0 (0)	1 (100)	1 (50)	1 (100)	2 (50)	14 (93)
Psoriasis-like lesions (n, %)	1 (17)	0 (0)	4 (100)	1 (20)	0 (0)	2 (67)	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	3 (20)
Photosensitivity (n, %)	1 (17)	0 (0)	2 (50)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7)
Viral cutaneous infections –Warts (n, %)	0 (0)	2 (50)	4 (100)	0 (0)	2 (29)	2 (67)	4 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	7 (47)
Viral cutaneous infections – Molluscum (n, %)	1 (17)	0 (0)	2 (50)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (20)
Skin abscesses (n, %)	2 (33)	1 (25)	3 (75)	0 (0)	7 (100)	3 (100)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (13)
Fungal cutaneous infections (n, %)	3 (50)	0 (0)	2 (50)	1 (20)	2 (29)	2 (67)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	6 (40)
Recurrent RTI (n, %)	6 (100)	4 (100)	3 (75)	5 (100)	5 (71)	3 (100)	0 (0)	0 (0)	1 (100)	0 (0)	1 (50)	1 (100)	2 (50)	10 (67)
Chronic diarrhea and IBD (n, %)	0 (0)	2 (50)	3 (75)	5 (100)	1 (14)	0 (0)	3 (75)	1 (100)	1 (100)	1 (100)	2 (100)	1 (100)	3 (75)	4 (27)
Asthma/chronic bronchitis (n, %)	3 (50)	0 (0)	4 (100)	1 (20)	2 (29)	2 (67)	2 (50)	1 (100)	0 (0)	0 (0)	2 (100)	0 (0)	1 (25)	8 (53)
Rhinitis/nasal congestion (n, %)	1 (17)	0 (0)	3 (75)	0 (0)	1 (14)	1 (33)	1 (25)	0 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (20)
Failure to thrive (n, %)	1 (17)	4 (100)	1 (25)	5 (100)	0 (0)	0 (0)	2 (50)	1 (100)	1 (100)	1 (100)	2 (100)	1 (100)	3 (75)	10 (67)
EBV-related smooth muscle tumor (n, %)	0 (0)	4 (100)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	2 (100)	0 (0)	0 (0)	2 (13)
EBV or CMV infection/viremia (n, %)	4 (67)	1 (25)	n.a.	n.a.	2 (29)	0 (0)	3 (75)	0 (0)	n.a.	n.a.	1 (100)	1 (100)	0 (0)	4 (27)

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Publications	Wang et al. (1)	Schober et al. (2)	Sorte et al. (11)	Magg et al. (12)	Alazami et al. (13)	Atschekzei et al. (14)	Shamriz et al. (15)	Shayegan et al. (16)	Kim et al. (17)	Kurolap et al. (18)	Yonkof et al. (19)	Maccari et al. (20)	Bosa et al. (28)	This Study
Eosinophilia (n, %)	1 (17)	n.a.	n.a.	n.a.	2 (29)	n.a.	n.a.	n.a.	1 (100)	1 (100)	1 (50)	1 (100)	n.a.	6 (40)
Normal/elevated IgG (n, %)	6 (100)	4 (100)	3 (75)	n.a.	7 (100)	3 (100)	4 (100)	n.a.	n.a.	1 (100)	2 (100)	0 (0)	2 (50)	10 (67)
Elevated IgE (n, %)	1 (17)	1 (25)	n.a.	n.a.	2 (29)	0 (0)	4 (100)	n.a.	n.a.	n.a.	0 (0)	0 (0)	n.a.	4 (27)
Elevated naïve $CD4^+$ T cells (n, %)	6 (100)	4 (100)	4 (100)	4 (100)	7 (100)	2 (67)	4 (100)	0 (0)	n.a.	1 (100)	2 (100)	0 (0)	2 (50)	12 (80)
Reduced memory B cells (n, %)	6 (100)	3 (75)	3 (75)	4 (100)	n.a.	3 (100)	n.a.	n.a.	n.a.	1 (100)	2 (100)	0 (0)	2 (50)	8 (53)
Reduced NK cells (n, %)	1 (17)	1 (25)	0 (0)	0 (0)	4 (57)	1 (33)	4 (100)	1 (100)	n.a.	0 (0)	1 (50)	0 (0)	1 (25)	11 (73)
Reduced Treg cells (n, %)	6 (100)	4 (100)	4 (100)	4 (100)	7 (100)	2 (67)	3 (100)	1 (100)	n.a.	1 (100)	2 (100)	1 (100)	n.a.	14 (93)
Mortality (n, %)	1 (17)	3 (75)	0 (0)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)

n.a.: Not available.

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