HHS Public Access

Author manuscript

J Allergy Clin Immunol. Author manuscript; available in PMC 2022 July 01.

Published in final edited form as:

J Allergy Clin Immunol. 2021 July ; 148(1): 256–261.e2. doi:10.1016/j.jaci.2020.12.629.

Two patients with chronic mucocutaneous candidiasis caused by *TRAF3IP2* deficiency

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Abstract

Background—TRAF3IP2 (Act1) is an adapter protein that interacts with IL-17R via its SEF/IL-17R (SEFIR) domain and coordinates two separate pro-inflammatory pathways following IL-17 cytokine stimulation.

Objective—To elucidate the immunological consequences of TRAF3IP2 homozygous mutations to improve treatments for immunodeficiency patients with chronic mucocutaneous candidiasis.

Methods—We describe two patients presenting with chronic mucocutaneous candidiasis that harbor biallelic nonsense mutations in TRAF3IP2. The cellular and molecular features of this genetic defect were assessed using *in vitro* cytokine assays and protein analysis.

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AUTHOR CONTRIBUTIONS

SS, YY, and WAC performed experiments and interpreted results. SS, SAC, WAC, and MJL wrote the paper. AO, EKA, and SB were responsible for care of Pt. 1 and AO contributed the patient history. YZ performed WGS analyzed variants for Pt. 1. HC, SA, and AK were responsible for care of Pt. 2 and AK provided the patient history. GY performed genetic workup for Pt. 2. MJL coordinated the overall direction of the study. All authors read and provided appropriate feedback on the submitted manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Results—We show the homozygous mutation causes complete loss of protein expression. We also show that the absence of TRAF3IP2 was associated with a defective response to combined IL-2/IL-25 (IL-17E) stimulation.

Conclusion—Failure to initiate normal signaling downstream of IL-17R engagement likely contributes to the patients' recurrent fungal infections. These findings add to our molecular understanding of genetic defects affecting this critical pathway of anti-fungal immunity.

Keywords

TRAF3IP2; Act1; IL-17; signaling; Chronic mucocutaneous candidiasis; anti-fungal immunity; recurrent fungal infections

Introduction:

Interleukin (IL)-17-mediated responses are necessary for protective mucosal immunity against fungal pathogens such as *Candida albicans*. Chronic mucocutaneous candidiasis (CMC) is a noninvasive and persistent *Candida* infection of the skin and oral surfaces that can occur when IL-17 signaling is deficient [1]. The *TRAF3IP2* gene is expressed ubiquitously in human tissues and encodes the 574 amino acid long TRAF3 interacting protein 2 (TRAF3IP2, previously known as CIKS or Act1) [2]. TRAF3IP2 is an adapter protein that interacts with IL-17R via its"similar expression to fibroblast growth factor genes and IL-17R (SEFIR) domain and coordinates two distinct pro-inflammatory pathways following IL-17 stimulation. The first pathway induces nuclear factor-kappaB (NF-κB) activity, while the second leads to activation of mitogen-activated protein kinases (MAPKs) p38 and ERK which stabilizes the *CXCL1* and *CXCL2* transcripts [2]. Given its critical role in multiple pro-inflammatory pathways, there is growing evidence that genetic changes in *TRAF3IP2* underlie human immune-mediated diseases. An early study showed that deleterious missense mutations in the SEFIR domain of *TRAF3IP2* lead to CMC in humans [3]. A more recent study reported biallelic nonsense *TRAF3IP2* variants in a patient with CMC but did not conclusively show their effects at the mRNA or protein level [4]. Here we describe two unrelated patients who presented with CMC and who harbor biallelic nonsense mutations in *TRAF3IP2*, enabling us to evaluate the physiological effects of complete loss of TRAF3IP2 protein expression.

Results and Discussion:

Patient (Pt. 1) is a 15-year-old Turkish male born to consanguineous parents who presented at 2 months of age with severe thrush and treated with a one-month course of intravenous antifungal treatment (Fig. 1a). At the time of active disease, direct microscopic examination of a smear from the oral monilial lesions demonstrated fungal hyphae. He was diagnosed with CMC and pityriasis lichenoides chronica. Recurrent episodes of candidiasis primarily affected the mouth and tongue and often affected the fingers, neck, or intertriginous areas (Fig. 1b). Over time, he was placed on prophylactic antifungal treatment with oral fluconazole, which led to amelioration of his symptoms. In addition to fungal infections, he had other cutaneous manifestations, including scanty psoriasis-like plaques and desquamation over the scalp, knee, and inguinal areas; erythematous lesions at axillary and

retroauricular locations; and livedo reticularis on the thighs (Fig. 2a). His hair was mildly discolored in some regions. From an unrelated family, Pt. 2 is a 10-year-old Turkish girl born to consanguineous parents who presented at 1 year of age with treatment-resistant oral thrush, angular cheilitis, and aphthous stomatitis (Fig. 1a,b). Pt. 2 also suffered from chronic intertrigo and folliculitis on her trunk but had no scalp, nail, or tooth abnormalities (Fig. 2b). Fluconazole treatment and prophylaxis was effective and did not lead to resistance. For both patients, moniliasis recurs upon interruption of prophylaxis. Neither patient exhibited increased susceptibility to bacterial or viral infections and immunophenotyping of peripheral blood mononuclear cells (PBMCs) revealed normal lymphocyte subsets and minor immune abnormalities in the patients (Table 1). Serum immunoglobulin levels were normal, and both patients are within normal height and weight ranges for their ages.

To investigate genetic contributions to CMC in these patients, we performed whole genome sequencing of DNA isolated from Pt.1 PBMCs, as well as DNA from his unaffected parents and brother. For Pt. 2, we used next-generation sequencing to target CMC-associated genes (*IL17F*, *IL17RA*, *IL17RC*, and *TRAF3IP2*) in DNA from patient PBMCs and carried out DNA sequencing by the Sanger method of the *TRAF3IP2* gene in DNA from her unaffected sister and health donors (Fig.1c). Both patients harbored rare, homozygous nonsense mutations in *TRAF3IP2* (NM_147686.3:c.559C>T,p.Arg187*) that segregated with disease in both kindreds that were heterozygous in the parents (Table 2). Homozygosity for this variant is not observed in healthy individuals reported in gnomAD, and this variant is not observed in a healthy Turkish population in the Greater Middle Eastern Variome database [5, 6]. It is worth noting that Pt. 1 also carried homozygous frameshift mutations in the second-to-last exon of the gene encoding THEMIS, a molecule that regulates TCR signal strength and is critical for thymocyte development and survival (Fig. 2c) [7, 8]. We confirmed the homozygous and heterozygous frameshift mutations in patient and sister, respectively (Fig. 2d). The levels of *THEMIS* transcripts were moderately reduced in Pt. 1 PBMCs, and lysates contained a slightly truncated protein product that could not be detected with a C-terminal targeted antibody (Fig. 2e,f). However, the patient did not display abnormal T cell subsets or increased sensitivity to restimulation-induced cell death (RICD) after TCR re-engagement, as would be expected for loss of THEMIS protein function, suggesting that this mutation may not account for the disease (Fig. 2g and Table 1).

We predicted that premature termination in the second exon of *TRAF3IP2* transcripts would lead to loss of TRAF3IP2 at the protein level. Indeed, immunoblot of lysates derived from Pt. 1 T cells revealed a complete loss of protein, and reduced mRNA levels suggesting nonsense-mediated decay (Fig. 1d,e). Due to sample unavailability, we were unable to perform direct tests on Pt. 2 cells, but we infer that since it was the same variant, there would be identical defects. We therefore investigated the effects of protein loss on IL-17 signaling in Pt. 1 lymphocytes. The p.T536I substitution in TRAF3IP2, reported by Boisson et al., was shown to impair IL-5 and IL-13 production after co-stimulation with IL-2 and IL-25 [3]. We observed this same defect in PBMCs from Pt. 1, which showed a good cytokine response to IL-2 stimulation alone but no synergistically enhanced response following combined IL-2/IL-25 stimulation (Fig. 1f). Consistent with the cell stimulation, IL-5 and IL-13 were present at a lower level in patient's serum (Fig. 1g) with relatively normal levels of other T helper (TH) cell cytokines (Fig. 2h). We noticed the amount of IL-22 in patient was much higher in

patient (Fig. 2h), this is may because TRAF3IP2 patient carried a higher IL-22 producing T cells [3]. We also observed a higher proportion of IL-17A producing TH17 cells in patients (Fig. 1h), which is consistant with previous TRAF3IP2 patient [3]. But we detected a lower proportion of IFN-γ producing TH1 cells in our patients, which may reflect a TH1/TH17 counterbalance *in vivo*. To further clarify the proportion of TH cells in our TRAF3IP2 patient, we also measured Treg cells and IL-13-producing TH2 cells. We found that the patient presented a higher proportion of Treg cells and a lower proportion of TH2 cells (Fig. 1h and Fig. 2i). Failure to initiate signaling downstream of IL-17R engagement likely underlies this defective IL-25 response, which could contribute to the patients' CMC. IL-25 signals though the IL-17 receptor which requires TRAF3IP2 to transmit a signal. This defect will be present for all IL-17R-mediated signaling. Defective IL-17 signaling in epithelial cells likely contributes importantly to susceptibility to candidiasis.

TRAF3IP2 has also been associated with immune-mediated diseases other than CMC (Table 3). One GWAS study associated a single nucleotide polymorphism (SNP) (rs33980500, p.D10N) in the TRAF6 binding site with increased risk of psoriasis and psoriatic arthritis [9]. A recent report of patients with biallelic missense mutations (p.T438N) in the SEFIR domain demonstrated a causal relationship between TRAF3IP2 loss-of-function and development of scalp discoid lupus erythematosus and folliculitis [10]. Interestingly, TRAF3IP2 knockout mice do not completely recapitulate phenotypes observed in human TRAF3IP2 deficiency (Table 3). For example, one of the earliest mouse models indicated that complete knockout of *TRAF3IP2* or B cell-specific deletion resulted in B cell abnormalities and severe autoimmunity, explained by hyperactive NF-κB activation upon CD40/BAFF-R stimulation [11]. More recent models differ in that the mice manifested either with no disease or with hyper IgE, lymphadenopathy and splenomegaly, and atopic dermatitis in the absence of overt B cell anomalies [12, 13].

In contrast to the first *TRAF3IP2* knockout mouse, our patients and those reported previously did not manifest B cell abnormalities or autoimmune features indicative of dysregulated CD40/BAFF-R signaling. Boisson et al. reasoned that the T536I point mutation in TRAF3IP2 specifically disrupts interaction with IL-17R, leaving CD40 and BAFF-R interactions intact. Bhattad et al. report premature stop mutations leading to CMC without B cell abnormalities but were unable to definitively show complete loss of TRAF3IP2 protein in patient cells. Our work reveals that even complete loss of TRAF3IP2 protein in humans does not impact B cell survival but manifests as an inborn error of IL-17 immunity leading to CMC due to insufficient antifungal immunity.

MATERIALS AND METHODS

Human subjects

Written informed consent was provided by all human subjects (or their legal guardians) in accordance with Helsinki principles for enrollment in research protocols that were approved by the Institutional Review Board of the National Institute of Allergy and Infectious Diseases, National Institutes of Health. Patient and healthy control blood was obtained at the National Institutes of Health under approved protocols. Mutations will be automatically

archived by Online Mendelian Inheritance in Man (OMIM) at time of publication and whole-genome data will be submitted in dbGaP.

Genetic analysis methods

For Patient 1 (Pt. 1) and family members, genomic DNA was obtained from probands and family members by isolation and purification from peripheral blood mononuclear cells (PBMCs) using Qiagen's DNeasy Blood and Tissue Kit. DNA was then submitted for whole-genome sequencing (WGS) through a collaboration with MERCK Pharmaceuticals. WGS was performed by massively parallel sequencing by Illumina HiSeq sequencing system on the collected DNA. For individual samples, WGS produced 30× to 60× average sequence coverage. All sequenced DNA reads were mapped to the hg19 human genome reference by Burrows-Wheeler Aligner with default parameters. Single nucleotide variant and indel calling were performed using the Genome Analysis Toolkit version 3.4 (the Broad Institute). Variants were then annotated by variant effect predictor and prioritized by GEMINI (GEnome MINIng) on the basis of population allele frequency, functional prediction, and potential genetic models (autosomal-recessive or de novo models). These methods were previously described.[14]

Primary Cells and Human Serum

Patient or control blood was subjected to a Ficoll density gradient centrifugation, after which PBMCs were collected from the interface and washed extensively in PBS. PBMCs were then used directly or pan T cells were expanded by the addition of 1 ug/mL anti-CD3 and 1 ug/mL anti-CD28 to PBMCs at 1×10^6 cells/mL in complete RPMI. After 72 hours, stimulatory antibodies were washed out and cells were placed in and maintained in fresh complete RPMI supplemented with 100 I.U./mL IL-2 resulting in activated, cycling T cells that we term, "T cell blasts." Human serum was collected from healthy donors at the NIH and pooled (at least three donors) and heat shocked (56°C for 30 minutes) prior to use.

FOXP3 and Intracellular cytokine staining

Isolated PBMCs were treated with PMA (100ng/ml) plus Ionomycin (500ng/ml) with the presence of monensin (BioLegend Cat. No. 420701) in the 5-6 hours of cell culture activation. The cells were suspended and stained with surface marker first for 30 minutes at room temperature. Intracellular cytokine staining was carried out by using Fixation/ Permeabilization Solution Kit (BD Biosciences, 554714). FOXP3 staining was done by using Foxp3 / Transcription Factor Staining Buffer Set (Thermo Fisher, 00-5523-00).

Media

Human T cell blasts were grown in RPMI 1640 (Gibco/Thermo Fisher, Waltham, Mass) supplemented with 10% heat-inactivated FBS (Gibco), 1% penicillin/streptomycin (Gibco), 1% L-glutamine (Gibco), 50 μM 2-mercaptoethanol (Sigma-Aldrich, St Louis, Mo), and either with or without 100 I.U./mL IL-2. Human PBMCs were stimulated with IL-2 and IL-25 in X-Vivo15 media supplemented with 1% penicillin/streptomycin (Gibco), 1% L-glutamine (Gibco), 50 μM 2-mercaptoethanol (Sigma-Aldrich, St Louis, Mo), and 5% human serum.

Antibodies and reagents

Anti-CD3 (clone: HIT3a) and anti-CD28 (clone: CD28.2) antibodies for T cell stimulation were purchased from Biolegend. Anti-TRAF3IP2 antibody was purchased from Ebioscience (Cat #14-4040-80), anti-THEMIS was from Thermo Fisher (Cat #PA5-56740), anti-β-tubulin was from Millipore Sigma (Cat #05-661), and anti-GAPDH was from Cell Signaling Technology (Cat #2118S). AlexaFluor-conjugated secondary antibodies were purchased from Thermo Fisher Scientific (Cat #32735 and Cat #32729).

Restimulation-induced cell death assays

Patient or control T cell blasts, between 2 and 3 weeks after initial activation, were collected and resuspended in fresh IL-2-containing media. Cells were then added to 96-well plates in triplicate and stimulated with the indicated dose of anti-CD3 (clone: HIT3a, BioLegend) in the presence of 1 ug/mL Protein A for 18 hours, as previously described.[15] Cells were then collected by repeat pipetting and stained with AnnexinV-APC and propidium iodide and analyzed by flow cytometry. The percentage of live cells was determined by AnnexinV and propidium iodide staining and expressed as a percentage of cells lost in the control condition due to TCR restimulation.

IL-25-induction of Th2 cytokines

IL-25-induced IL-5 and IL-13 production was measured as previously described.[3, 16] Briefly, PBMCs from the patient or healthy controls were resuspended to 1×10^6 cells/mL in complete X-VIVO15 media and 1×10^5 cells were transferred in triplicate to 96-well round bottom plates. Cells were then left untreated or treated at a final concentration of 0.5×10^5 cells/mL with 50 I.U./mL IL-2 with or without recombinant human IL-25 (IL-17E) (R&D systems, 8134-IL) at the indicated final concentrations for 7 days at 37°C. Supernatants were then analyzed using the LEGENDplex™ Human Th Panel (13-plex) to assess IL-5 and IL-13 levels. Values are normalized to the average control level after IL-2 stimulation.

Quantitative RT-PCR

Quantitative RT-PCR was performed as described previously.[14] Briefly, total RNA was isolated with the RNeasy kit (Qiagen, Gaithersburg, MD). cDNA was synthesized from 1 μg of total RNA using the SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific). cDNA was brought to a total volume of 100 μL in double-distilled H_2O and either immediately used or stored at −80°C for future use. Quantitative RT-PCR for TRAF3IP2, THEMIS, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was performed using the Power SYBR Green (Thermo Fisher) method on a 7900HT machine (ABI) using 2.5 μL of cDNA reaction product. Results were analyzed by using the $\Delta\Delta C_t$ method and normalized to the first healthy normal donor values.

GAPDH Forward	GATGACATCAAGAAGGTGGTG
GAPDH Reverse	ACCACCTGGTGCTCAGTGTAG
Themis Forward	CAGGCATCTATCTTGAAGGCTCT

Themis Reverse	GAAGCAAGGATGCCCTAGTCT
TRAF3IP2 Forward	TGGATCTAAGCCTCATCTGTATCC
TRAF3IP2 Reverse	AACCACAGACTCAGACGCAG
TRAF3IP2 N-terminal Forward	GAACCGAAGCATTCCTGTGGAGG
TRAF3IP2 N-terminal Reverse	CTGTTGGGTGCCATGTTCCTTATATTTGG

Immunoblotting

To analyze total TRAF3IP2 and THEMIS protein levels, T cell blasts were washed in PBS and lysed in 1× Triton lysis buffer with complete protease inhibitor cocktail (Roche, Basel, Switzerland) on ice for 20 minutes. The lysates were then cleared at 14,000 × *g* at 4°C for 15 minutes. Lysates were then diluted with 2X SDS sample buffer (Quality Biologicals, Gaithersburg, MD) supplemented with 10% BME. Approximately 2×10^6 cell equivalents were separated by SDS-PAGE on 4% to 20% precast tris-glycine gels (Invitrogen) and transferred to a nitrocellulose membrane (Invitrogen). Membranes were blocked with 3% BSA in Tris-buffered saline with 0.01% Tween-20 (TBST) for 30 minutes at room temperature before incubating with primary antibody overnight at 4°C. After 3× 5-minute washes with TBST at room temperature with rocking, fluorescent secondary antibody was added for 2 hours at room temperature. After 5× 5-minute washes in TBST and 1 wash in PBS, membranes were analyzed on the LI-COR Odyssey imaging system or the Azure c300 imaging system.

Statistical Analysis

Graphs are generated by averaging at least 3 different experiments. Comparative statistics were not performed between patients and controls because patient samples represent biological repeats, not independent biological samples. To compare cytokine production between control samples a paired non-parametric Wilcoxon matched-pairs signed rank test was used to compare treated and untreated samples. $*p < 0.05$, $**p < 0.01$.

ACKNOWLEDGMENTS

The authors would like to thank Andrew Oler, Ke Huang, Sandhya Xirasagar, Darrell Hurt, and other members of the Bioinformatics and Computational Biosciences Branch (BCBB), NIAID for variant assessment and bioinformatics support. We thank MERCK Pharmaceuticals for performing whole genone sequencing and allowing us to access to their patient information. Finally, we thank the patient, family members, and healthy controls for participating in this study, and without whom it would not be possible to conduct this research.

FUNDING

This work was supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health. This work was also funded by a fellowship grant (1-16-PDF-025, to Dr. Comrie) from the American Diabetes Association, a F12 postdoctoral fellowship (1FI2GM119979-01, to Dr. Comrie) from the National Institute of General Medical Sciences, NIGMS, NIH, The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Abbreviations

NF-κB	Nuclear Factor-kappaB

TRAF3IP2	TRAF3 interacting protein 2
SEFIR	“similar expression to fibroblast growth factor genes and IL-17R”
CMC	Chronic Mucocutaneous Candidiasis
PBMCs	Peripheral Blood Mononuclear Cells
RICD	Restimulation-Induced Cell Death
SNP	Single Nucleotide Polymorphism
IL-25	IL-17E
PMA	Phorbol myristate acetate

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Capsule Summary

We show that complete loss of TRAF3IP2 protein in humans does not impact B cell survival but manifests as an inborn error of IL-17 immunity leading to chronic mucocutaneous.

Clinical Implications

Our aim is to help with the diagnosis and treatment of immunodeficiency patients by elucidating the molecular mechanisms leading to chronic fungal infections.

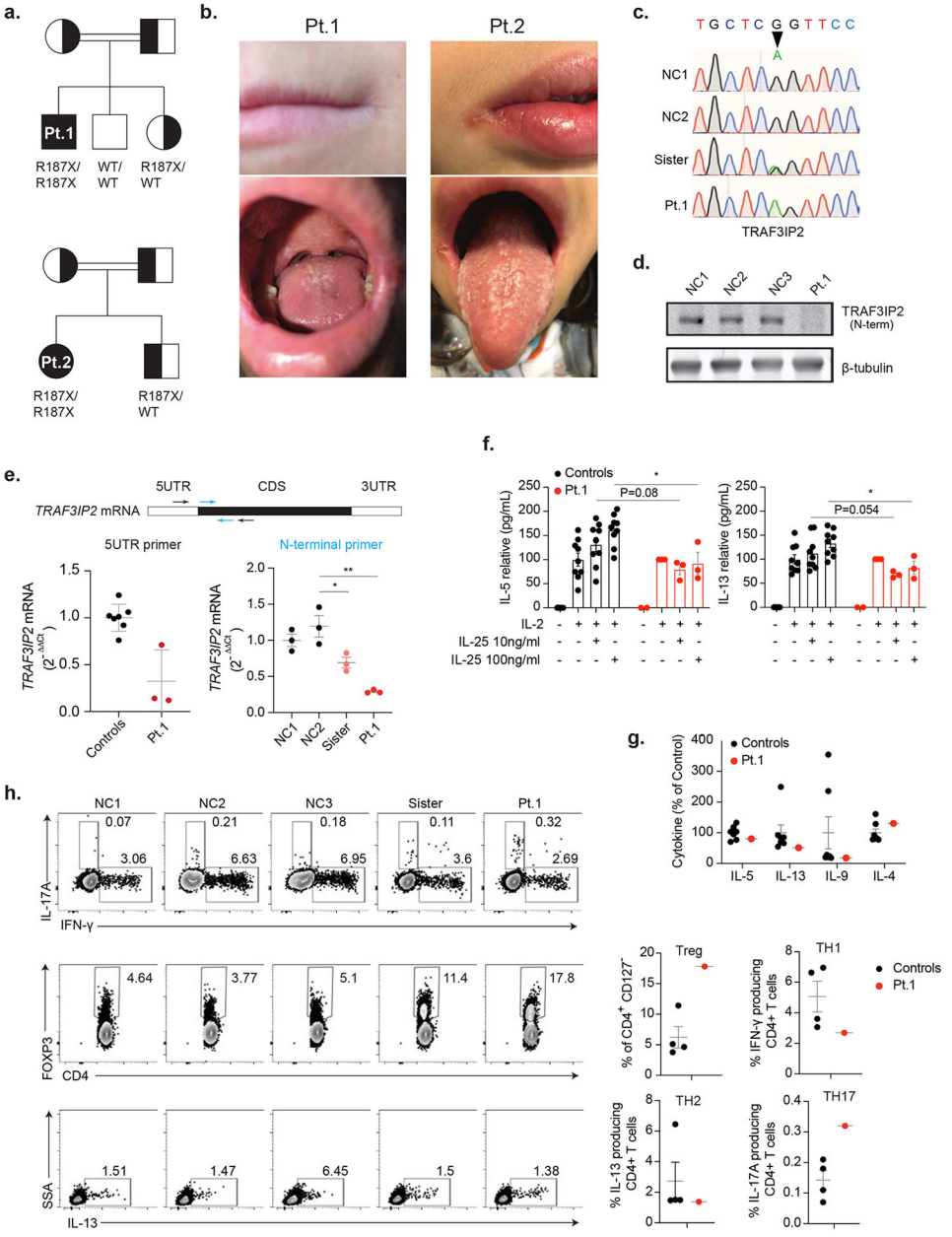

Figure 1.

(a) Pedigrees for Patient 1 (Pt. 1) and Pt. 2 showing *TRAF3IP2* allele inheritance. Double lines indicate consanguinity. X indicates stop codon; WT is wild-type sequence. **(b)** Angular cheilitis (top row) and oral thrush (bottom row) in Pt. 1 and Pt. 2. **(c)** TRAF3IP2 genomic DNA sequence electropherograms, for two normal controls (NC), sister and patient. Arrow shows the missense mutation. **(d)** Immunoblot showing TRAF3IP2 protein expression in T cell blasts with β-tubulin loading control. **(e)** The N-terminal primer set to show the *TRAF3IP2* mRNA levels in control and Pt. 1 T cell blasts. Each control dot represents an individual donor while each patient dot represents a repeat measurement from an independent experiment. **(f)** Peripheral blood mononuclear cells (PBMC) were cultured for 7 days with exogenous IL-2 and IL-25 at the indicated concentrations and were measured

using a multiplex cytokine assay. Cytokine concentrations were normalized to 100 pg/ml with IL-2 only stimulation. **(g)** The indicated cytokines from controls and patient were measured using a multiplex cytokine assay. **(h)** Flow cytometric analysis of Treg cells, IL-17A, IFN-γ and IL-13 producing CD4+ T cells. Statistical comparison is shown on the right. n.s.: not significant, *$p < 0.05$, **$p < 0.01$.

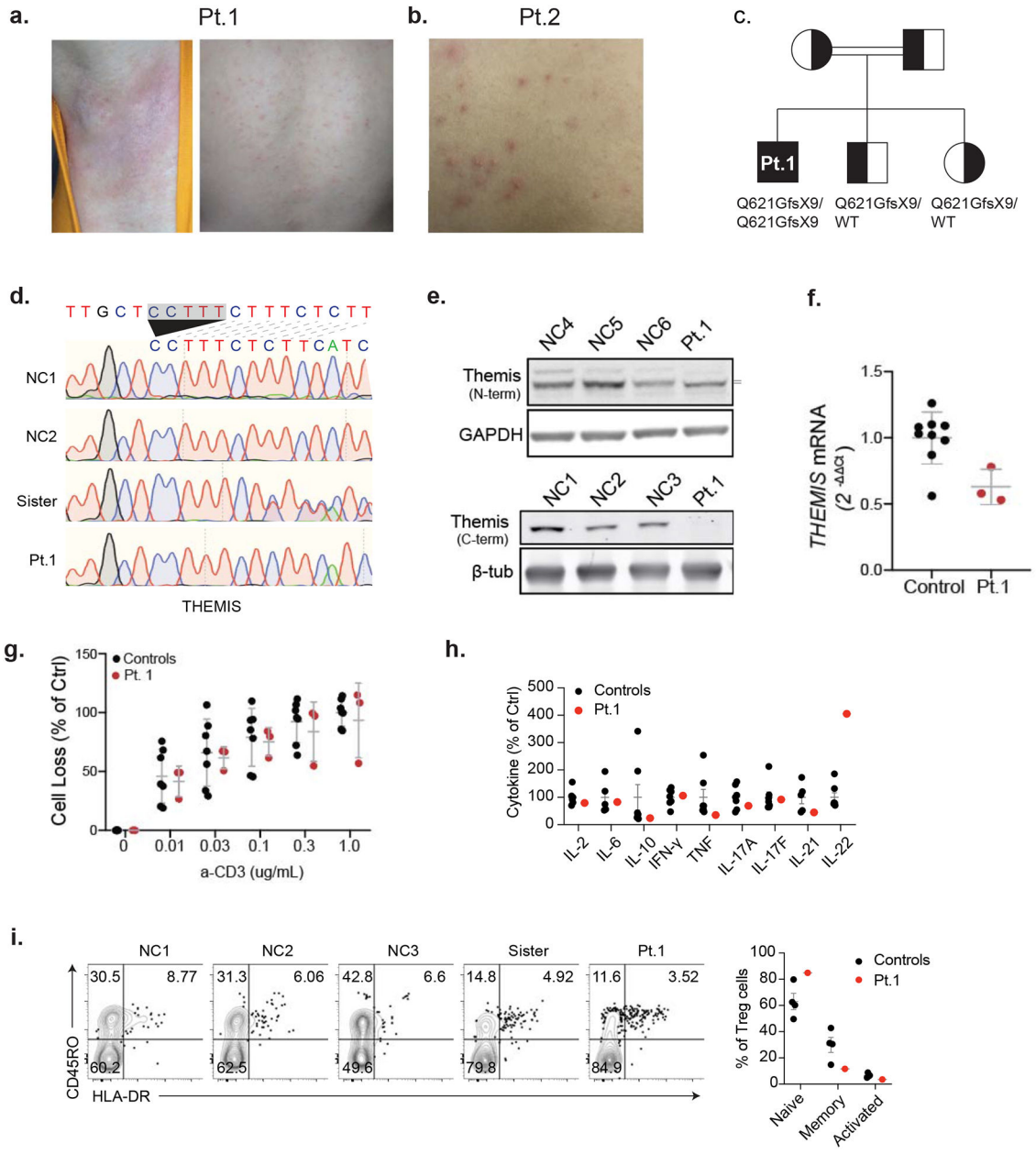

Figure 2.

(a,b) Erythematous lesions on axillary skin (left) and pityriasis lichenoides (right) in Patient 1 (Pt. 1). Folliculitis on the trunk of Pt. 2. **(c)** Family pedigree for Pt. 1 showing *THEMIS* allele inheritance. Double lines indicate consanguinity. **(d)** THEMIS genomic DNA sequence electropherograms for normal controls (NC), sister and patient. Arrow shows the deletion. **(e)** Immunoblot of lysates derived from T cell blasts using an N-terminal THEMIS antibody protein with GAPDH loading control (Top). Immunoblot using C-terminal directed antibody with β-tubulin loading control (bottom). **(f)** *THEMIS* mRNA levels in control and Pt. 1 T cell blasts. Each control dot represents an individual donor while each patient dot represents a repeat measurement from an independent experiment**. (g)** Cell death of T cell blasts proliferating in IL-2 following restimulation with the indicated dose of soluble cross-

linked anti-CD3 for 18 hours. Each control dot represents an individual donor while each patient dot represents a repeat measurement from an independent experiment**. (h)** The indicated cytokines in serum from NC, healthy sister, and patient were measured using a multiplex cytokine assay. **(i)** Isolated PBMCs were stained with FOXP3 and surface maker CD45RO and HLA-DR. Statistical comparison is shown on the right.

Table 1

Key features of patients' clinical disease and immunological workup.

Demographics	**Pt. 1**	**Pt. 2**
Y.O.A. (as of 10/2019)	15	10
Sex	male	female
Ethnic background of parents	caucasian	caucasian
Primary Diagnosis	**Chronic mucocutaneous candidiasis, recurrent skin rash, pityriasis lichenoides**	**Chronic mucocutaneous candidiasis**
Age at onset/presentation	2 months	1 year
Secondary diagnoses	Moniliasis episodes recur as soon as prophylaxis is interrupted. Experiences cutaneous rash at extremities, neck and intertriginous skin.	Chronic intertrigo, folliculitis
Lymphadenenopathy	no	no
Splenomegaly	no	no
Hematologic abnormalities	no	no
Malignancy/lymphoma	no	^no
Autoimmunity or autoinflammatory disease	no	no
Immunodeficiency or susceptibility to infection	yes, chronic mucocutaneous candidiasis, recurrent/ destructive Otitis Media	yes, chronic mucocutaneous candidiasis
Liver abnormalities	no	no
Antibody Abnormalities	no	no
Lung involvement	no	no
Height (percentile)	10–25th	25–50th
Weight (percentile)	50th	25–50th
Other significant symptoms	no	no
Immunological Workup		
Quantitative Immunoglobulins (mg/dl)	IgG: 1260 (876–2197) IgA: 73 (108–447) IgM: 75 (75–448) IgG1: 758 (547–1474) IgG2: 363 (77–706) IgG3: 35 (36–238) IgE: 686 ng/ml	IgG: 1490 (431–1803) IgM: 87 (126–449) IgA: 138 (45–164)
CBC with differential	WBC: 10800 (4500–13000) ANC: 6000 (1500–8000) ALC: 2800 (1400–4500) Eo: 1000 (44– 400) Hb: 11.4 (11– 16) Plt: 338000 (150000– 400000)	WBC: 8500 (4500–13800), ANC: 2700 (1500–8600), ALC 4900 (1800–5600), Eo: 100, Hb 9.6 (11–14), Plt 335000 (182000–564000)
Flow Cytometry	CD3: 50%(47–76) CD3+4: 33% (23–48) CD3+8: 27% (25–50) CD19: 30% (14–44) CD16+56: 22% (4–23)	$CD3^+$: 50% (55–88), $CD3^+4^+$: 43% (24–50), $CD3^+8^+$:16% (17–42), $CD19^+$:14% (7–22), $CD3^-16/56^+$:14% (4–29)

Table 2

Rare homozygous variants in Pt. 1, identified by WGS using a recessive model of disease inheritance from a consanguineous family.

Gene	Chr	Exon	Ref	Alt	DNA change	Protein Change	Worst Annotation
RNF223	1:1007306	Exon 2/2	G	A	c.641 C>T	p.Pro214Leu	missense
RNF223	1:1007730	Exon 2/2	C	T	c.217G>A	p.Val73Ile	missense
ATPSCKMT (FAM173B)	5:10239295	Exon 2/5	G	A	c.190C>T	p.Arg64Ter	nonsense
TRAF3IP2	*6:111912731*	*Exon 2/9*	*G*	*A*	*c.559C>T*	*p.Arg187Ter*	*nonsense*
TBC1D32	6:121602737	Exon 14/32	T	C	c.1561A>G	p.Ile521 Val	missense
THEMIS	6:128040861	Exon 6/7	CCTTT	C	c.1979_1982delAAAG	p.Glu621GlyfsTer9	frameshift/stop
USPL1	13:31195297	Exon 2/9	T	C	c.20T>C	p.Ile7Thr	missense
HHIPL1	14:100118886	Exon 2/8	G	A	c.581 G>A	p.Arg194His	missense
GPR132	14:105517528	Exon 3/3	G	A	c.946C>T	p.Arg316Cys	missense
MYBPC2	19:50939323	Exon 4/28	C	T	c.251 C>T	p.Pro84Leu	missense
MYBPC2	19:50946971	Exon 11/28	C	T	c.1031 C>T	p.Pro344Leu	missense
POLA1	X:24757653	Exon 20/37	A	G	c.2184A>G	p.Ile728Met	missense
WNK3	X:54278026	Exon 14/24	T	A	c.2462A>T	p.His821 Leu	missense
THOC2	X:122747528	Exon 35/39	G	A	c.4481 C>T	p.Pro1494Leu	missense

Gene	Exac Freq.	Patient	Dad	Mom	Brother	OMIM
RNF223	0.00186776	G/A	G/A >	G/G	G/G	none
RNF223	0	C/T	C/C	C/T	C/T	none
ATPSCKMT (FAM173B)	0.00026499	A/A	G/A	G/A	G/A	none
TRAF3IP2	*8.28E-06*	*A/A*	*G/A*	*G/A*	*G/G*	*615527, familial Candidiasis*
TBC1D32	0.00018284	C/C	T/C	T/C	T/T	none
THEMIS		C/C CC	TTT/C	CCTTT/C	CCTTT/C	none
USPL1	0.00094032	C/C	T/C	T/C	T/C	none
HHIPL1	0.00062418	A/A	G/A	G/A	G/A	none
GPR132	0.00010728	A/A	G/A	G/A	G/G	none
MYBPC2	0.00011153	C/T	C/C	C/T	C/T	none
MYBPC2	7.46E-05	C/T	C/T	C/C	C/C	none
POLA1	0	G/G	A/A	A/G	A/A	301220,301030 Van Esch-O'Driscoll syndrome
WNK3	0	A/A	T/T	T/A	T/T	none

Gene	Exac Freq.	Patient	Dad	Mom	Brother	OMIM
THOC2	0	A/A	G/G	G/A	G/G	300957, Mental Retardation- X linked

Table 3.

Summary of literature related to TRAF3IP2 deficiency, including mouse models and previously reported cases of humans harboring deleterious mutations. KO: knockout, Hypergam: hypergammaglobulinemia, LAD/SM: lymphadenopathy and splenomegaly, CMC: chronic mucocutaneous candidiasis, PNA: pneumonia.

Cited	Organism Studied	TRAF3IP2 Protein Alteration	Variant effect on TRAF3IP2 protein	Increased B cells	Autoimmunity	Hypergam	Hyper IgE	LAD/ SM	Atopic dermatitis	CMC	Bronchiectasis / PNA	Scalp lesions +/− alopecia	Folliculitis	Psoriasis (or psoriasis-like skin disease)
Qian, 2004	mouse	full KO and B-cell specific KO	no protein (not shown)	+	+	+		+						
Matsushima, 2010	mouse	C-term truncation	no protein				+	+	+					
Claudio, 2009	mouse	full KO	no protein (not shown)											
Huffmeier, 2010	human	D10N missense in TRAF6 binding	full-length protein (not shown)											+
Boisson, 2013	human	T583I missense in SEFIR domain	full-length protein						+	+				
Bhattad, 2019	human	R283X	truncated protein (not shown)			+ (from infections)				+	+			
Nemer, 2020	human	T438N missense in SEFIR domain	full-length protein									+	+	
Pt. 1 (this report)	human	**R187X**	**no protein**							+		+		+
Pt. 2 (this report)	human	**R187X**	**no protein (not shown)**							+			+	