Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

in comparison with the intraparticipant variance of 15.3, as shown in sequential Bland-Altman plots in Fig E1 in this article's Online Repository at www.jacionline.org. This is consistent with low variability of periostin levels within subjects in a clinical sample representing a well-controlled adult asthmatic population. The 95% CI for individual predictions of periostin levels were plus or minus 7.7 ng/mL. The mean coefficient of variation for periostin for the 60 participants was 6.3% (95% CI, 5.7% to 7.0%). There was no evidence of monthly ($P = .56$, see Table E4 in this article's Online Repository at www.jacionline.org) or seasonal ($P = .90$. see Table E5 in this article's Online Repository at www.jacionline.org) variation between participants. Five participants had a severe asthma exacerbation during the study and required systemic corticosteroids (Fig 2) with a mean dose of 35 mg/d (range, 20-60 mg/d; courses lasting from 1-16 days; see Table E6 in this article's Online Repository at www.jacionline.org). The mixed linear model estimate of the difference in periostin levels between the first measurement after a severe exacerbation requiring systemic corticosteroids and the pre-exacerbation measurements for these 5 participants was −5.4 ng/mL (95% CI, −8.3 to −2.6 ng/mL; $P = .002$). The difference in periostin levels between the postexacerbation and pre-exacerbation levels was −4.5 ng/mL (95% CI, −7.0 to −1.9 ng/mL [$P < .001$]; which is smaller than the 95% CI for an individual prediction of periostin levels).

In summary, we found little variability in periostin measurements within subjects over an 8-week period. The within-participant variance was 15.3, with a 95% CI for periostin levels of ±7.7 ng/mL. These findings in adults receiving step 4 treatment according to Global Initiative for Asthma guidelines[9] are consistent with those of studies performed in populations with severe asthma over a period of 2[4] and 5[1] weeks, respectively. These studies reported a coefficient of variation rather than variance components, with point estimates of 5.0% and 5.3%, respectively. This is consistent with the low intraparticipant variability in periostin measurements observed in this study, in which the coefficient of variation was 6.3%. We found no evidence of a monthly or seasonal fluctuation in serum periostin levels between participants who were recruited over a 12-month period. Therefore it might not be necessary to consider seasonality when assessing periostin levels in candidates for treatment with the novel asthma biologic therapies. Periostin levels are modestly reduced by systemic corticosteroid treatment of a severe asthma exacerbation, but the effect lies within the level of variation expected in otherwise stable adults with asthma.

In conclusion, we propose that the stability of periostin measurements supports its use as a biomarker in asthmatic patients, predicting responsiveness to biologic therapy directed against type 2 disease.

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Morpholino-based correction of hypomorphic *ZAP70* mutation in an adult with combined immunodeficiency

To the Editor:

The ζ-associated protein of 70 kDa (ZAP-70) is a cytoplasmic tyrosine kinase critical for intracellular signaling downstream of the T-cell receptor (TcR). Biallelic mutations in *ZAP70* cause combined immunodeficiency (CID). Classically, ZAP-70 deficiency results from null alleles and presents with failure to thrive and severe/recurrent infections in infancy (ie, severe CID) that is marked by absent circulating $CD8^+$ T cells, quantitatively normal yet dysfunctional $CD4^+$ T cells accounting for hypo-/dysgammaglobulinemia, with quantitatively intact B cells and natural killer cells and requires hematopoietic stem cell transplant for survival beyond early childhood[1-5] (see Table E1 in this article's Online Repository at www.jacionline.org). "Leaky" ZAP-70 deficiency has been reported

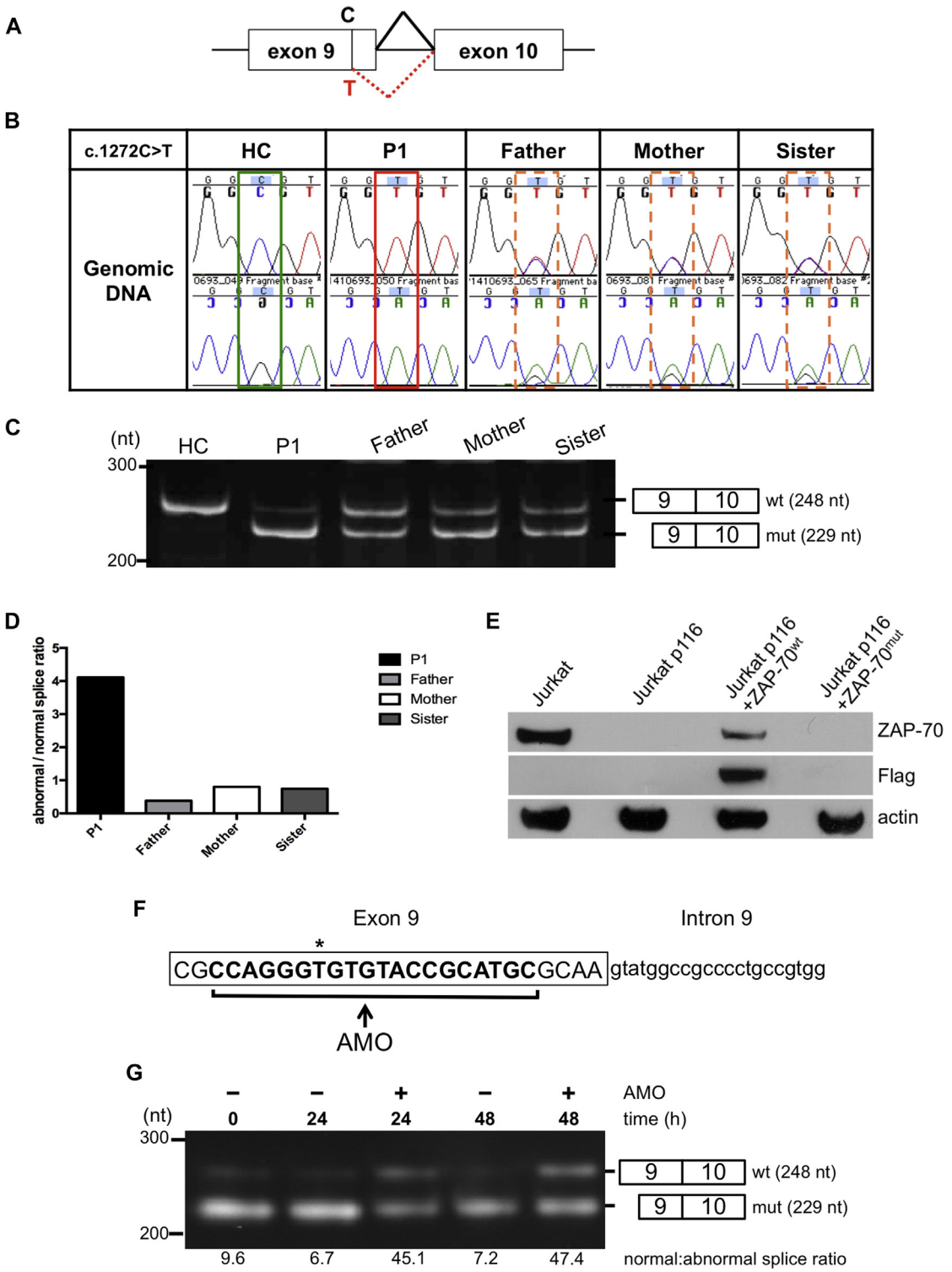

FIG 1. **A,** The c.1272C>T ZAP-70–altered splice site. **B,** Electropherograms from a healthy control (HC), P1, and P1's family. **C,** RT-PCR products from an HC, P1, and P1's family. **D,** Summary of densitometry analysis. **E,** Immunoblot of ZAP-70^{wt} or ZAP-70^{mut} transfected cells. **F,** The AMO binding site within the ZAP-70 pre-mRNA. An asterisk denotes the c.1272C>T mutation. **G,** RT-PCR products of AMO-treated P1 PBMCs.

only once: a 9-year-old boy with nonsevere skin and lung infections had a homozygous intronic mutation producing a novel splice acceptor site, resulting in predominant expression of a frame-shifted mutant protein but with residual levels of wild-type (WT) protein.[6] Recently, a sibling pair manifesting only early-onset autoimmune diseases, without opportunistic infections, was shown to be compound heterozygous for a hypoactive allele and a weakly hyperactive allele; the specific

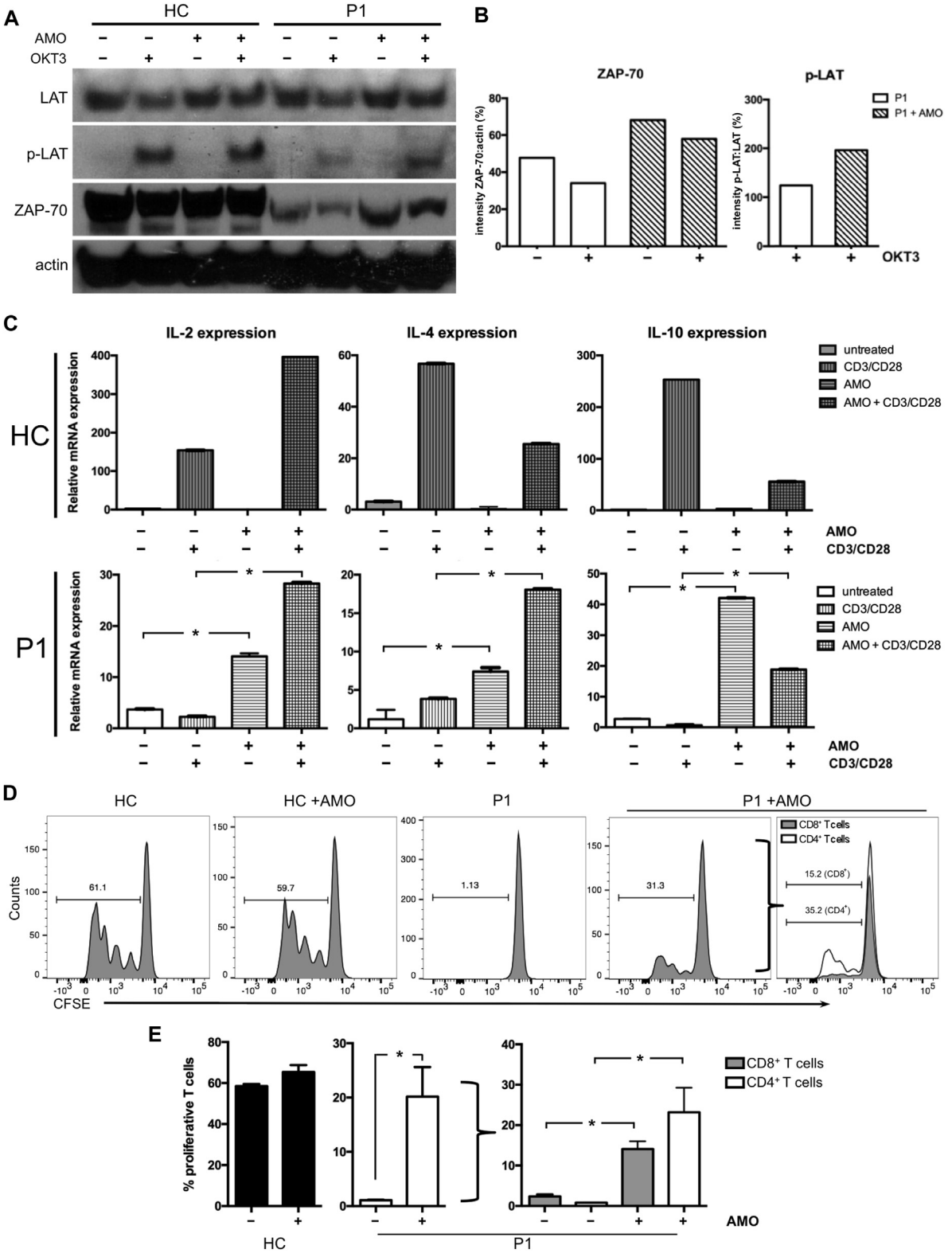

FIG 2. A, AMO-treated PBMCs from a healthy control (HC) or P1 stimulated with OKT3, with densitometry analysis summarized in **B**. **C,** AMO-treated HC or P1 PBMCs stimulated with CD3/CD28 as indicated. **D,** CFSE profiles of HC or P1 CD3/CD28-stimulated T cells, with percentages summarized in **E**. One experiment representative of at least 2 independent experiments is shown. *CFSE*, Carboxyfluorescein succinimidyl ester. $*P < .05$.

combination of these paired alleles was required for disease.[7] Collectively, these cases show that residual levels of functional protein regulate the clinical phenotype of ZAP-70 deficiency. It further suggests that, in distinction to null alleles, different therapeutic approaches may be needed for "leaky" mutations that permit survival beyond the typical time frame and for whom hematopoietic stem cell transplant at older age may be associated with excess risk.[8] We expand the clinical phenotype of hypomorphic ZAP-70 deficiency and provide proof-of-concept that mutation-targeted therapy can improve immune function.

We identified a 33-year-old male (P1) born from consanguineous Coptic parents (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). An older brother died at 16 months from suspected viral encephalitis; an older sister and parents were well. P1 had a history of failure to thrive, adrenal insufficiency, and inflammatory enterocolitis starting in infancy, recurrent respiratory tract infections starting at 9 months, and intermittent mucocutaneous candidiasis, recurrent herpes simplex virus stomatitis, and varicella-zoster virus infections 3 times. Investigations at the time revealed a visible thymus, normal immunoglobulin isotypes with poor response to vaccines, and persistent $CD8^+$ lymphocytopenia (1% to 3% of total T cells age-adjusted reference range). Beginning at approximately 3 years of age, serial testing demonstrated increasing proportion of $CD8^+$ T cells (8% to 10% of total lymphocytes). Further investigations were suggestive of ZAP-70 deficiency (Fig E1, *B* and *C*). However, a reference laboratory reported identifying no mutation in *ZAP70*. Thus, bone marrow transplantation was not pursued. IgA nephropathy at age 16 years eventually required renal transplant from his mother at age 30 years. The subsequent years were marked by EBV viremia/lymphoproliferative disorder (with recurrence of viremia ~6 months after completing rituximab), cytomegalovirus viremia (with recurrence following cessation of (val)ganciclovir), polyomaviremia (BK and JC viruses), and epidermodysplasia verruciformis-like lesions (due to human papilloma virus-23) (see Fig E2 in this article's Online Repository at www.jacionline.org), which prompted reevaluation for an underlying immunodeficiency. He is currently aged 35 years, on immunoglobulin replacement for respiratory tract infection prophylaxis, and his viral infections are managed conservatively.

Whole-exome sequencing revealed no known etiology for the immunodeficiency, other than a homozygous c.1272C>T variant in *ZAP70* (NM_001079; hg19). The germline c.1272C>T variant, while synonymous (p.G355G), is predicted *in silico* (Human Splicing Finder) to create a novel donor splice site within exon 9 (DSS^{mut}) that is stronger than the native junctional one (DSS^{wt}) (Fig 1, *A*). This new splice site is predicted to create a 19-bp deleted product with a premature stop codon in exon 10. Sanger sequencing confirmed the homozygous c.1272C>T variant in P1; the unaffected family members were heterozygous (Fig 1, *B*). RT-PCR from P1's PBMCs confirmed the predicted splicing effect, demonstrating a predominant mutant isoform with trace levels of WT isoform (Fig 1, *C* and *D*). The heterozygous family members possessed both isoforms but with higher WT levels; unrelated healthy controls expressed only the WT isoform. Sequencing of gel-eluted bands confirmed the 19-bp deletion in the mutant isoform (data not shown). Thus, c.1272C>T variant creates a mutant mRNA isoform through aberrant splicing; in homozygous state, the mutant isoform predominates over WT in P1.

To determine the impact of the predominant aberrant *ZAP70* mRNA isoform on protein production, we performed immunoblot analysis on P1's PBMCs and confirmed reduced ZAP-70 levels, as was seen in the original childhood investigations (Fig E1, *C*). Plasmids encoding the open-reading frame of WT ZAP-70 ($ZAP\text{-}70^{wt}$) or the 19-bp deleted variant ($ZAP\text{-}70^{mut}$) were stably transfected into ZAP-70–deficient Jurkat P116 cells, and immunoblot of cell lysates confirmed the absence of ZAP-70 protein with the mutant isoform (Fig 1, *E*). TcR signaling downstream of ZAP-70, assessed by phosphorylation of linker for activation of T cells, was absent following OKT3 stimulation of $ZAP\text{-}70^{mut}$–transfected Jurkat P116, but intact with $ZAP\text{-}70^{wt}$ (see Fig E3, *B*, in this article's Online Repository at www.jacionline.org). These findings confirm that the mutant *ZAP70* mRNA resulting from the c.1272C>T variant is null.

Although the mutant donor splice site (DSS^{mut}) is stronger than the native WT site (DSS^{wt}), the latter is still intact and functionally able to generate WT *ZAP70* mRNA and protein. In P1's homozygous mutant state, this is clearly inadequate for T-cell homeostasis. However, in heterozygous family members, ZAP-70 WT protein is sufficiently produced to maintain an otherwise well state. We hypothesized that blocking the stronger DSS^{mut} in P1 would favor usage of DSS^{wt} to increase WT ZAP-70 levels and consequently, T-cell function. To prove this, we designed an antisense morpholino oligonucleotide (AMO) to target the c.1272C>T variant while sparing the native exon-intron junction so as to block the mutant splice effect during processing of *ZAP70* pre-mRNA (Fig 1, *F*). To confirm that the AMO specifically inhibited expression of the mutant *ZAP70* isoform (ie, blocked its transition from pre-mRNA to mRNA), we treated P1's PBMCs and demonstrated correction of splicing (Fig 1, *G*). Optimization studies demonstrated maximal corrective effect starting at 48 hours using 5 μM AMO, producing approximately 50% WT mRNA (confirmed by sequencing; data not shown). A similar AMO-induced effect on mutant mRNA was seen on the sister's cells (data not shown); there were insufficient number of cells from P1's parents for testing. To verify the specificity of this effect, we treated cells from healthy controls and observed no effect (data not shown). Immunoblot analysis of P1's AMO-treated PBMCs demonstrated increased ZAP-70 expression, confirming that the correction of aberrant splicing results in augmented production of ZAP-70 protein (Fig 2, *A* and *B*). AMO-treated PBMCs demonstrated increased linker for activation of T cells activation following OKT3 stimulation compared with untreated cells (Fig 2, *A* and *B*), indicating that this restored ZAP-70 retains functionally intact TcR signaling. To determine whether the AMO could improve cellular function, we evaluated the expression of prototypical T-cell cytokine genes, *IL2*, *IL4*, *IL10*, and *IFNG* (IFN-γ) (Fig 2, *C*). AMO treatment alone increased *IL2*, *IL4*, and *IL10* expression; *IL2* and *IL4* expression was further enhanced by CD3/CD28 stimulation (Fig 2, *C*). Treatment with AMO had no effect on IFN-γ expression (data not shown), likely due to the reestablished T_H2-driven responses in the PBMCs polarizing away from T_H1 responses. Last, AMO treatment reestablished the proliferative response to CD3/CD28 costimulation in P1's primary T cells, an effect seen in both $CD4^+$ and $CD8^+$ T cells (Fig 2, *D* and *E*; see Fig E4 in this article's Online Repository at www.jacionline.org). Collectively, these findings demonstrate that AMO treatment increases the

expression of WT ZAP-70 protein, which is molecularly intact and capable of restoring key T-cell functional responses.

Analysis of this hypomorphic ZAP-70–deficient patient uniquely illustrates several key points: (1) Clinically, the morbid natural history suggests a role for ZAP-70 in the functional control of double-stranded DNA viruses beyond the generation of $CD8^+$ T cells, which requires further elucidation. Interestingly, the autoimmune diseases here (colitis; nephropathy) were also reported by Chan et al,[1] who elegantly demonstrated that a fine molecular balance in ZAP-70 effect on T-cell signaling can be associated with autoimmunity only. (2) Genetically, it shows that synonymous genetic variants may be deleterious by modifying splicing, with hypomorphism resulting from a skewed net balance of amorphic versus residual WT protein. (3) Immunotherapeutically, it demonstrates that the accurate identification of causal genetic lesions permits a mutation-silencing approach that leads to molecular correction and recuperation of fundamental T- cell cytokine responses, at least *in vitro*. Although the breadth of T-cell functions could not be fully investigated pragmatically, to our knowledge this represents the first example in which AMO therapy successfully restored immune function in primary cells from a human with a genetically defined immunodeficiency. This approach could also extend to therapeutically modulate functional single-nucleotide polymorphisms in immune genes. Clearly, additional parameters will need to be addressed before this approach can be used in clinical practice (eg, pharmacokinetics and safety), although this strategy is in use for inborn errors of muscle or metabolism.[9] Overall, the insight gained from this work opens new avenues for individualized, mutation-targeting therapy for CID.

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Cross-talk between human mast cells and epithelial cells by IgE-mediated periostin production in eosinophilic nasal polyps

To the Editor:

Periostin, an extracellular protein, has emerged as a novel mediator of allergic diseases and plays an important role in tissue remodeling. Serum periostin, as a biomarker, is elevated in severe eosinophilic asthma and aspirin-exacerbated respiratory disease[1] and levels can predict the development of airflow limitation in patients with asthma on an inhaled corticosteroid[2] as well as responsiveness to omalizumab or lebrikizumab in uncontrolled severe allergic asthma.[3,4] However, despite its clinical significance, the mechanism by which periostin regulates inflammation remains poorly understood in the pathogenesis of airway inflammatory diseases.

Chronic rhinosinusitis (CRS) is one of the most common chronic rhinologic diseases and can significantly reduce the quality of life of affected subjects. Increased periostin production in sinonasal tissues occurs in subjects with CRS, particularly

METHODS

Subjects

Subjects and the patient's family members provided informed consent on McGill University Health Centre institutional review board–approved research protocol (GEN10-256). Comprehensive medical histories, including review of all available outside records and serial clinical evaluations, and clinical immunologic laboratory testing were performed at the McGill University Health Centre.

T_H17 *ex vivo* enumeration

T_H17 enumeration from whole blood was performed as previously described.[E1]

Sequencing and bioinformatics analysis

For whole-exome sequencing, exome enrichment was conducted on genomic DNA using the SeqCap EZ Exome v3+ UTR kit (64Mb sequence capture) from Roche-Nimblegen (Madison, Wis). The enriched genomic DNA fragments were sequenced (PE100) on an Illumina HiSeq 2000.The whole-exome sequencing data were analyzed following the GATK Best Practices recommendations for variant discovery in DNA-Seq, with GATK version 3.[E2-E8] After verifying the quality of the reads with FastQC,[E8] the reads were aligned with the Burrows-Wheeler Aligner (v0.7.12-r1039)[E5] to the GRCh37 reference genome. Duplicate reads were marked with Picard tools.[E7] Reads were realigned around indels with GATK IndelRealigner. Base quality scores were recalibrated with GATK BaseRecalibrator. Variant calling was performed with GATK HaplotypeCaller. Variant annotation was performed with SnpSift[E6] and SnpEff.[E9] Only variants with coverage of at least 10 reads were kept for downstream analysis. To identify rare variants, the frequency of the variants in the Exome Aggregation Consortium[E10] database was verified. For Sanger sequencing, the *ZAP70* gene (NG_007727.1) was PCR amplified from genomic DNA using primers designed to flank the respective regions (primers and sequencing conditions available on request). Sequencing was performed at the McGill University and Génome Québec Innovation Centre. Sequencing analyses were performed on Sequencher sequence analysis software (Gene Codes Corporation, Ann Arbor, Mich). Potential splice sites were predicted using the online tool Human Splicing Finder (www.umd.be/HSF/).

Cell culture

The Myc-Flag–tagged ZAP-70 plasmid was from OriGene (Rockville, Md). Site-directed mutagenesis was used to create a 19-bp deletion at the end of exon 9 (New England Biolabs, Whitby, Ontario, Canada). Jurkat ZAP-70–deficient (p116) Jurkat cells were stably transfected with either WT or mutant ZAP-70 plasmid by electroporation followed by G418 selection (1 mg/mL).

Antisense morpholino oligonucleotide

A 20-mer AMO was designed to target the de novo mutant donor splice site generated by the c.1272C>T mutation in the pre-mRNA of *ZAP70*, while sparing the native donor splice site. The AMO sequence was as follows: 5′-GCATGCGGTACACACCCTGG-3′. Vivo-morpholinos were used at a concentration of 5 μM. AMOs were designed and synthesized by Gene-Tools, LLC (Philomath, Ore).

Cell stimulation experiments

PBMCs were isolated as previously described.[E11,E12] PBMCs were cultured in Opti-MEM Reduced Serum Media (Thermo Scientific, Waltham, Mass) and stimulated with 1 μg/mL OKT3 followed by 3 μg/mL secondary antibody or added to 96-well round-bottom plates coated with 3 μg/mL anti-CD28 and 1 μg/mL OKT3. There were insufficient number of cells from the sister and parents for functional testing.

Flow cytometry

Samples were acquired on a BD FACSCANTO II flow cytometer. Single-color compensation samples were prepared using AbC Total Antibody Compensation Bead Kit (Thermo Scientific). Cells were labeled with CellTrace CFSE Cell Proliferation Kit (Thermo Scientific) and LIVE/DEAD Fixable Cell Stain Kit (Thermo Scientific) as per manufacturers' instructions. Compensation and analysis were performed on FlowJo version V10.2

Immunoblot

Samples were separated on Bis-Tris Polyacyrlamide Gels (Thermo Scientific), transferred onto polyvinylidene difluoride membranes, and immunoblotted. Band densities were measured with ImageJ software (http://imagej.nih.gov/ij/, 1997-2014).

Antibodies

Antibodies against phosphorylated linker for activation of T cells (Tyr171), and β-actin were from Cell Signaling Technology (Danvers, Mass). Total linker for activation of T cells antibody was from Santa Cruz Biotechnology (Dallas, Texas). Anti-Flag was from OriGene. Antibodies anti-CD3 (OKT3) and anti-CD28 were from Biolegend (San Diego, Calif). Antibodies against ZAP-70, Lck, Fyn, and Csk were as described previously.[E13,E14] Allophycocyanin-conjugated anti-human CD3 was from Thermo Scientific. Phycoerythrin-conjugated anti-human CD4 and APC-Cy7–conjugated CD8 were from BD Biosciences (Mississauga, Ontario, Canada).

Quantitative real-time PCR

Total RNA was isolated using the RNeasy kit (Qiagen, Mississauga, Ontario, Canada) and reverse transcribed with the Maxima cDNA synthesis kit for RT-quantitative PCR (Thermo Scientific). Quantitative real-time PCR was performed using the Taqman quantitative PCR Gene Expression assay system with probes directed against *IL2* (assay ID Hs00174114_m1), *IL4* (assay ID Hs00174122_m1), *IL10* (assay ID Hs00961622_m1), *UBASH3A* (assay ID Hs00955170_m1), and *CD28* (assay ID Hs01007422_m1) (Thermo Scientific) on Applied Biosystem 7500 real-time PCR system. The mRNA input was normalized to the expression of the T-cell–specific housekeeping gene *UBASH3A* to account for any variability in T-lymphocyte composition. One experiment representative of 3 independent experiments performed is shown.

Statistical analysis

Graphs and statistical analyses were generated with GraphPad Prism Version 6.00. Statistical significance was calculated according to an unpaired 2-tailed ratio student *t* test, with $P < .05$ considered significant.

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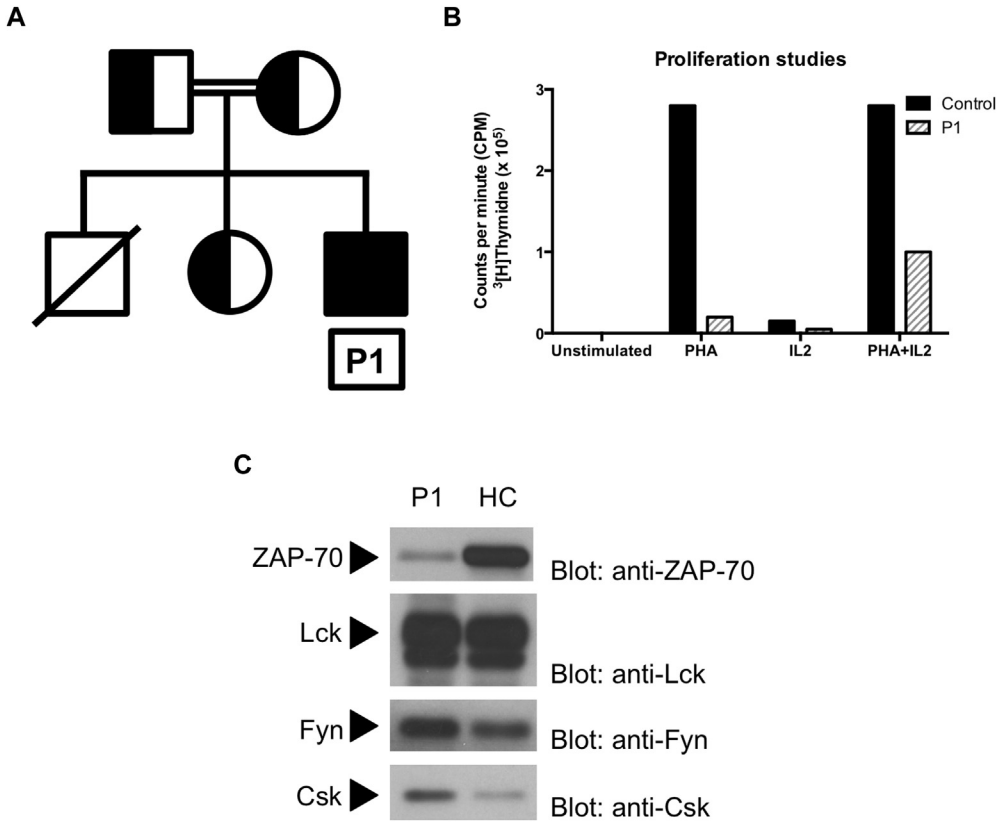

FIG E1. A, P1 pedigree. An older brother had a papulo-squamous rash at age 1 month, recurrent otitis media, and died at 15 months following recurrent seizures, suspected to be viral encephalitis by imaging, electroencephalogram, and autopsy. **B,** During P1's first 2 years of life, summaries of investigations revealed decreased CD8+ T lymphocytes with very low T-cell mitogen (PHA and concanavailin A) proliferative responses. B lymphocytes were quantitatively normal with preserved proliferation in response to pokeweed mitogen. Circulating immunoglobulin concentrations (IgG, IgA, IgM) were normal, with IgE occasionally being slightly elevated. Subsequent investigations found no evidence of adenosine deaminase (ADA) or purine nucleoside phosphorylase (PNP) deficiency with normal expression of HLA on B cells. **C,** T-cell lysates from P1 and a healthy control (HC) were analyzed by immunoblot with indicated antibodies.

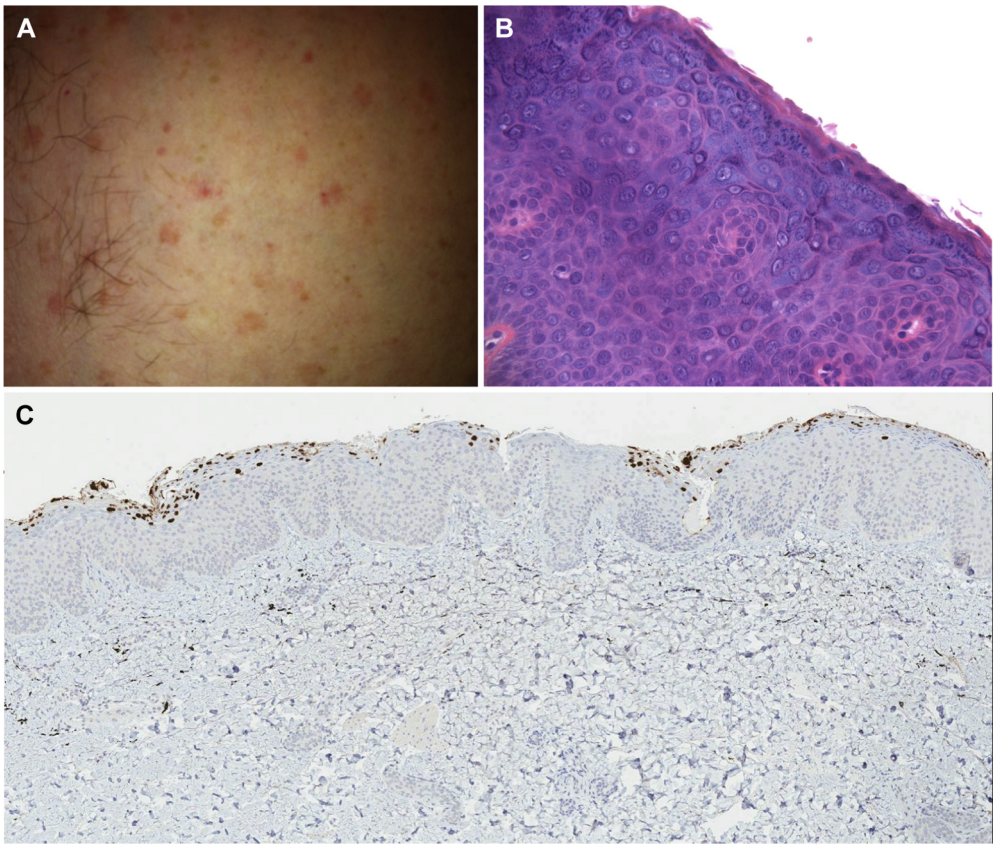

FIG E2. A, Well-circumscribed pink macules on the trunk torso. **B,** Hematoxylin and eosin stain demonstrating enlarged superficial keratinocytes with distinctive blue-gray cytoplasm in association with prominent keratohyaline granules, consistent with HPV infection. **C,** Immunohistochemical pan-HPV stain.

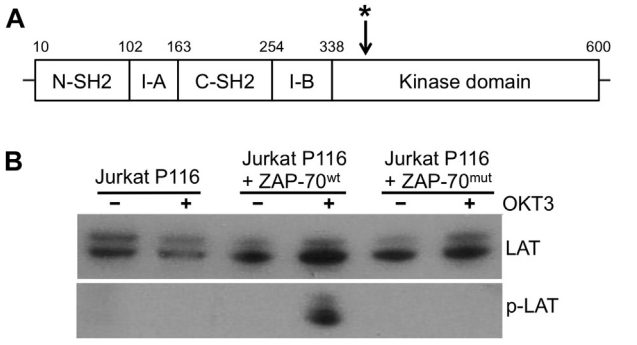

FIG E3. A, A schematic representation of the ZAP-70 protein, with the asterisk denoting the location of the c.1272C>T mutation. **B,** Jurkat P116 cells were stimulated with OKT3 antibody and cell lysates were analyzed by immunoblot using antibodies specific for LAT and phospho-LAT. *LAT,* Linker for activation of T cells.

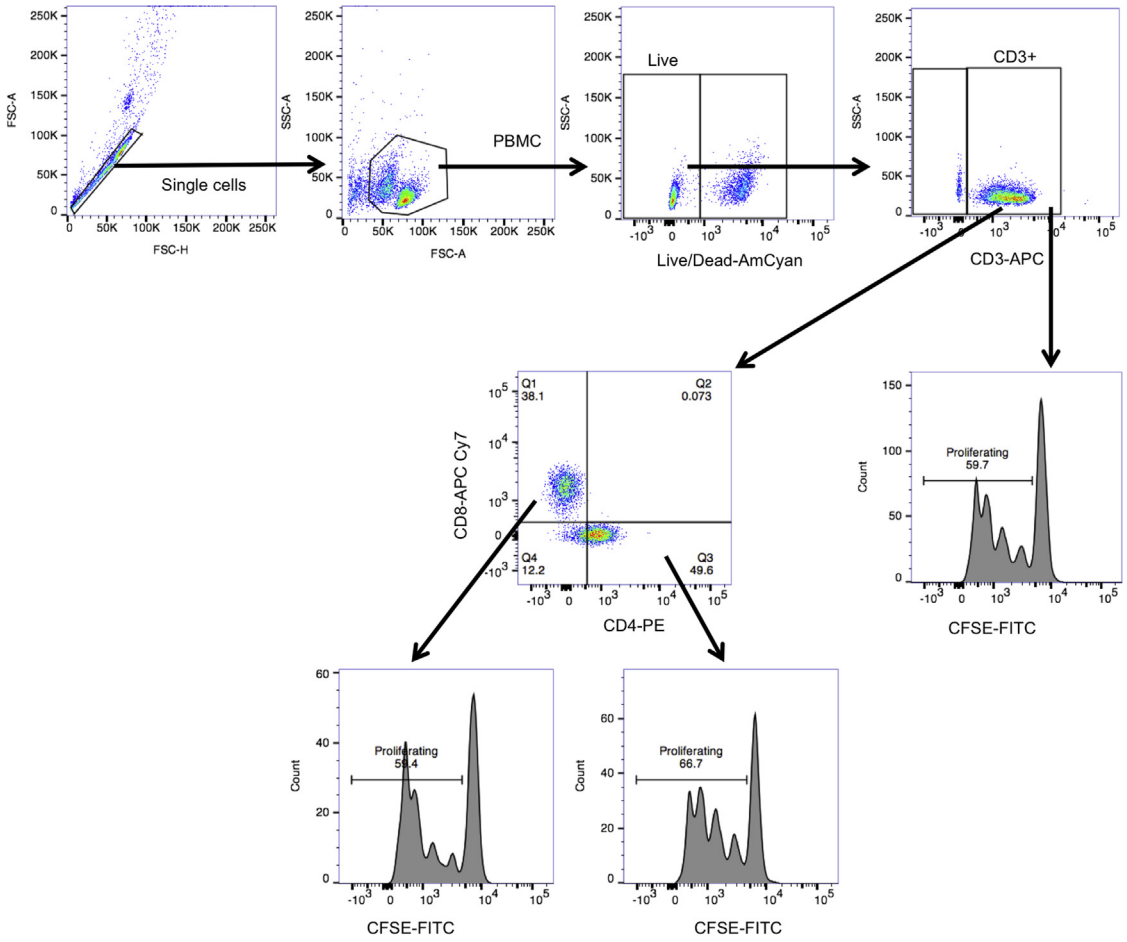

FIG E4. Gating strategy for assessment of T-cell proliferation of CFSE-labeled, CD3/CD28-stimulated cells. *APC*, Allophycocyanin; *CFSE*, carboxyfluorescein succinimidyl ester; *FITC*, fluorescein isothiocyanate; *PE*, phycoerythrin.

TABLE E1. Reports of ZAP-70 deficiency

Case	Reference	Mutation	Effect on protein	Age of onset; sex	Infections	Autoimmune/ dysregulated inflammation	Immunologic phenotype	Outcome
1	E15-E17	Compound heterozygous		<1 y-old M; Mennonite	Upper respiratory tract Infections; Pneumonia; Oral ulcerations; PCP*	Diarrhea, weight loss	Total CD3: normal. $CD4^+$ T: normal. $CD8^+$ T: absent. B: elevated. Immunoglobulin: IgG (↑); IgA (↑); IgM (N); IgE (NR)	HLA-matched unrelated BMT at 20 mo with successful engraftment but died at 22 mo (unclear etiology)
		c.1763C>A	p.S518R. No protein detected in patients' cells.					
		c.1624-11G>A [NM_001079.3]; G to A transition in intron 12 [g.98354447G>A; assembly GRCh37]	Creation of stronger splicing acceptor site leading to addition of 3 amino acids (LEQ) in the catalytic domain. No protein detected in patients' cells					
2	E15-E17			Neonate M (brother of above case); identified by immunophenotypic screen; Mennonite	None (kept in protective isolation). (Had CMV in urine and rotavirus in stool, but was asymptomatic)	NR	Total CD3: decreased. $CD4^+$ T: normal. $CD8^+$ T: absent. B: elevated. Immunoglobulin: NR	HLA-matched unrelated BMT at 4 mo; alive at age 2 y
3	E15-E17			6 mo F; Mennonite; unrelated to above cases	Otitis media with perforation; PCP	NR	Total CD3: normal. $CD4^+$ T: elevated. $CD8^+$ T: absent. B: normal. Immunoglobulin: IgG (↓); IgA (↑); IgM (N); IgE (NR)	HLA-matched related BMT at 11 mo; alive at 4 y
4	E15-E18			5 mo F (sister of case 3); Mennonite; identified by immunophenotypic screen	Oral ulcerations with eczematous rash; PCP; chronic diarrhea with reovirus; oral thrush	NR	Total CD3: normal. $CD4^+$ T: elevated. $CD8^+$ T: absent. B: normal. Immunoglobulin: IgG (N); IgA (N); IgM (N); IgE (NR)	Partial HLA-matched unrelated BMT at age 24 mo; alive at 3 y
5	E19	Homozygous: 1719_1931del13 with predicted frameshift	No protein detected in patient's cells	NR	NR	NR	$CD4^+$ T: normal. $CD8^+$ T: absent. B: normal. Immunoglobulin: NR	NR
6	E20,E21	Mutation not defined but no mRNA detected	No protein	4.5 mo M; not Mennonite	Multiple respiratory infections; laryngomalacia; enteritis due to coronavirus; pneumonitis due to human parainfluenza virus 3	Diarrhea, failure to thrive	$CD4^+$ T: normal. $CD8^+$ T: absent. B: elevated. Immunoglobulin: pan-hypo-gamma globulinemia	BMT at 7 mo from mother with survival

(Continued)

TABLE E1. *(Continued)*

Case	Reference	Mutation	Effect on protein	Age of onset; sex	Infections	Autoimmune/ dysregulated inflammation	Immunologic phenotype	Outcome
7	E22,E23	Compound heterozygous		2 mo M; Japanese	None (kept in isolation because of skin lesions in sister, who died of CMV pneumonia at 6 mo)	Infiltrative skin lesions on face & extremities; No diarrhea	Total CD3: NR. $CD4^+$ T: NR. $CD8^+$ T: absent. B: normal. Immunoglobulin: IgG (N); IgA (N); IgM (N); IgE (↑)	Well at 17 mo
		c.C448A	p.P80Q					
		c.A1923T	p.M572L					
			Temperature-sensitive instability of proteins: degradation at 37°C; detectable protein at 30°C					
8	E24	Homozygous: 1719_1931del13 with predicted frameshift	No protein detected in patient's cells	1-y-old F (unrelated to case 5)	PCP at age 3 mo; extensive varicella infection at 6 mo	NR	Total CD3: NR. $CD4^+$ T: NR. $CD8^+$ T: absent. B: normal. NK: normal. Immunoglobulin: NR	NR
9	E25	Homozygous: c.C1729T	p.A507V. No protein detected in patient's cells	2 siblings NOS	NR	NR	Total CD3: NR. $CD4^+$ T: normal. $CD8^+$ T: severely low (<3%). B: NR Immunoglobulin: NR	NR
10	E26	Homozygous: c.C1602T	p.R465C. This mutation does not impair ZAP-70 expression or autophosphorylation but it impairs its kinase activity	10 mo M; white	PjP at 7 mo	NR	Total CD3: NR. $CD4^+$ T: NR. $CD8^+$ T: few (<170 cells/μL). B: normal. Immunoglobulin: IgG (↓); IgA (N); IgM (N); IgE (NR)	T-cell–depleted BMT from mother at 1 y, complicated by non-EBV large B-cell lymphoma that was treated. Subsequently underwent peripheral SCT from father
11	E27	Homozygous: g.G1603A	p.R465H	8 mo F; NOS	Recurrent respiratory tract infections	NR	Total CD3: NR. $CD4^+$ T: NR. $CD8^+$ T: absent. B: NR. Immunoglobulin: NR	Haplo-identical HSCT (from father) at 9 and 10 mo, but engraftment not successful

(Continued)

TABLE E1. *(Continued)*

Case	Reference	Mutation	Effect on protein	Age of onset; sex	Infections	Autoimmune/ dysregulated inflammation	Immunologic phenotype	Outcome
12	E28	Homozygous: 836+121G>A	Creation of a new splicing acceptor site in intron 7 (78 bp upstream of the normal splice site), creating an in-frame product with a stop codon 61 bp after the new splice site. Decreased expression of WT protein (hypomorphic)	9 y M; NOS	Skin infections; lung infections; no opportunistic infections	No autoimmunity. No lymphoproliferative disease	Total CD3: ↓. $CD4^+$ T: ↓. $CD8^+$ T: ↓. B: normal. Immunoglobulin: IgG (N); IgA (N); IgM (N); IgE (↑)	Alive
13	E29	Homozygous: c.1520C>T	p.A507V; absence of protein detection	4 mo F	Recurrent lower respiratory tract infections	None reported	Total CD3: N. $CD4^+$ T: N. $CD8^+$ T: ↓. B: NR. Immunoglobulin: IgG (N); IgA (↑); IgM (N); IgE (NR)	Awaiting HSCT at time of reporting
		Homozygous: c.1010T>G	p.L337R; absence of protein detection	3 mo F	Recurrent gastroenteritis. Recurrent lower respiratory tract infections. Oral candidiasis. Failure to thrive	Secondary hemophagocytic syndrome	Total CD3: ↓. $CD4^+$ T: N. $CD8^+$ T: ↓. B: NR. Immunoglobulin: IgG (↑); IgA (N); IgM (↑); IgE (NR)	Died of multiorgan failure awaiting HSCT
		Homozygous: c.1690T>C	p.C564R; absence of protein detection	2 mo F	Recurrent pneumonia. Oral candidiasis. Probable BCGosis	Exfoliative dermatitis. Subcutaneous nodules (probable BCGogsis). Generalized erythodermia with eosinophilia (Omenn)	Total CD3: ↑. $CD4^+$ T: ↑. $CD8^+$ T: ↓. B: NR. Immunoglobulin: IgG (↑); IgA (↑); IgM (N); IgE (NR)	Awaiting HSCT at time of reporting
14	E30	Homozygous c.836_837delAT	Absence of protein detection	11 mo F	EBV-associated diffuse large B-cell lymphoma	NR	Total CD3: N. $CD4^+$ T: N. $CD8^+$ T: ↓ (<1%). B: N. NK: N. Immunoglobulin: (NR)	Died of pneumonitis, disseminated intravascular coagulation, and multiorgan failure
15	E31	Homozygous: c.C1153T	p.R385C	5 mo M; Turkish (sibling 1)	PjP at 5 mo	Cholestatic liver disease due to toxic cholangitic hepatitis with portal fibrosis	Total CD3: (NR). $CD4^+$ T: (NR). $CD8^+$ T: (NR). B: (NR). NK: (NR). Immunoglobulin: (NR)	BMT from matched, unrelated female donor at 8 mo. Required liver transplant 2 y after BMT. Alive

(Continued)

TABLE E1. *(Continued)*

Case	Reference	Mutation	Effect on protein	Age of onset; sex	Infections	Autoimmune/ dysregulated inflammation	Immunologic phenotype	Outcome
				4-wk-old M; Turkish (sibling 2)	NR	NR	Total CD3: N. $CD4^+$ T: N. $CD8^+$ T: absent. B: N. NK: N. Immunoglobulin: (NR)	Combined transplant: $CD34^+$ stem cells from same donor of sibling 1, plus peripheral blood lymphocytes of sibling 1
16	E32	Homozygous c.C1747>T	p.R514C	5 mo F; Turkish	Pneumonia	Icthyosiform skin rash at 2 mo. Severe erythrodermia at 5 mo. High IgE	Total CD3: N. $CD4^+$ T: N. $CD8^+$ T: ↓. B: N. NK: ↓. Immunoglobulin: IgG (N); IgA (N); IgM (N); IgE (↑)	Died because of CMV pneumonitis (age NR)
17	E33	Compound heterozygous		9 mo M; 1 mo F; white	None	9 mo M: Nephrotic syndrome (minimal change disease); bullous pemphigoid (20 mo); acquired hemophilia from autoantibody to factor VIII (2 y); inflammatory colitis (3 y) 1 mo F: Bullous pemphigoid; inflammatory colitis (NOS); nephrotic syndrome (NOS)	9 mo M: Total CD3: ↓. $CD4^+$ T: (NR). $CD8^+$ T: ↓. B: ↓. NK: (NR). Immunoglobulin: IgG (↓); IgA (NR); IgM (NR); IgE (NR) 1 mo F: Total CD3: (NR). $CD4^+$ T: (NR). $CD8^+$ T: ↓. B: ↑. NK: (NR). Immunoglobulin: (NR)	Male: HSCT at 5 y with resolution of autoimmune manifestations Female: HSCT at 6 mo with resolution of pemphigoid; HSCT at 28 mo because of declining donor chimerism and autoimmune hypothyroidism, with resolution of disease
		c.574C>T c.1079G>C	p.R192W p.R360P					

BCGosis, Disseminated BCG infection; *BMT*, bone marrow transplant; *CMV*, cytomegalovirus; *F*, female; *HSCT*, hematopoietic stem cell transplant; *M*, male; *NK*, natural killer; *NOS*, not otherwise specified; *NR*, not reported; *PCP*, *Pneumocystis carinii* pneumonia; *PjP*, *Pneumocystis jirovecii* pneumonia; *SCT*, stem cell transplant.