article's Methods section in the Online Repository). These showed an irregular distribution of LEKTI and filaggrin within the granular and upper spinous layers of the patient's skin, which contrasted with the well-demarcated localization seen in control skin (Fig 2, *C* and *D*). *In situ* zymographies demonstrated a moderate increase in protease activity in the patient's epidermis compared with the LEKTI$^{420K/420K}$ healthy control skin but less than in the LEKTI-negative skin of a patient with NS (Fig 2, *E*). In line with these results, immunoblotting and quantitative real-time PCR demonstrated a strong reduction of LEKTI protein and mRNA and *FLG* mRNA in the patient's keratinocytes compared with that seen in control cells (Fig 2, *F* and *G*). The decrease in LEKTI expression combined with the heterozygous variant p.E420K results in a strong reduction in the LEKTI proteolytic fragment (D6D9), which was associated with susceptibility to atopic dermatitis.[8] The interpretation of these observations is challenging, but they suggest that interactions between mutant ichthyin, LEKTI, and filaggrin exist. The atypical ultrastructure of the patient's skin, lacking the characteristic features confined by ichthyin mutations, strongly supports the modifying influence of the additional LEKTI and filaggrin variants (see Fig E1).

The complexity of this molecular interplay cannot be fully addressed here. However, our global genetic analysis points to this "personalized" genetic constellation as the cause for high IgE levels and allergic sensitizations and suggests that *SPINK5* and *FLG* functional variants become relevant in the presence of additional mutations, leading to clear keratinization defects, as seen with *NIPAL4* in our patient.

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Hematopoietic stem cell transplantation rescues the immunologic phenotype and prevents vasculopathy in patients with adenosine deaminase 2 deficiency

To the Editor:

Recently, recessively inherited loss-of-function mutations in *CECR1* (cat eye syndrome chromosome region, candidate 1), which encodes adenosine deaminase 2 (ADA2), were identified in patients with a complex immunologic and vascular phenotype.[1,2] Possible mechanisms for this disorder are proinflammatory polarization and disturbed endothelial integrity.[1,2] Zhou et al[1] reported that aggressive systemic immunosuppressive treatment was not effective in controlling inflammation but hypothesized that hematopoietic stem cell transplantation (HSCT) might be curative given that bone marrow–derived monocytes and macrophages are the main source of secreted ADA2.

Here we report on 2 related patients with homozygous p.Arg169Gln missense mutations in ADA2 located within the putative receptor-binding domain.[3] Our observations in these siblings demonstrate the clinical heterogeneity associated with ADA2 deficiency and show that HSCT can be an effective therapy. In the index patient the clinical course was dominated by autoimmunity and lymphoproliferation with a combined immunodeficiency–like phenotype, which prompted HSCT

TABLE I. Plasma ADA2 activity in the affected pedigree

Sample	Age (y)	Plasma ADA2 activity (mU/mL)
Patient 1 after HSCT	8	22.07
Patient 2	3	0.11
Healthy sibling (= HSCT donor)	10	19.14
Father	43	7.20
Mother	40	2.91

Reference values for plasma ADA2 activity (mU/mL), mean ± SD (min-max)	
ADA2 deficient (n = 4)	1.0 ± 0.4 (0.6-1.4)
ADA2 carriers (n = 4)	4.9 ± 0.3 (4.6-5.3)
Control subjects (n = 5 + pooled human plasma)	14.0 ± 6.1 (4.8-21.3)

from a healthy sibling. Despite early complications, transplantation was successful both in rescuing the immunologic phenotype and in preventing vascular disease; at 5 years after HSCT, the patient remains off treatment.

The index patient (P1) was the second child of a father of Moroccan descent and a white mother. He was first admitted at age 6 months for complicated human respiratory syncytial virus infection. At this time, hypogammaglobulinemia was noted (see Table E1 in this article's Online Repository at www.jacionline.org). At age 12 months, P1 presented with fever, lymphadenitis, generalized lymphadenopathy, and hepatosplenomegaly. *Staphylococcus aureus* was cultured from the lymph nodes, and fever resolved within 24 hours of starting amoxicillin–clavulanic acid treatment. Pancytopenia, hypogammaglobulinemia, and the absence of specific antibodies were found (see Table E1). Results of blood PCRs for EBV, cytomegalovirus, human herpesvirus (HHV) 6, HHV-8, and adenovirus were negative. However, adenovirus and norovirus were detected in the stool. Computed tomographic scans confirmed generalized lymphoproliferation with mediastinal and intra-abdominal lymphadenopathy and splenomegaly. Lymphoma was suspected, but the results of lymph node biopsy and bone marrow examination were normal. Macrophage activation syndrome as the cause of the pancytopenia and lymphoproliferation was excluded based on serum markers (including soluble IL-2 receptor) and the absence of hemophagocytosis on bone marrow examination. A primary immune deficiency (PID) with predominant lymphoproliferation and autoimmunity was suspected, and known genetic causes were excluded. Prednisone (2 mg/kg) led to resolution of the thrombocytopenia and splenomegaly. However, attempts to taper led to a relapse of thrombocytopenia. Despite the addition of mycophenolate mofetil, sirolimus, tacrolimus, cyclosporine, and mercaptopurine, the cytopenia and lymphoproliferation persisted.

Because of growth failure secondary to chronic corticosteroid treatment, HSCT was considered at the age of 3 years. The patient's HLA-identical healthy elder brother was chosen as the donor. After conditioning with oral busulfan and cyclophosphamide, 7.5×10^6 $CD34^+$ bone marrow–derived hematopoietic stem cells per kilogram were infused. Anti–graft-versus-host-disease (GvHD) prophylaxis consisted of cyclosporine, whereas steroids were slowly tapered. Antiviral prophylaxis consisting of acyclovir and intravenous immunoglobulin (IVIG) administration and antifungal prophylaxis with fluconazole was added. The transplantation was complicated by late engraftment of neutrophils (day 26 $<1.5 \times 10^9$/L) and persistent severe thrombocytopenia ($<10 \times 10^9$/L) refractory to transfusion, although at day 28, whole blood chimerism was greater than 95%. At day 36, magnetic resonance imaging (MRI) of the brain, which was performed because of severe sudden-onset headache, identified a pineal gland hemorrhage (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). The thrombocyte level was 2×10^9/L but increased to greater than 50×10^9/L at day 40 after 2 infusions of rituximab. Veno-occlusive disease (VOD) was diagnosed according to the Seattle criteria at day 60 and was accompanied by a relapse of thrombocytopenia. VOD responded well to fluid restriction. Platelet levels of greater than 100×10^9/L were reached at day 111. Adenovirus reactivation was found at day 40, with accompanying intestinal GvHD grade III, which responded to corticosteroids. Cyclosporine was stopped at day 150. IVIG was discontinued at day 180. Immunoreconstitution at day 360 was excellent, including normal antibody levels, normal numbers of B- and T-lymphocytes, and normal T-cell proliferation in response to PHA. Moreover, response to polysaccharide vaccine was normal (data not shown).

Five years after transplantation, P1 is clinically well and off all medication. No more lymphoproliferation has occurred, and the most recent MRI of the brain 5 years after HSCT did not show any signs of acute or chronic small infarcts.

Two years after transplantation of P1, his younger brother (P2) presented at age 5 months with profound Coombs (−) anemia (hemoglobin, 2 g/dL), which was attributed to PCR-verified HHV-6–associated erythroblastopenia. At this time, immunologic analysis of P2 was normal. Several episodes of PCR-verified facial herpes simplex virus infection followed. At age 23 months, P2 was admitted with abdominal pain and ileus refractory to conservative treatment. He had generalized lymphadenopathy and hepatosplenomegaly, as well as hypogammaglobulinemia and intermittent lymphopenia and neutropenia (see Table E1). Results of blood polyomavirus PCR were positive. Bone marrow examination was normal. Partial enterectomy was performed; biopsy showed an atypical ulcerative bowel disease devoid of plasma cells (see Fig E1, *B*), as can be seen in patients with common variable immunodeficiency.[4] No cytomegalovirus, EBV, herpes simplex virus, HHV-6, polyomavirus, or adenovirus could be detected in the biopsy specimen, and no signs of vasculitis could be observed in the entire surgical specimen.

Obstruction persisted despite aggressive systemic immunosuppressive treatment and was only relieved after treatment with sirolimus. At this time, IVIG was started, and sirolimus was slowly tapered without clinical relapse. Subsequently, P2 did not receive any immunosuppressive treatment for a period of 13 months but was solely treated with IVIG. At 3.5 years of age, P2 had neurologic manifestations in the form of 2 episodes of acute loss of balance in the absence of fever or signs of systemic inflammation on blood analysis, Repeated MRI of the brain did not reveal any lesions compatible with ischemic or hemorrhagic stroke. A transient ischemic attack (TIA) was suspected, and treatment with sirolimus was restarted.

Whole-exome sequencing was performed on the untreated patient (P2), the parents, and the healthy sibling (for details, see the Methods section in this article's Online Repository at www.jacionline.org). We hypothesized a recessive model of inheritance. After filtering out common polymorphisms, we identified

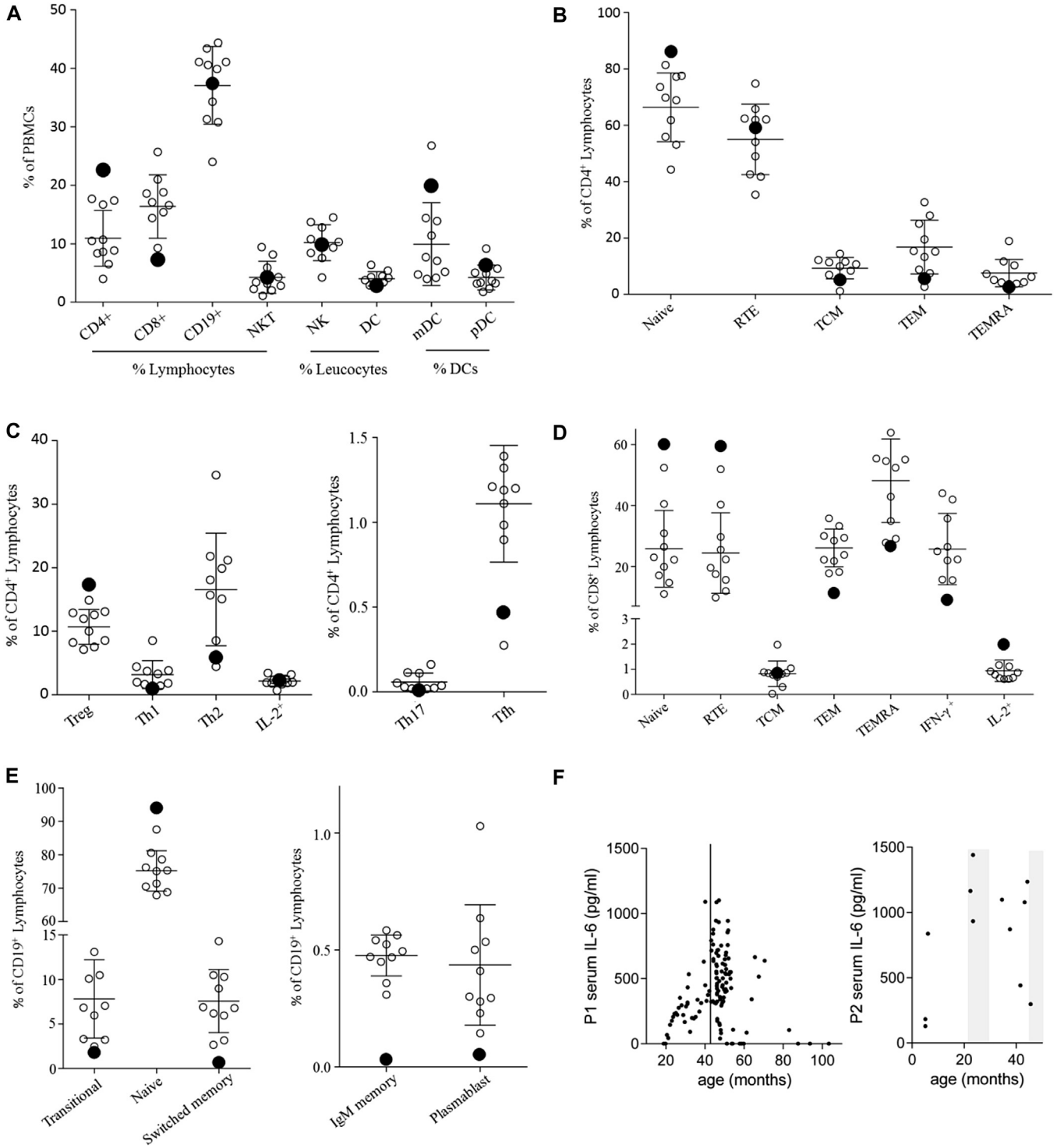

FIG 1. Serum IL-6 levels and immunoprofiling in ADA2-deficient patients. **A,** Major blood leukocyte subsets. **B,** $CD4^+$ T-lymphocyte subsets. **C,** T_H cell lineages. **D,** $CD8^+$ T-lymphocyte subsets. **E,** B-cell subsets. P2's values are shown as *filled circles*, and values of healthy age-matched control subjects are indicated by *open circles*. Means and SDs *(error bars)* shown exclude values for the patient. **F,** IL-6 levels in sera of P1 and P2. The *vertical line* indicates the moment of HSCT followed by pineal stroke in P1. The *gray shading* indicates the periods in which P2 was treated with sirolimus. *DC*, Dendritic cell; *mDC*, myeloid dendritic cell; *NK*, natural killer cell; *NKT*, natural killer T cell; *pDC*, plasmacytoid dendritic cell; *RTE*, recent thymic emigrant; *TCM*, central memory T cell; *TEM*, effector memory T cell; *TEMRA*, CD45RA-expressing effector memory T cell; *Tfh*, follicular T cell; *Th17*, IL-17–expressing helper T cell; *Treg*, regulatory T cell.

a homozygous c.G506A variant in *CECR1*, resulting in a p.Arg169Gln missense mutation in ADA2. Sanger sequencing on DNA obtained from the cheek swab of the patient who underwent transplantation confirmed that he was also homozygous for this variant. Both parents were carriers, whereas the sibling donor was homozygous for the wild-type form of *CECR1* (see Fig E2 in this article's Online Repository at www.jacionline.org).

ADA2 enzyme activity in plasma (Table I) was essentially absent in P2, the patient who did not undergo transplantation, whereas in post-HSCT plasma from P1, ADA2 activity was comparable with that of his healthy donor and in the range for healthy control subjects. Both parents have intermediate plasma ADA2 activity. Of note, neither adenosine nor deoxyadenosine levels were increased (<0.4 μmol/L) in plasma of P2 (these levels have not been measured in previous patients). Both P1 and P2 had normal ADA1 activity in dried blood spots, and deoxyadenosine nucleotides were undetectable.

Although it has been speculated that the clinical consequences of ADA2 deficiency might be due to increased extracellular adenosine, our findings suggest this is not the case and that ADA2 actually has a minimal role compared with ADA1 in adenosine metabolism *in vivo*, which is consistent with the very different substrate affinities of the 2 ADA enzymes (see the Methods section in this article's Online Repository).

Because of the observed immunodeficiency, we performed extensive profiling of peripheral immune cells of P2 (for details, see the Methods section in this article's Online Repository). Of the major mononuclear leukocyte cell types surveyed, $CD4^+$ T-cell numbers were increased and $CD8^+$ T-cell numbers were reduced in P2 compared with those in healthy age-matched control subjects. B-cell, natural killer cell, and dendritic cell numbers were within 1 SD of the mean of the healthy control subjects (Fig 1, *A*). Within the T-lymphocyte population, we found defective T-cell activation, with increased naive and low effector and memory subsets (Fig 1, *B* and *D*). Within the T_H cell population, numbers of regulatory T cells were increased, whereas T_H1, T_H2, and follicular helper $CD4^+$ T-cell numbers were low (Fig 1, *C*). T-cell proliferation in response to *Candida* species, tetanus, and PHA was normal (data not shown). Within the B-lymphocyte population, naive B-cell numbers were increased at the expense of memory and plasmablasts (Fig 1, *E*), which is suggestive of a defect in B-lymphocyte differentiation or T-cell provision of help. Limited immunoprofiling performed before HSCT showed similar findings in P1 (see Table E2 in this article's Online Repository at www.jacionline.org).

Because of the presence of severe inflammation in P1, serum IL-6 levels were measured from initial evaluation to last follow-up (Fig 1, *F*). IL-6 levels were persistently high before HSCT and before engraftment, but after HSCT IL-6 levels slowly decreased and were undetectable at 3 years post-HSCT. In P2 serum IL-6 levels were extremely increased, despite the absence of clinical signs of inflammation, with levels peaking at the time of bowel obstruction and at the time of the suspected TIAs. IL-6 was undetectable in the healthy sibling and in healthy control subjects. Moreover, TNF-α was not detectable in the serum of P1 and P2 at the time of the highest IL-6 levels. The immune profile of the other family members was normal (data not shown). Together, these data demonstrate a profound defect in T cell–dependent antibody-mediated responses and a failure to regulate normal inflammatory cytokine production in ADA2-deficient patients, adding to the previously identified function of ADA2 in *in vitro* stimulation of T_H cells.[5]

PIDs with autoimmunity and lymphoproliferation dominated the clinical image in our patients. The index patient P1 presented with persistent autoimmune pancytopenia and lymphoproliferation, whereas P2 had an episode of lymphoproliferation, bowel involvement, and 2 possible TIAs. Both patients only had fever during infectious episodes, and unlike previously reported patients, neither showed skin involvement or clear signs of vasculitis. P1 had a stroke as an apparent early complication of HSCT in the context of prolonged and severe thrombocytopenia. Only 3 years after initial presentation, P2 presented with 2 potential TIAs, although transient labyrinthitis caused by a viral infection could not be excluded. Therefore in retrospect vasculitis and inflammation might have been present at a subclinical level in both patients, but vasculopathy and inflammation did not dominate the clinical presentation, as is the case in the patients reported by Zhou et al[1] and Elkan et al.[2] Interestingly, serum IL-6 levels were increased in both patients in the absence of clinical and (routine) biochemical signs of inflammation. This suggests that ADA2 deficiency might lead to a subclinical state of inflammation. This phenotypic discrepancy cannot be explained entirely by *CECR1* genotype because the p.Arg169Gln mutation was previously observed in hemizygous and homozygous form.[1,2] The ADA2-deficient patients previously described had decreased serum immunoglobulin levels and enhanced B-cell apoptosis *in vitro*.[1] By contrast, our patients had abnormalities suggesting an *in vivo* defect in T-cell activation and proliferation, corresponding to their increased susceptibility to viral infections and combined immunodeficiency–like phenotype. Taken together, these observations suggest that ADA2 deficiency has a more varied clinical phenotype than initially reported and that the diagnosis should be considered in cases of undiagnosed PID characterized by lymphoproliferation and autoimmunity, even in the absence of overt vasculopathy or inflammation.

As reported by Zhou et al,[1] we found that treatment with a variety of immunosuppressive medications resulted in poor disease control in P1. However, both at the time of bowel obstruction and at the time of potential TIA, P2 seemed to respond well to sirolimus treatment. Sirolimus reduces M1 macrophage differentiation and IL-6 production.[6] Because ADA2 deficiency drives macrophages toward a more proinflammatory M1 profile,[1] we present sirolimus as a potential therapeutic option to at least temporarily control inflammatory complications in ADA2-deficient patients. TNF-α was undetectable in the serum of our patients. However, this finding does not at all exclude a role for this cytokine in disease pathogenesis. Indeed, etanercept led to a significant response in all patients reported by Elkan et al[2] and should therefore be considered as a potential treatment.

In the index patient P1 we successfully performed an allogeneic HSCT. At 5 years after HSCT, consecutive clinical and biochemical investigations in P1 have shown no signs of immunologic disorder and no additional strokes. This result supports the potential of HSCT as a long-term treatment strategy for ADA2 deficiency. However, caution is warranted because the HSCT procedure in P1 was characterized by severe early complications. Indeed, ADA2-deficient patients might present as high-risk candidates for HSCT. First, the inflammatory response associated with conditioning is superimposed on the inflammatory state intrinsic to ADA2 deficiency, which might negatively affect engraftment. Second, the compromised

endothelial integrity observed in patients with ADA2 deficiency could predispose to development of VOD, a potentially fatal complication of HSCT. This combination of inflammation and endothelial injury might further increase the risk of stroke in the pre-engraftment and early postengraftment phases,[7] as observed in P1. It is reasonable to hypothesize that ADA2-deficient patients might benefit from VOD prophylaxis with defibrotide, as well as from pretreatment with anti–IL-6 mAbs, rituximab, or both. Moreover, treatment with etanercept peri-HSCT could be considered in the context of ADA2 deficiency, especially given its usefulness in preventing and treating acute GvHD. However, given the underlying immunodeficiency, the risk of infection needs to be carefully balanced when using anti–IL-6 and anti–TNF-α mAbs.

Allogeneic HSCT restored normal plasma ADA2 activity in P1, which is consistent with bone marrow–derived monocytes and macrophages being the main sources of secreted ADA2. Whether ADA2 plays a role in other tissues and the effect of this on long-term prognosis remains unclear. A recent report on HSCT in a patient with ADA2 deficiency with a 9-year follow-up is promising and supports our findings.[8] However, it is plausible that the benefit from HSCT to our patient is entirely due to restoration of normal plasma ADA2 levels. If true, future treatment with exogenous ADA2 might provide an alternative therapy for ADA2 deficiency in patients in whom allogeneic HSCT is contraindicated.

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METHODS

The study was performed in accordance with the modified version of the Declaration of Helsinki. The study was approved by the Ethics Committee of UZ Leuven. Written informed consent was obtained before DNA isolation from blood of all family members and from cheek epithelium of the transplanted patient.

Functional assays

PBMCs were isolated from heparinized blood of patients, family members, and control subjects and analyzed by using flow cytometry, as previously described.[E1] Serum IL-6 levels were measured by means of ELISA, according to the manufacturer's instructions (BD Bioscience, San Jose, Calif).

Whole-exome sequencing

We performed whole-exome sequencing on the untreated patient and on the unaffected parents and sibling. Genomic DNA samples for whole-exome sequencing were prepared from heparinized peripheral blood by using the QIAamp DNA Blood Midi Kit (QIAGEN, Hilden, Germany). Exome sequence libraries were prepared with a SeqCap EZ Human Exome Library v3.0 kit (Roche NimbleGen, Madison, Wis). Paired-end sequencing was performed on the Illumina HiSeq2000 (Genomics Core Facility, University of Leuven, Leuven, Belgium). BWA software was used to align the sequence reads to the Human Reference Genome Build hg19. The GATK Unified Genotyper was used to identify single nucleotide variants and insertions/deletions. ANNOVAR was used for annotation.

Sanger sequencing

A somatic DNA sample of the patient undergoing transplantation was obtained from a cheek swab by using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich, St Louis, Mo). The region of interest in exon 2 of *CECR1* was sequenced with the primers 5′-GTTTGTACCAAGGGAGACACCTACC-3′ and 5′-CTGGCTGGTGAGGAATGTCAC-3′. Sanger sequencing was performed on an ABI 3730 XL Genetic Analyzer (Applied Biosystems, Foster City, Calif) at the LGC Genomics Facility in Berlin, Germany. Sequencing data were analyzed by using DNADynamo (Blue Tractor Software, Llanfairfechan, United Kingdom).

Flow cytometry

PBMCs were isolated from heparinized blood of patients and control subjects by using lymphocyte separation medium (MP Biomedicals, Solon, Ohio) and frozen in 10% dimethyl sulfoxide (Sigma). Thawed cells were stained with antibodies (from eBioscience [San Diego, Calif], unless stated otherwise) against CD11c (3.9), CD3 (SK7), CD4 (RPA-T4), CD8α (RPA-T8), CD19 (HIB19), CD45RA (HI100), CD56 (MEM188), HLA-DR (LN3), forkhead box protein 3 (FOXP3; 206D; BioLegend, San Diego, Calif), IFN-γ (4S.B3 IL-17, eBio64DEC17), IL-2 (MQ1-17H12), CXCR5 (IgG23; R&D Systems, Minneapolis, Minn), CD31 (WM-59), CCR7 (3D12), IgM (MHM-88, BioLegend), CD27 (O323), IgE (IgE21), CD24 (eBioSN3, SN3 A5-2 H10), CD38 (HIT2), γδ T-cell receptor (B1.1), CD56 (MEM188), CD14 (61D3), CD123 (6H6), and IL-4 (8D4-8). For cytokine staining, T cells were stimulated *ex vivo* for 5 hours in 50 ng/mL phorbol 12-myristate 13-acetate (Sigma) and 500 ng/mL ionomycin (Sigma) in the presence of GolgiStop (BD Biosciences) before staining. Before intracellular staining, cells were first surface stained as described, fixed, and permeabilized with fixation/permeabilization buffer (eBioscience) for forkhead box protein 3 staining or Cytofix/Cytoperm (BD) for other intracellular stainings. All data were acquired on BD FACSCanto II and analyzed with FlowJo (Tree Star, Ashland, Ore).

ELISA for measurement of IL-6 levels in serum

An in-house validated ELISA was used based on a commercially available antibody pair (BD Biosciences).

Measurements of ADA1 and ADA2 activity in plasma

ADA2 activity in plasma was measured by using the HPLC method described by Zhou et al.[E2] ADA1 activity and concentrations of total adenosine and deoxyadenosine nucleotides in extracts of dried blood spots were measured, as previously described.[E3,E4] The concentrations of adenosine and deoxyadenosine in plasma were determined by means of HPLC analysis of a neutralized perchloric acid extract of plasma. In brief, 200 μL of plasma was acidified with 40 μL of 5 N perchloric acid and centrifuged, and the supernatant was neutralized with 3 N KOH and 1 M $KHCO_3$. After centrifugation, 100 μL of the supernatant was analyzed on a C18 μBondapak column (Waters Corporation, Milford, Mass) by using 0.05 mol/L $NH_4H_2PO_4$, 8% methanol, and 1% acetonitrile (pH 5.2; flow rate, 0.5 mL/min) as the mobile phase and monitoring absorbance at 260 and 280 nm with a diode array detector. The lower limit of quantitation for adenosine and deoxyadenosine in this assay was 0.8 μmol/L; the lower limit of detection was taken as half the lower limit of quantitation or 0.4 μmol/L.

RESULTS

X-linked lymphoproliferative disease type I and II, Wiskott-Aldrich syndrome, autoimmune lymphoproliferative syndrome, ADA1 deficiency, purine nucleoside phosphorylase deficiency, and immune dysregulation–polyendocrinopathy–enteropathy–X-linked syndrome were excluded by means of functional and genetic analyses.

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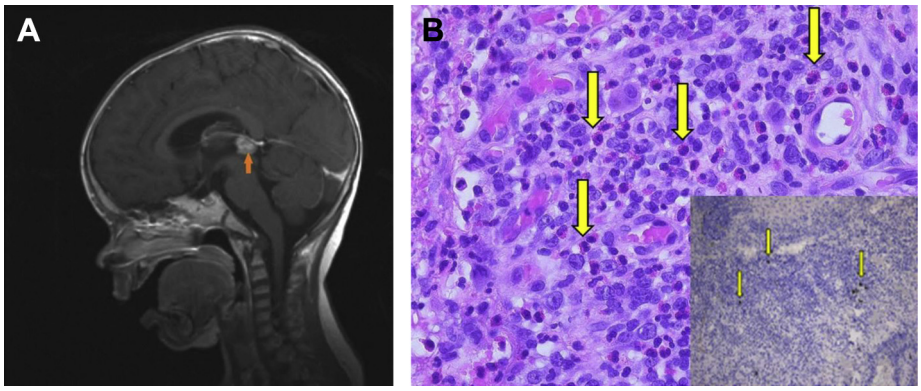

FIG E1. Vasculopathology and immunopathology in patients with ADA2 deficiency. **A,** Sagittal T1-weighted MRI of P1 showing pineal gland hemorrhage *(arrow)*. **B,** Hematoxylin and eosin staining of jejunal ulceration in P2 showing chronic ulcer with predominant eosinophils *(arrows)*, some neutrophils and lymphocytes, and very few plasma cells. Plasma cells stained by means of CD138 staining are indicated by *arrows* in the *inset*.

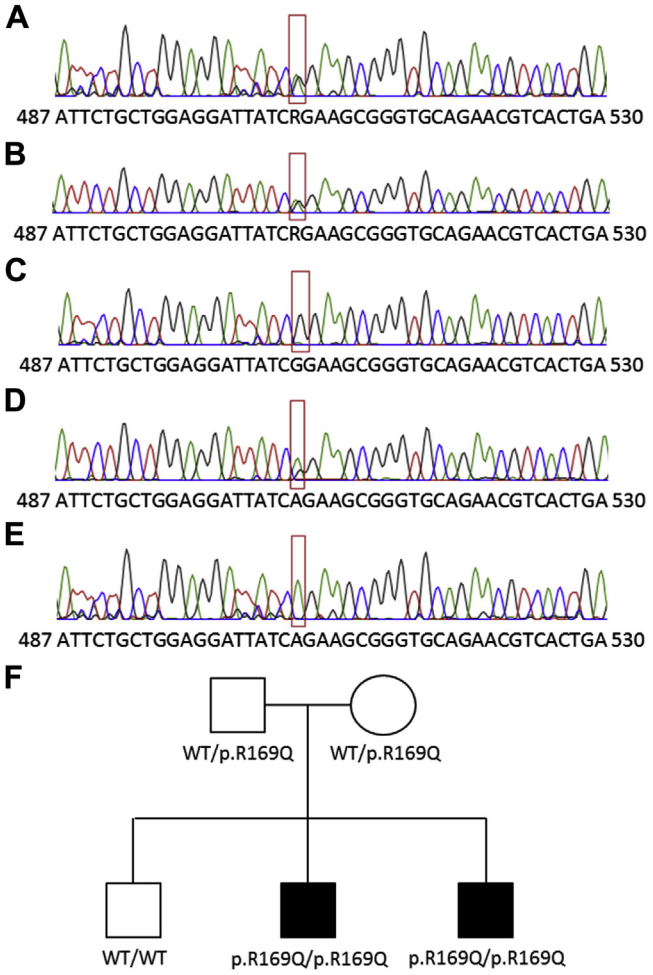

FIG E2. Familial inheritance of *CECR1* mutation. The region of interest in exon 2 of *CECR1* was sequenced by means of Sanger sequencing. **A-E,** Sequence reads for the father (Fig E2, *A*), mother (Fig E2, *B*), healthy sibling (HSCT donor; Fig E2, *C*), patient 1 after HSCT (chimerism accounts for presence of a minor G peak; Fig E2, *D*), and patient 2 (Fig E2, *E*). **F,** Family tree of the affected pedigree, indicating affected patients and *CECR1* genotype.

TABLE E1. Clinical presentation, laboratory values, and therapeutic history of ADA2-deficient patients

	Patient 1	Patient 2
Clinical phenotype		
Clinical presentation	Hypogammaglobulinemia, pancytopenia, lymphoproliferation	Hypogammaglobulinemia, (intermittent) lymphopenia and neutropenia, lymphoproliferation
Viral infections confirmed by means of PCR	RSV, adenovirus, norovirus	HHV-6, HSV, polyomavirus
Stroke	Hemorrhage in the pineal gland	None
Laboratory values*		
White blood cell count (kU/μL)	2.02	9.08
Neutrophil count (kU/μL)	0.3	6.4
Lymphocyte count (kU/μL)	1.0	1.7
Hemoglobin (g/dL)	8.8	9.7
Thrombocytes (kU/μL)	25	300
ALT (5-38 U/L)	44	15
AST (0-41 U/L)	64	6
IgG (3.02-9.85 g/L)	<1.00	2.77
IgA (0.13-1.08 g/L)	<0.07	0.11
IgM (0.26-1.60 g/L)	0.09	0.27
IgE (0-91 IU/mL)	<2	30
IgD (<10 U/mL)	0	0
ANA	Negative	Not determined
ANCA	Negative	Not determined
Thrombocyte autoantibodies	Anti-gpIIB-IIIa antibody present	Not determined
Erythrocyte autoantibodies	Anti-MNS1 antibody present	Not determined
Lymphocyte count (kU/μL) [at moment of immunophenotyping]	0.39	3.0
Therapeutic history		
Immunosuppressive medication	Corticosteroids, sirolimus, mycophenolate mofetil, tacrolimus, cyclosporine, mercaptopurine	Corticosteroids, azathioprine, sirolimus
Immunoglobulin substitution	Yes (before HSCT)	Yes
Allogeneic HSCT	Yes	No

ALT, Alanine aminotransferase; *ANA*, antinuclear antibody; *ANCA*, antineutrophil cytoplasmic antibody; *AST*, aspartate aminotransferase; *HSV*, herpes simplex virus; *RSV*, respiratory syncytial virus.

*Values were obtained at initial clinical presentation unless stated otherwise.

TABLE E2. Relative frequencies of peripheral blood leukocyte populations in P1 before HSCT compared with those in healthy age-matched control subjects

			Healthy volunteers (%)
Subset	**Defining surface markers**	**Patients (%)**	**Range (minimum-maximum)**
T cells	$CD3^+$	80.6	52.9-65.2
$CD4^+$ T cells	$CD4^+$ $CD8^-$	63.8	29.4-65.2
→ Treg	$CD25^+Foxp3^+$	10.0	
$CD8^+$ T cells	$CD4^-$ $CD8^+$	0.83	17.6-23.2
B cells	$CD19^+$	5.56	11.8-30.4
→ Transitional	$CD38^{high}CD24^{high}$	0.2	
→ Naive	$CD27^-IgD^+$	97.8	
→ Immature	$CD27^+IgD^+$	0.1	
→ Switched memory	$CD27^+IgD^-$	0.3	

$CD3^+$, $CD4^+$, and $CD8^+$ T cells and $CD19^+$ B cells are shown as percentages of total lymphocytes. Regulatory T *(Treg)* cells are shown as percentages of $CD4^+$ T cells. B-cell subsets are shown as percentages of $CD19^+$ B cells.
Foxp3, Forkhead box protein 3.