

Letter to the Editor

Adenosine deaminase type 2 deficiency masquerading as GATA2 deficiency: Successful hematopoietic stem cell transplantation*To the Editor:*

The clinical diagnosis of certain primary immunodeficiency diseases (PIDs) may be challenging due to the narrow phenotypes initially published. With the advent of whole-exome sequencing (WES), accurate diagnosis of PID and extension of published phenotypes are more easily accomplished.

We describe a young woman initially diagnosed with common variable immunodeficiency disease who developed monocytopenia and *Mycobacterium avium* complex infection (MonoMAC) syndrome. Before identification of her molecular diagnosis, she underwent a haploidentical hematopoietic stem cell transplant (HSCT) from her clinically healthy sister. Following HSCT, complete reversal of the hematological, immunological, and clinical manifestations of disease occurred. She was subsequently found to have mutations in cat eye syndrome chromosome region, candidate 1 (*CECR1*) (encoding adenosine deaminase type 2 [ADA2]) by WES.¹⁻³ This case illustrates the phenotypic overlap among several PIDs, the role for WES in establishing the correct diagnosis, and the efficacy of HSCT in reversing the phenotype.

A 20-year-old Hispanic female presented to the National Institutes of Health Clinical Center with an absolute neutropenia, anemia, and an invasive *Fusarium proliferatum* sinusitis. She had been diagnosed with common variable immunodeficiency disease at age 7 years with low IgG levels and frequent respiratory infections. She had frequent infections throughout childhood and received intravenous immunoglobulin on 2 occasions at age 15 years. At age 19 years, she was hospitalized for febrile neutropenia, right maxillary sinusitis secondary to *Fusarium* species, sepsis, and respiratory failure due to an extended spectrum β -lactamase producer *Escherichia coli*. She required intensive care unit care, mechanical ventilation, and treatment with broad-

spectrum antibiotics, liposomal amphotericin B, posaconazole, granulocyte colony stimulating factor-1, and corticosteroids.

Upon admission to the National Institutes of Health, she had right maxillary *F proliferatum* sinusitis. Her absolute neutrophil count was 0 cells/ μ L, monocytes 1 cell/ μ L, natural killer cells 32 cells/ μ L, and CD20+ B cells 0 cells/ μ L (Table I). The bone marrow was mildly hypocellular for age with no neutrophils or neutrophil precursors (promyelocytes, myelocytes, and/or metamyelocytes), erythroid predominance, myeloid hypoplasia, very low CD20+ B cells, and increased T cells with lymphohistiocytic aggregates (Fig 1). There was no evidence of dysplasia. Consistent with GATA2 deficiency,⁴ flow cytometric analysis of the marrow aspirate showed 4% myeloblasts, absence of maturing neutrophil precursors, monocytopenia, severe B lymphopenia, and increased T cells with a CD4:CD8 ratio of 0.48. The patient's clinical history, peripheral blood, and bone marrow findings were compatible with GATA2 deficiency, although no mutation in *GATA2* was identified.^{1,5} Bone marrow cytogenetic analysis was normal. Unlike GATA2-deficient marrow, both dendritic cells and B-cell precursors were present.

Because of her recent intensive care hospitalization, invasive fusariosis, and 3-month history of refractory neutropenia, the patient underwent haploidentical HSCT from her 17-year-old sister, who had normal blood cell counts (Table I) and no history suggestive of immunodeficiency. Her immediate posttransplant course was complicated by methicillin-resistant *Staphylococcus aureus* pneumonia and bacteremia on day +8 requiring intubation and mechanical ventilation. She had neutrophil engraftment on day +19 and was extubated on day +20. Her invasive fungal infection resolved. By day +30 her CD3+ and myeloid chimerisms were 100% donor and her monocyte count and natural killer cell counts returned to normal. CD19+ B cells required 1 year to normalize. Immunosuppression was tapered after day +180. More than 2 years posttransplant she is off all immunosuppression with complete normalization of her hematologic and clinical abnormalities (see this article's Methods section in the Online Repository at www.jacionline.org).

TABLE I. Clinical characteristics of complete *CECR1* deficiency and her kindred

Characteristic	Patient ^c	Father ^a	Mother ^b	Sister ^d	Sister ^e	Sister ^f	Sister ^g	Brother ^h	Reference range
Clinical									
Age (y)	20	39	40	17	12	8	8	5	
<i>CECR1</i> status	—/—	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/+
Infection	<i>Fusarium proliferatum</i> sinusitis	None	None	None	None	None	None	None	None
Vasculitis	No	No	No	No	No	No	No	No	No
Laboratory									
WBC	0.7 ↓	3.6 ↓	6.8	4.2	6.8	5.1	4.8	ND	4.2-9.0 × 10 ³ cells/ μ L
ANC	0 ↓	1.69 ↓	4	2.4	4.3	2.2	2.1	ND	1.7-5.3 × 10 ³ cells/ μ L
AMC	0 ↓	0.22 ↓	0.43	0.24 ↓	0.41	0.26 ↓	0.12 ↓	ND	0.3-0.8 × 10 ³ cells/ μ L
Hb	10.2 ↓	16.5	12.3	13.5	13.6	13.4	13.4	ND	11.2-15.7 g/dL
Platelets	125 ↓	155 ↓	183	188	194	143	268	ND	173-369 × 10 ³ cells/ μ L
ABS CD19+	0 ↓	126	224	145	250	242	223	ND	61-321 cells/ μ L
ABS NK (CD56+)	32 ↓	140	213	175	426	416	488	ND	126-729 cells/ μ L
ABS CD3+	645 ↓	1190	1860	939	1279	1887	1633	ND	714-2266 cells/ μ L

ABS, Absolute number cells/ μ L; AMC, absolute monocyte count/ μ L; ANC, absolute neutrophil count/ μ L; Hb, hemoglobin; ND, not done; NK, natural killer; WBC, white blood cell. Superscript letters in column headers correspond to individual in pedigree, Fig E1. Boldface numbers indicate values outside the reference range listed in last column.

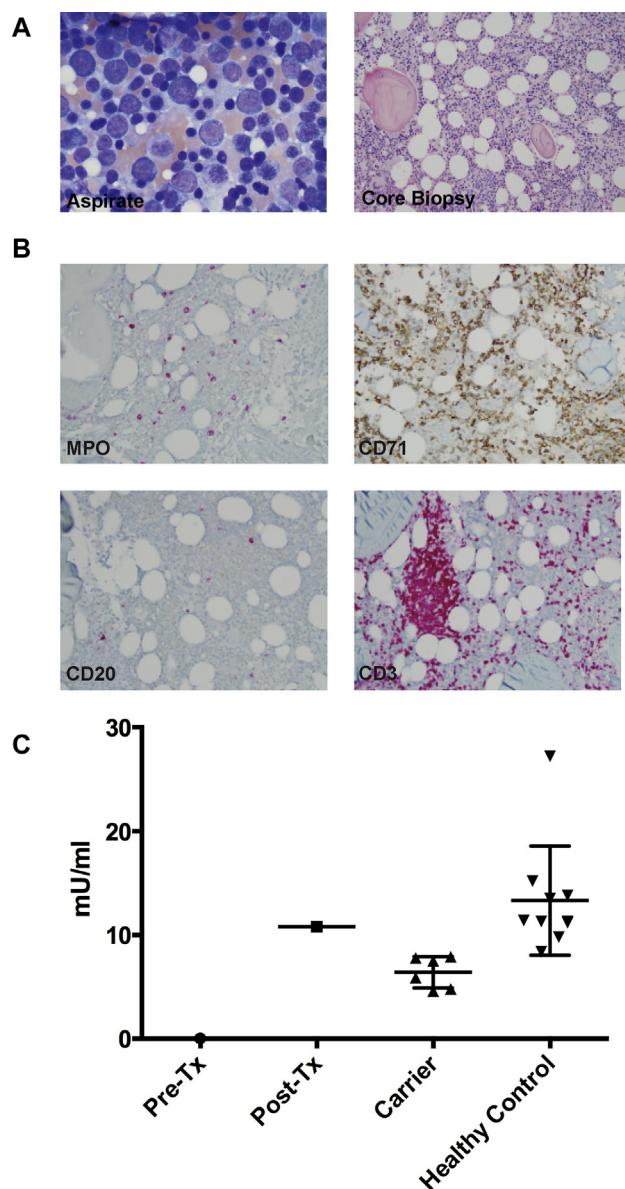


FIG 1. Bone marrow findings and ADA2 enzyme activity. **A**, Left: Bone marrow aspirate. Right: Bone marrow core biopsy. **B**, Immunohistochemistry: Myeloperoxidase (MPO); Erythroid (CD71); B cells (CD20), T cells (CD3). **C**, ADA2 plasma enzyme activity levels from the patient, pretransplant and posttransplant, her first-degree carrier relatives, and 10 healthy, nonrelated controls. Tx, Transplant.

WES was pursued because of the failure to detect a mutation in *GATA2*. After filtering out common variants, we identified a novel, homozygous change leading to a premature stop codon, c.794C>G, p.Gln265Stop, in *CECR1* encoding ADA2. Sanger sequencing of parents and siblings demonstrated all to be heterozygous for *CECR1* c.794C>G (see Fig E1 in this article's Online Repository at www.jacionline.org). No consanguinity is reported by the family; however, they are from a rural village and there is a homozygous region of 650kb surrounding *CECR1*, suggesting a common ancestral allele. No other family member had any clinical manifestations of disease. However, both the father and 2 twin sisters had slightly low neutrophil counts (Table I). Plasma ADA2 levels were undetectable in this patient and intermediate

in both her parents and siblings; the patient's plasma ADA2 activity normalized posttransplant (Fig 1). ADA2 enzyme activity has been shown to be elevated in several primary immunodeficiencies.⁶ The posttransplant ADA2 activity level could be the result of a single time point sample or due to underlying interactions with nonhematopoietic host cells, consistent with other immunodeficient patients.

In 2014, mutations in *CECR1*/ADA2 were shown to underlie a complex autoinflammatory syndrome of intermittent fevers, early-onset lacunar strokes, and other neurovascular manifestations including livedo rash, hepatosplenomegaly, and systemic vasculopathy.^{2,3} In the first study, 6 patients were compound heterozygous for 8 *CECR1* mutations, while 3 patients with polyarteritis nodosum or small vessel vasculitis were homozygous for a p.Gly47Arg mutation.³ All 9 patients had low ADA2 enzyme activity in the blood. Skin, liver, and brain biopsies revealed vasculopathic changes. These patients had modest decreases in B lymphocytes, with 2 patients treated with intravenous immunoglobulin. Knockdown of the zebrafish ADA2 homologue caused intracranial hemorrhage and neutropenia. The second study described 6 families with inherited polyarteritis nodosum with homozygous or compound heterozygous missense mutations in *CECR1*.² A subsequent report emphasized B-cell deficiency and hypogammaglobulinemia in 2 siblings with compound heterozygous, missense *CECR1* mutations.⁷

The initial report of ADA2 deficiency noted that systemic immunosuppressive treatment did not control the inflammatory state.³ However, they suggested that HSCT might reverse the phenotype because monocytes and macrophages are the 2 main cell types that secrete ADA2. Subsequently, Van Eyck et al⁸ reported the successful use of HLA-matched sibling HSCT in a 3-year-old boy with homozygous ADA2 p.Arg169Gln mutation who had lymphadenopathy, splenomegaly, and autoimmunity. Five years posttransplant the patient has had complete reversal of the ADA2 immunologic phenotype and is off all medications. A 4-year-old patient with ADA2 also underwent a successful transplant from an unrelated donor after myeloablative conditioning.⁹

As more ADA2-deficient patients are identified, the phenotype will continue to expand. ADA2 deficiency should be considered in the setting of immunodeficiency, even without vasculopathy. Interestingly, it is possible that whereas aberrant ADA2 proteins (due to missense mutations) may cause additional complications, the complete absence of ADA2, as in this case, may lead to profound cytopenias rather than the vascular complications seen with missense mutations. This case highlights the need for WES analysis in patients with a phenotype suspicious for a known genetic disorder yet no mutation identified in the candidate gene.

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METHODS

Patients provided written informed consent and were investigated under National Institute of Allergy and Infectious Diseases and National Cancer Institute Institutional Review Board–approved research protocols. Written informed consent was obtained before DNA isolation from blood of all family members.

Whole-exome sequencing

Genomic DNA samples for WES were prepared from peripheral blood by using the PureGene DNA extraction kit (QIAGEN, Valencia, Calif). 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 (NIAID Genomics Core Facility, Rocky Mount, Mont). 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

Pretransplant DNA of the region of interest in *CECR1* was amplified with the primers 5'-AGCAGCAGAGACAATGCCCAAGGCCGTAGAG-3' and 5'-ACGTGCCAGGCCACGCACATGGTACTACTG-3'. Resulting PCR products were treated with ExoSAP-IT (USB/Affymetrix, Cleveland, Ohio) and sequenced using the primer 5'-CAGAGCTCGTTGATGGCTGGGAAGGAGTTGACT-3' and Big Dye Terminators v3.1 (Applied Biosystems, Foster City, Calif) chemistry. The reactions were purified with DTR spin plates (EDGE Biosystems, Gaithersburg, Md) and run on an ABI 3730 XL Genetic Analyzer (Applied Biosystems). Sequencing data were analyzed using Sequencher (GeneCodes Corporation, Ann Arbor, Mich).

Flow cytometry

PBMCs were isolated from heparinized blood of patients and control subjects using lymphocyte separation medium (MP Biomedicals, Solon, Ohio) and frozen in 10% dimethyl sulfoxide (Sigma, Allentown, Pa). Thawed cells were stained with antibodies (from eBioscience, San Diego, Calif, unless stated otherwise) against CD3 (SK7), CD19 (HIB19), CD56 (MEM188), and CD14 (61D3). All data were acquired on BD FACSCanto II and analyzed with FlowJo (Tree Star, Ashland, Ore).

Measurements of ADA2 activity in plasma

ADA2 activity in plasma was measured at Duke University by using the HPLC method described by Zhou et al^{E1} and Van Eyk et al.^{E2}

Chimerisms

The Department of Laboratory Medicine at the National Institutes of Health Clinical Center is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) and performed donor chimerism assays. Donor chimerism was measured using predetermined differences in short tandem repeats to quantitate the proportion of donor nucleated cells in samples obtained from transplant recipients. The assay can consistently measure donor chimerism ranging from 5% to 95% with an SD of $\pm 5\%$.

Transplant regimen

The patient was conditioned with fludarabine 24 mg/m²/d on days –6 to –2, cyclophosphamide 14.5 mg/kg on days –6 and –5, busulfan 3.2 mg/kg/d on days –4 and –3, and 200 cGy total body irradiation on day –1. On day 0 she received 1.24×10^8 total nucleated cells/kg and 0.48×10^6 CD34+ cells/kg from her donor. Because of low donor bone marrow cell dose, the donor was remobilized with filgrastim for 4 days along with a single dose of plerixafor 20 mg/kg subcutaneously 15 hours before apheresis. The patient received 5.7×10^6 mobilized CD34+ peripheral blood stem cells/kg on day +3. Posttransplant immunosuppression consisted of cyclophosphamide 50 mg/kg recipient body weight on days +3 and +4, followed by tacrolimus starting at day +5 until day +180, and mycophenolate mofetil on day +5 until day +35.

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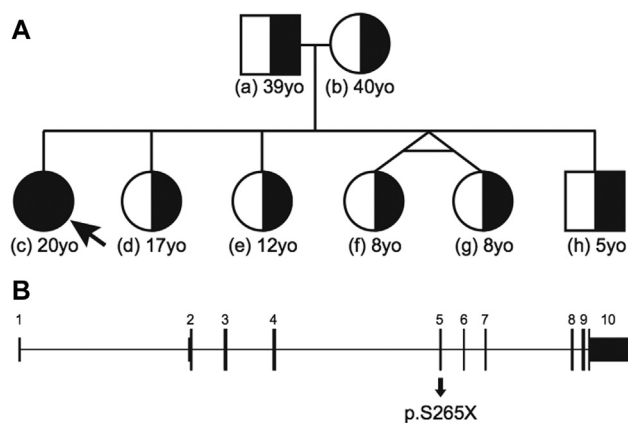


FIG E1. Familial inheritance of *CECR1* mutation. **A**, Pedigree with carriers designated by *half-filled boxes* and *solid circle* for the homozygous proband (*arrow*). Ages at the time of transplant are shown; lowercase letters correspond to [Table 1](#) designations. **B**, Diagram of the *CECR1* gene (NG_033943.1) isoform a (NM_001282225). The arrow indicates the location of the C>G mutation in exon 5 of the *CECR1* gene causing the substitution of a stop codon (X) for serine (S). Wide bars indicate coding sequence, narrow bars indicate untranslated cDNA, and thin line indicates intron.