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Adenosine deaminase type 2 deficiency (ADA2) masquerading as GATA2 deficiency: successful hematopoietic stem cell transplantation

Amy P. Hsu, BA[a], **Robert R. West, PhD**[b], **Katherine R. Calvo, MD, PhD**[c], **Jennifer Cuellar-Rodriguez, MD**[d], **Mark Parta, MD**[e], **Susan J. Kelly, PhD**[f], **Nancy J. Ganson, PhD**[f], **Michael S. Hershfield, MD**[g], **Steven M. Holland, MD**[a,*], and **Dennis D. Hickstein, MD**[b,*]

[a]Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

[b]Experimental Transplantation and Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD

[c]Hematology Section, Department of Laboratory Medicine, National Institutes of Health Clinical Center, Bethesda, MD

[d]Department of Infectious Diseases, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubiran, Mexico City, Mexico

[e]Clinical Research Directorate/Clinical Monitoring Research Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland 21702

[f]Department of Medicine, Duke University School of Medicine, Durham, NC 27710

[g]Department of Medicine and Department of Biochemistry, Duke University School of Medicine, Durham, NC 27710

Keywords

To the Editor

The clinical diagnosis of certain primary immunodeficiency diseases (PID) may be challenging due to the narrow phenotypes initially published. With the advent of whole

Corresponding author: Dennis D. Hickstein, MD, Experimental Transplantation and Immunology Branch, Division of Intramural Research, National Cancer Institute, National Institutes of Health, Building 10/CRC, Room 3E-3142, 10 Center Dr., MSC 1203, Bethesda, MD 20892-1456, USA. hicksted@mail.nih.gov; Phone: 301-594-1718; FAX: 301-402-5054.
*These authors contributed equally to this work.

exome sequencing (WES), accurate diagnosis of PID and extension of published phenotypes is more easily accomplished.

We describe a young woman initially diagnosed with common variable immunodeficiency disease (CVID), who developed monocytopenia and *Mycobacterium avium* complex infection (MonoMAC) syndrome. Prior to 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 *CECR1* (encoding 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.

Case Description

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 CVID at age 7 with low IgG levels and frequent respiratory infections. She had frequent infections throughout childhood and received intravenous immune globulin (IVIG) on two occasions at age 15. At age 19 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 ICU care, mechanical ventilation, and treatment with broad-spectrum antibiotics, liposomal amphotericin B, posaconazole, granulocyte colony stimulating factor-1, and corticosteroids.

Upon admission to the NIH she had right maxillary *Fusarium proliferatum* sinusitis. Her absolute neutrophil count was 0 cells/μl, monocytes 1 cell/μl, NK cells 32 cells/μl, and CD20+ B-cells 0 cells/μl (Table 1). 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.

Due to 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 1), and no history suggestive of immunodeficiency. Her immediate post-transplant course was complicated by methicillin-resistant *Staphylococcus aureus* (MRSA) 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 NK cells returned to normal. CD19+ B-cells required one year to normalize. Immunosuppression was tapered after day +180. More than 2 years post-transplant she is off all immunosuppression with complete normalization of her hematologic and clinical abnormalities (see Methods in this article's Online Repository).

WES was pursued due to 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 adenosine deaminase 2 (ADA2). Sanger sequencing of parents and siblings demonstrated all to be heterozygous for *CECR1* c.794C>G (Supplemental Figure 1). 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 two twin sisters had slightly low neutrophil counts (Table 1). Plasma ADA2 levels were undetectable in this patient and intermediate in both her parents and siblings; the patient's plasma ADA2 activity normalized post-transplant (Figure 1). ADA2 enzyme activity has been shown to be elevated in several primary immunodeficiencies.[6] The post-transplant ADA2 activity level could be the result of a single time point sample or due to underlying interactions with non-hematopoietic 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 (PAN) 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 IVIG. Knockdown of the zebrafish *ADA2* homologue caused intracranial hemorrhage and neutropenia. The second study described 6 families with inherited PAN with homozygous or compound heterozygous missense mutations in *CECR1*. [2] A subsequent report emphasized B-cell deficiency and hypogammaglobulinemia in two 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 since monocytes and macrophages are the two main cell types that secrete ADA2. Subsequently, Van Eyck, et. al [8] 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 post-transplant the patient has had complete reversal of the ADA2 immunologic phenotype and is off all medications. A 4 year-old patient with ADA2 was also successfully transplanted with 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations used

CECR1	cat eye syndrome chromosome region, candidate 1)
ADA2	adenosine deaminase type 2
MonoMAC	monocytopenia with atypical mycobacterial infection
GVHD	graft versus host disease
HSCT	hematopoietic stem cell transplantation
ND	not done
NIH	National Institutes of Health

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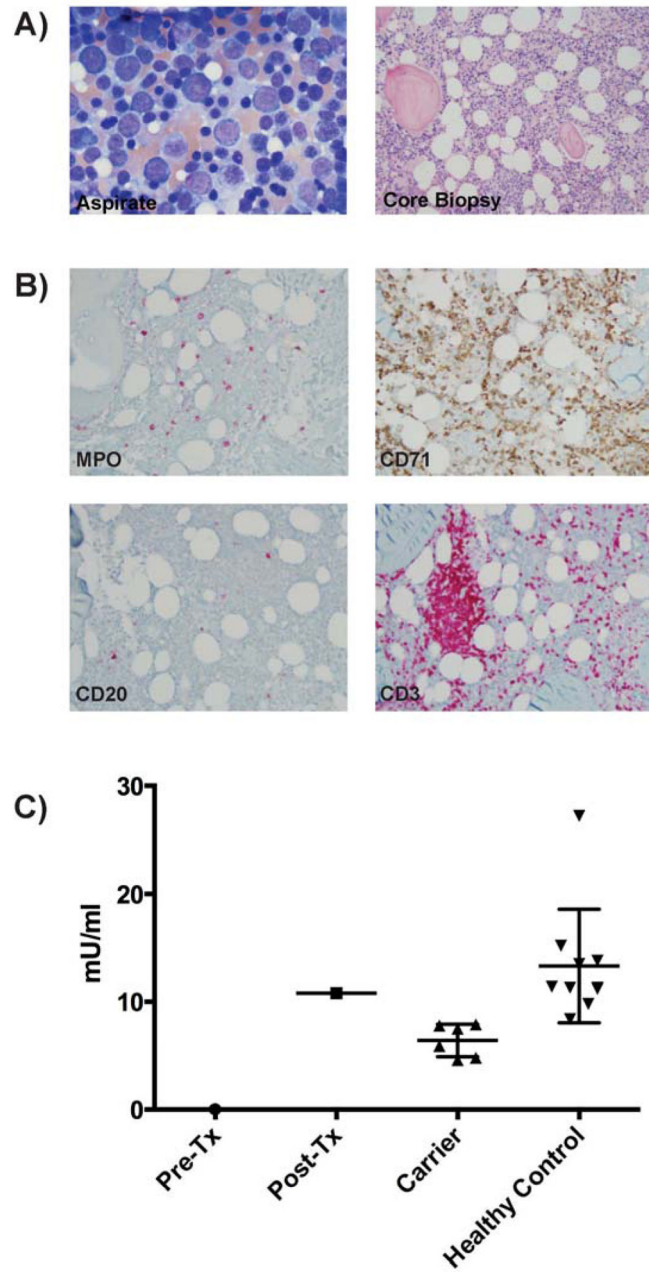
FIG. 1.

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, pre and post transplant, her 1st degree carrier relatives, and 10 healthy, non-related controls.

Table I

Clinical Characteristics of complete *CECR1* Deficiency and Her Kindred

	patient[c]	father[a]	mother[b]	sister[d]	sister[e]	sister[f]	sister[g]	brother[h]	reference range
Clinical									
Age (years)	20	39	40	17	12	8	8	5	
CECR1 status	−/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/+
infection	*Fusarium proliferatum* sinusitis	none	none	none	none	none	none	none	none
vasculitis	no	no	no	no	no	no	no	no	no
Lab									
WBC	**0.7** ↓	**3.6** ↓	6.8	4.2	6.8	5.1	4.8	ND	4.2–9.0 × 10³ cells/μl
ANC	**0** ↓	**1.69** ↓	4	2.4	4.3	2.2	2.1	ND	1.7–5.3 × 10³ cells/μl
AMC	**0** ↓	**0.22** ↓	0.43	**0.24** ↓	0.41	**0.26** ↓	**0.12** ↓	ND	0.3–0.8 × 10³ cells/μl
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 × 10³ cells/μl
ABS CD19+	**0** ↓	126	224	145	250	242	223	ND	61–321 cells/μl
ABS NK(CD56+)	**32** ↓	140	213	175	426	416	488	ND	126–729 cells/μl
ABS CD3+	**645** ↓	1190	1860	939	1279	1887	1633	ND	714–2266 cells/μl