Arthritis & Rheumatology
Vol. 71, No. 10, October 2019, pp 1747–1755
DOI 10.1002/art.40913

American College of Rheumatology
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Identification of Novel Adenosine Deaminase 2 Gene Variants and Varied Clinical Phenotype in Pediatric Vasculitis

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Objective. Individuals with deficiency of adenosine deaminase 2 (DADA2), a recently recognized autosomal recessive disease, present with various systemic vascular and inflammatory manifestations, often with young age at disease onset or with early onset of recurrent strokes. Their clinical features and histologic findings overlap with those of childhood-onset polyarteritis nodosa (PAN), a primary "idiopathic" systemic vasculitis. Despite similar clinical presentation, individuals with DADA2 may respond better to biologic therapy than to traditional immunosuppression. The aim of this study was to screen an international registry of children with systemic primary vasculitis for variants in *ADA*2.

Methods. The coding exons of *ADA*2 were sequenced in 60 children and adolescents with a diagnosis of PAN, cutaneous PAN, or unclassifiable vasculitis (UCV), any chronic vasculitis with onset at age 5 years or younger, or history of stroke. The functional consequences of the identified variants were assessed by ADA2 enzyme assay and immunoblotting.

Results. Nine children with DADA2 (5 with PAN, 3 with UCV, and 1 with antineutrophil cytoplasmic antibody–associated vasculitis) were identified. Among them, 1 patient had no rare variants in the coding region of *ADA*2 and 8 had biallelic, rare variants (minor allele frequency <0.01) with a known association with DADA2 (p.Gly47Arg and p.Gly47Ala) or a novel association (p.Arg9Trp, p.Leu351Gln, and p.Ala357Thr). The clinical phenotype varied widely.

Conclusion. These findings support previous observations indicating that DADA2 has extensive genotypic and phenotypic variability. Thus, screening *ADA*2 among children with vasculitic rash, UCV, PAN, or unexplained, early-onset central nervous system disease with systemic inflammation may enable an earlier diagnosis of DADA2.

INTRODUCTION

Deficiency of adenosine deaminase 2 (DADA2) is a recently characterized autosomal recessive genetic disease that was first reported in 2 independent cohorts of children with early-onset vasculopathy resembling polyarteritis nodosa (PAN) (1,2). All children in the described cohorts harbored rare, biallelic variants in the adenosine deaminase 2 (*ADA2*) locus (formerly known as Cat Eye Syndrome candidate region 1, or *CECR1*), which encodes the enzymatic protein ADA2. The absence of ADA activity in the plasma of all patients substantiated the notion of a damaging effect of the identified variants.

Supported by a Canadian Institute of Health Research (CIHR) grant for the PedVas Initiative (TR2-119188 to Dr. Cabral). Dr. Cabral's work was supported by The Arthritis Society (TAS) Canada through the Ross Petty Arthritis Society Chair. Dr. Brown's work was supported by the Michael Smith Foundation for Health Research and a Cassie & Friends Society Scholar Award. Dr. Drögemöller's work was supported by a CIHR Postdoctoral Fellowship and the Michael Smith Foundation for Health Research Trainee Award.

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No potential conflicts of interest relevant to this article were reported.

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Submitted for publication September 28, 2018; accepted in revised form April 16, 2019.

The most prevalent mutation in the first reports of DADA2 was the p.Gly47Arg variant, having an estimated carrier frequency of 10% in the Georgian-Jewish population—a population known to have high rates of PAN (1). More than 60 disease-causing variants, mostly missense single-nucleotide variants, have now been described across the entire coding region of *ADA2*, including the catalytic, dimerization, and secretion domains (3). The most frequently reported variants, in addition to p.Gly47Arg, are p.Gly47Ala, p.Arg169Gln, Tyr453Cys, and p.Thr360Ala, with the latter being most common in Italian patients (4).

Despite conservation across species (5), there is considerable variation within the human *ADA2* locus. In parallel, there is large phenotypic heterogeneity associated with DADA2. Initial cases were notable for early-onset disease as well as recurrent strokes, fever, and livedo reticularis rash associated with vasculopathy; more recent case descriptions have been extended to include an autoimmune phenotype (6–8), bone marrow deficiencies (4,9), and adult-onset disease (2,10,11). With accumulating reports, the characteristic phenotypic spectrum of DADA2 is widening and there appears to be little correlation between these widening phenotypic clinical features and *ADA2* genotype (4). Patients with DADA2, including those with life-threatening disease, may respond more favorably to treatment with anti–tumor necrosis factor (anti-TNF) blocking agents (12) than with the "traditional" treatments for chronic primary vasculitides (CPVs). It is critical to distinguish patients with DADA2 from those with other types of CPV to enable earlier and more effective interventions; identifying all of the deleterious *ADA2* gene variants and the associated pathogenic mechanisms may further guide both prognostication and therapy.

Because vasculitis remains a predominant feature in most cases of DADA2 described to date (3), we retrospectively and selectively screened an international cohort of children with CPV for DADA2 by targeted Sanger sequencing and assessment of ADA2 enzyme activity in the patients' serum. We identified 9 children with DADA2 with known pathogenic and novel variants in *ADA2*. Based on our observations, we propose additional clinical features that should be considered in screening criteria for DADA2.

PATIENTS AND METHODS

Participants. Patients described in this study were enrolled in the Pediatric Vasculitis Initiative (PedVas), an international study on pediatric CPV. Eligibility criteria for PedVas have been described previously (13). The study protocol was approved by the Children's and Women's Research Ethics Board of the University of British Columbia (approval no. H12-00894) and the respective ethics committees or IRBs at participating PedVas sites. Written informed consent was obtained between February 2013 and July 2018 from children or adolescents age 18 years and younger who were diagnosed as having a systemic CPV, including PAN, cutaneous PAN (cPAN), granulomatosis with polyangiitis (GPA), microscopic polyangiitis, eosinophilic granulomatosis with polyangiitis, Takayasu arteritis, unclassified vasculitis (UCV), or suspected DADA2. Study visits coincided with times of routine clinical care (13). Healthy children with sleep apnea (n = 4), who were enrolled in the BCCH BioBank, were used as pediatric controls for the ADA2 activity assay (approval no. H13-03111).

Clinical data. At each study visit, as described previously, patients contributed data that included demographics, clinical features, medical history, diagnostic data, treatment, and clinical laboratory results (14). Data were entered by participating sites into A Registry of Childhood Vasculitis (ARChiVe), the RedCap (15) data collection platform for PedVas. Clinical data were reviewed in Vancouver for errors and completeness. Patients were formally classified into CPV subtypes by the on-site rheumatologist as well as by using a pediatric-modified algorithm of the European Medicines Agency. Generation of a pediatric vasculitis activity score (PVAS) (16) was a component of data entry to ARChiVe; active and inactive disease was defined as a PVAS of >2 and PVAS of ≤2, respectively.

Biosample collection and processing. Participants contributed blood in serum separation tubes and/or K2 EDTA vacutainers (BD Biosciences) and Tempus RNA tubes (Applied Biosystems). RNA tubes and serum/plasma aliquots were stored at –80°C upon receipt in Vancouver. DNA was isolated from whole blood (collected in K2 EDTA tubes) or saliva (collected in OG-500, OG-575, or OCR-100 Oragene•DNA collection kits; DNA Genotek Inc.) using QIAsymphony SP, in accordance with the manufacturer's protocol (Qiagen). Isolated DNA was quantitated using a Quant-iT PicoGreen double-stranded DNA assay kit (ThermoFisher) and stored at –20°C prior to sequencing.

Sanger sequencing of *ADA2*. Targeted Sanger sequencing was implemented for the coding exons of *ADA2* (exons 2–10) (for primer sequences, see Supplementary Table 1, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40913/abstract). Total genomic DNA (50 ng) was combined with 1× AmpliTaq Gold 360 buffer, 2.0 m*M* magnesium chloride, 3 μl of 360 GC Enhancer, 200 μ*M* dNTPs, 0.5 μ*M* forward primer, 0.5 μ*M* reverse primer, and 0.625 units/reaction AmpliTaq Gold 360 DNA Polymerase in a total volume of 20 μl in a 96-well plate. Initial denaturation was at 95°C for 5 minutes, followed by 40 cycles at 95°C for 50 seconds, 59°C for 35 seconds, 72°C for 60 seconds, and a final extension step at 72°C for 7 minutes. Polymerase chain reaction products were cleaned with ExoSAP-IT Express (ThermoFisher) and analyzed using an ABI Genetic Analyzer (Applied Biosystems). Sequences were aligned to the *ADA2* transcript (ENST00000399839.1, Ensembl GRCh37) and analyzed using CodonCode Aligner version 3.03.

Assay for ADA2 enzyme activity. Extracellular ADA2 enzyme activity was quantified in the patients' serum (patients 1, 2, 4, 6, 7, 8, 10, and 11) and plasma (patient 3) using an ADA assay (Diazyme Laboratories) with some modification to the manufacturer's protocol. Briefly, 10 µl of serum/plasma was incubated with 5 µl of an ADA1-specific inhibitor, erythro-9-(2-hydroxy-3-nonyl) adenine-HCl (100 µ*M*; Millipore Sigma), for 5 minutes at 37°C. Reagent 1 (180 µl) and Reagent 2 (90 µl) were added (reaction volume of 285 µl), and the absorbance at 556 nm was read every 10 minutes over 3 hours, using an Infinite M200 microplate reader and Magellen analysis software (TECAN).

ADA2 enzyme activity in the samples was calculated using a calibrator of known ADA activity and using 0.9% saline as a blank. ADA2 activity in patient 9 was quantified at the local site, using a dried plasma spot analysis. No serum samples were reserved from patient 5 prior to death.

Western blot analysis of ADA2 protein. ADA2 protein in the patients' serum (patients 1, 2, 4, 6, 7, 8, 10, and 11) and plasma (patient 3) was analyzed by immunoblotting. Briefly, nonreduced samples (1:50 dilution) and prestained protein ladder (no. 26619; PageRulerPlus) were resolved on a 10% NuPage sodium dodecyl sulfate–polyacrylamide electrophoresis gel. Separated proteins were transferred to a 0.45-µm PVDF membrane (Immobilon-P; Millipore Sigma) that was blocked with phosphate buffered saline/5% skim milk and probed with an anti-*CECR1* polyclonal antibody (1/1,000 dilution, PA5-30635) followed by a horseradish peroxidase–conjugated goat anti-rabbit IgG (H+L) antibody (1/10,000 dilution, A16104) and SuperSignal West Pico PLUS Chemiluminescent Substrate. The membrane was exposed to autoradiography film (Diamed) and imaged with an Alphalmager 2200 (Alpha Innotech). All reagents were from ThermoFisher, unless specified otherwise.

Statistical analysis. Analysis of variance, followed by a 2-tailed Tukey's test, was used to analyze group differences, and the results were analyzed using GraphPad Prism statistical software version 7.0. For all analyses, 95% confidence intervals were used, and *P* values less than or equal to 0.05 were considered significant.

RESULTS

Identification of novel and rare variants in *ADA2*. Among 542 pediatric CPV patients in our ARChiVe registry, DNA was available from 138 individuals. Of these, Sanger sequencing for variants in the splice-site and coding regions (exons 2–10) of *ADA2* was performed on samples from 60 patients classified as having PAN, cPAN, or UCV (n = 44), suspected DADA2 (n = 2), a disease onset at age ≤5 years (n = 22), and/or stroke-like episodes (n = 7) (see Supplementary Table 2, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40913/abstract). For 3 individuals (patients 5, 6, and 9), *ADA2* variants were identified by whole exome sequencing that was initiated at their respective health care center, and results in 2 samples (patients 6 and 9) were confirmed by Sanger sequencing. Eight individuals carried novel or rare variants in *ADA2* with a minor allele frequency

Table 1. Pediatric vasculitis patients with rare *ADA2* mutations*

Patient	Ethnicity†	Sex	Age at onset‡	Age at diagnosis§	Initial diagnosis	*ADA2* mutation (coding sequence)
1	South Asian	F	11 years	12 years	PAN	c.[139G>C];[139G>C]
2	South Asian	F	11 years	12 years	PAN	c.[139G>A];[139G>A]
3	East Asian	M	16 years	16 years	Unclassified vasculitis	c.[25C>T];[140G>C]¶
4	South Asian	M	11 years	12 years	PAN	c.[1069G>A];[1069G>A]¶
5	White	F	1 week	NA#	Undiagnosed	c.[1052T>A];[1052T>A]¶
6	White	F	10 months	3 years#	Undiagnosed (suspected DADA2)	c.[1052T>A];[1052T>A]¶
7	East Asian	F	4 years	4 years	PAN	c.[139G>A];[139G>A]
8	South Asian	F	3 years	5 years	GPA	No identified variants in coding or splice-site regions
9	White	F	6 months	8 months	Unclassified vasculitis	c.[139G>C]; deletion
10	South Asian	M	11 years	11 years	cPAN	c.927G>A
11	White	F	2 years	NA	Undiagnosed	c.1252G>T

* PAN = polyarteritis nodosa; NA = not applicable; GPA = granulomatosis with polyangiitis; cPAN = cutaneous PAN.
† Self-reported.
‡ At first associated symptoms.
§ At diagnosis of systemic vasculitis.
¶ Variant showing a novel association with deficiency of adenosine deaminase 2 (DADA2).
# In sibling patients 5 and 6, a diagnosis of systemic vasculitis was not made; the diagnosis of DADA2 was made at age 3 years in patient 6 and postmortem in patient 5.

of <0.01, as defined by the Exome Aggregation Consortium (Tables 1 and 2, and Supplementary Figure 1 available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40913/abstract).

Three patients (patients 1, 2, and 7) carried the known pathogenic variant within the dimerization domain of *ADA2*, p.Gly47Arg. Patient 3 was compound heterozygous (confirmed by targeted sequencing of the affected exons in the parents) for a known pathogenic variant, p.Gly47Ala, and a variant showing a novel association with DADA2, p.Arg9Trp, that is located in the signal sequence domain of *ADA2* (see Supplementary Figures 1 and 2 at http://onlinelibrary.wiley.com/doi/10.1002/art.40913/abstract). Patient 4 was homozygous for a variant of novel association, p.Ala357Thr, within the catalytic domain of ADA2 (Supplementary Figure 2). Patients 5 and 6 were siblings; in patient 5, whole exome sequencing (GeneDx) identified a novel variant of unknown significance, p.Leu351Gln, that was subsequently identified in patient 6 through this study. Both parents were heterozygous for the p.Leu351Gln variant (Supplementary Figure 1). Patient 8 had no rare variants in the splice-site or coding regions of *ADA2*. Patient 9 was compound heterozygous (confirmed by trio sequencing [data not shown]) for the known pathogenic variant, p.Gly47Arg, and for a deletion that has not yet been fully characterized. Patients 10 and 11 were heterozygous for rare variants, a known one, p.Met309Ile, and one predicted to be pathogenic, p.Val418Leu. The identified variants showing a novel association with DADA2 (p.Arg9Trp, p.Leu351Gln, and p.Ala357Thr) were predicted to be damaging, by computational modeling (Table 2).

Compromising effects of biallelic *ADA2* variants on ADA2 enzyme activity. The impact of the identified variants on ADA2 enzyme function was quantified in the patients' serum (patients 1, 2, 4, 6, 7, 8, 10, and 11) and plasma (patient 3) using an ADA assay. Samples obtained from patients with rare, biallelic *ADA2* variants (patients 1, 2, 3, 4, 6, and 7) had a significant loss of ADA2 enzyme activity (Figure 1B) compared to healthy children (n = 4; $P < 0.0001$) and other children with vasculitis that met *ADA2* screening criteria but did not carry rare *ADA2* variants, which included children with PAN (n = 4; $P < 0.0001$) and children with Takayasu arteritis (n = 7; $P < 0.0001$), a large-vessel vasculitis that may or may not be associated with stroke (Figure 1A). ADA2 enzyme activity was also diminished in patient 8 despite a lack of rare variants in the coding and splice-site regions of *ADA2,* suggesting that this patient may harbor damaging noncoding or structural variants. ADA2 enzyme activity in the serum from patients with a single, rare, known pathogenic variant (patient 10) or a single, rare, predicted pathogenic variant (patient 11) was similar to that in the control groups (Figures 1A and B).

To confirm that ADA2 protein was present in patient samples (thereby confirming that loss of ADA2 enzyme activity could not be attributable to the absence of ADA2 protein), samples of

Table 2. Characteristics of the identified *ADA2* variants and the predicted consequence in silico*

rsID	Coding sequence	Predicted consequence	ExAC MAF	ExAC GMAF	CADD	PolyPhen predicted consequence	ClinVar annotation
rs202134424	c.139 G>C	Missense (p.Gly47Arg)	0.001	<0.01	26	Probably damaging	Pathogenic
rs202134424	c.139G>A	Missense (p.Gly47Arg)	7.40×10^{-5}	<0.01	26	Probably damaging	Pathogenic
rs200930463	c.140G>C	Missense (p.Gly47Ala)	6.60×10^{-5}	<0.01	26	Probably damaging	Pathogenic
rs753994372	c.25C>T†	Missense (p.Arg9Trp)	8.20×10^{-6}	<0.01	18	Benign	NA
rs374974565	c.1069G>A†	Missense (p.Ala357Thr)	1.20×10^{-4}	<0.01	31	Probably damaging	NA
NA	c.1052>A†	Missense (p.Leu351Gln)	NA	NA	25	Probably damaging	NA
rs146597836	c.927G>A	Missense (p.Met309Ile)	0.002	<0.01	3	Benign	Pathogenic in Behçet's syndrome; likely benign in PAN
rs142726959	c.1252G>T	Missense (p.Val418Leu)	7.52×10^{-5}	<0.01	29	Probably damaging	NA

* For each variant, the predicted consequence is shown, along with the following characteristics: ethnicity-matched minor allele frequency (MAF) as reported by the Exome Aggregation Consortium (ExAC), global MAF (GMAF) as reported by the ExAC, combined annotation-dependent depletion score (CADD) based on the conservation and consequence of the affected amino acid, consequence of the amino acid substitution as predicted by PolyPhen, and the ClinVar annotation. NA = not applicable; PAN = polyarteritis nodosa.
† Variant showing a novel association with deficiency of adenosine deaminase 2 (DADA2).

patients' serum/plasma were resolved by gel electrophoresis, and ADA2 protein was detected by immunoblotting. ADA2 protein in all serum/plasma samples was detected at levels similar to those in a patient without variants in ADA2 (a patient with PAN) and was detected in the dimeric form (~120 kd), even in those patients carrying pathogenic variants in the dimerization domain (patients 1–3 and patient 7) (Figure 1C). Furthermore, using paired serum samples from patients with PAN who were negative for rare *ADA2* variants (n = 3), we confirmed that disease activity did not influence the results, as ADA2 activity was similar in samples obtained at a time when the disease was active (PVAS scores of 7, 12, and 17 in the 3 patients) and in samples obtained when it was inactive (PVAS scores of 0 in each patient) ($P = 0.2324$) (data not shown).

Variation in clinical phenotypes associated with rare, biallelic *ADA2* variants. The age range at the time of symptom onset in patients in our cohort with rare or novel *ADA2* variants was 1 week to 16 years (Table 1). Clinical manifestations in the 9 patients with DADA2 are summarized in Table 3. Cutaneous involvement was present in 8 of the 9 patients and included livedo reticularis/racemosa, nodular lesions, soft tissue/subcutaneous edema, and ulcers. Neurologic involvement, a common cardinal feature that has been reported in other DADA2 patient cohorts (1,2,4), was also present in 8 of 9 patients. Five of these patients had central nervous system (CNS) involvement in the form of stroke (patients 3, 4, 6, 7, and 9) and 1 had stroke along with diffuse cerebral atrophy (patient 5) (Figure 2A), while 4 had peripheral nervous system (PNS) involvement, of whom 1 had mononeuritis multiplex (patient 2) and 3 had cranial nerve involvement (patients 6, 7, and 9).

Sibling patients 5 and 6 had severe disease. Both had gastrointestinal disease resulting in bowel perforations, and significant hematologic disease. Patient 5 at age 1 week was diagnosed as having presumed Diamond-Blackfan anemia, but subsequent testing excluded the diagnosis. She later developed severe neutropenia, with the absence of neutrophils on a complete blood cell count, and had a bone marrow biopsy that showed no neutrophils or precursors (Figure 2B). She died at the age of 17 months due to complications of the bowel perforation. Patient 6, the older sister of patient 5, was treated for urosepsis at age 4 months and was noted to have persisting, unexplained elevated liver transaminase levels. At age 10 months, she developed a third nerve palsy. She was asymptomatic until age 3 years, when she presented, shortly after the death of her younger sibling, with fevers, raised levels of inflammation markers, neutropenia, and evidence of prior stroke.

Four patients had low IgM levels despite having normal levels of IgA and IgG. Blurred vision (defined in the registry as "altered measurement of best visual acuity from previous or baseline, requiring specialist opinion for further evaluation") was noted in 4 patients, in the absence of other diagnoses such as ocular inflammation (uveitis, scleritis, episcleritis) or any underlying retinal pathology. No patient with PAN who was not later diagnosed as having DADA2 had this symptom. All patients were negative for antinuclear antibodies. One patient (patient 8) who was positive for antineutrophil cytoplasmic antibodies (ANCAs), specifically anti–proteinase 3 ANCAs, was diagnosed as having GPA (fulfilling classification criteria), having both multiple pulmonary nodules

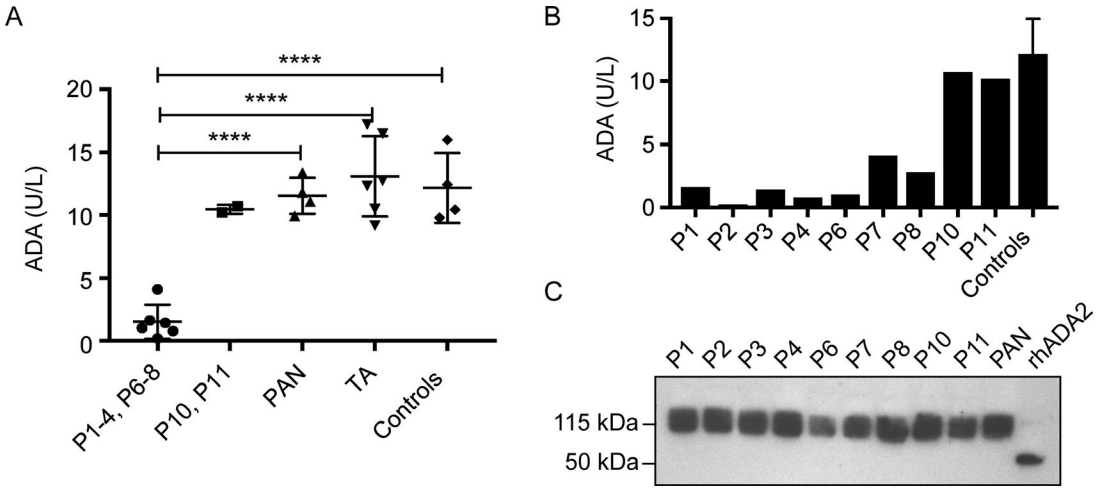

Figure 1. **A**, Adenosine deaminase 2 (ADA2) activity (measured in units/liter) in the serum or plasma of healthy children (controls; n = 4), children with vasculitis and overlapping clinical symptoms (polyarteritis nodosa [PAN] n = 4; Takayasu arteritis [TA] n = 6), and children with biallelic variants in *ADA2* (patients P1–4 and P6–8; n = 7) or with single, heterozygous variants in *ADA2* (patients P10 and P11; n = 2). **B**, ADA2 activity in the serum or plasma of children with biallelic variants in *ADA2* (patients P1–P4, P6, and P7), children with no identified variants in *ADA2* (patient P8), children with a single variant in *ADA2* (patients P10 and P11), and pediatric controls (n = 4). Results in **A** and **B** are the mean ± SD. **C**, Immunoblot analysis of ADA2 protein in the serum of pediatric patients with diminished ADA2 enzyme activity (patients P1–4, P6–8, P10, and P11) compared to a pediatric patient with PAN who had no rare *ADA2* variants and to 10 ng recombinant human ADA2 (rhADA2). **** = $P < 0.0001$.

Table 3. Clinical manifestations in the patients with deficiency of adenosine deaminase 2

	Fever	Cutaneous	Nervous system	Other organ systems	Vascular imaging	Hypogammaglobulinemia
Patient 1	No	Nodules; livedo reticularis	None	Blurred vision; anemia	Microaneurysms (hepatic and splenic artery)	Not determined
Patient 2	Yes	Nodules; diffuse finger and toe swelling	Motor mononeuritis multiplex	Oral ulcers; abdominal pain	Microaneurysms (splanchnic vessels)	Not determined
Patient 3	Yes	Painful subcutaneous nodules	Meningitis/encephalitis; brainstem infarct	None	No abnormal findings	Not determined
Patient 4	Yes	Ulcers; livedo reticularis; superficial infarctions	Midbrain infarcts	Blurred vision	Aneurysms (renal, splanchnic, vertebral arteries)	Low IgM
Patient 5	Yes	Livedo racemosa or cutis marmorata (age 1 week)	Diffuse cerebral atrophy	Oral ulcers; bloody diarrhea; perforated bowel; anemia; neutropenia	No abnormal findings	Low IgM
Patient 6	Yes	None	Stroke; cranial nerve involvement	Blurred vision; chronic liver disease; perforated bowel; neutropenia	No abnormal findings	Low IgM
Patient 7	Yes	Subcutaneous edema; nodules	Stroke; cranial nerve involvement	Blurred vision	Restricted diffusion on left thalamus	Low IgM
Patient 8	Yes	Subcutaneous edema; polymorphous rash	Decreased tendon reflexes	Oral ulcers; anal fissures; saddle nose deformity; glomerulonephritis	No vascular imaging performed	Normal
Patient 9	Yes	Livedo reticularis; Raynaud's phenomenon	Oculomotor nerve involvement; lacunar cerebral infarctions	Oculomotor palsy; ileitis; splenic infarction; hypertension	Decreased diameter of thoracic aorta and right radial artery; irregularities of the abdominal aorta	Not determined

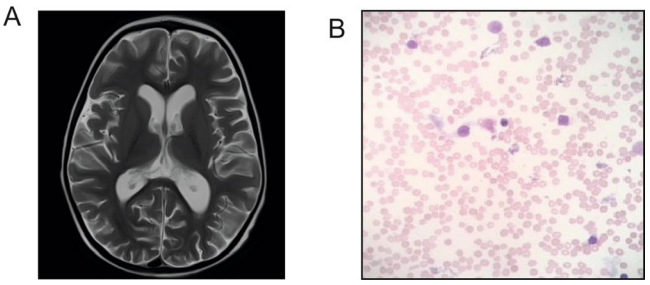

Figure 2. P5 magnetic resonance imaging of the brain (**A**) and bone marrow aspirate (**B**) from representative children with deficiency of adenosine deaminase 2 (DADA2). **A**, T2-weighted axial image of the brain of a patient with DADA2 demonstrates diffuse white matter volume loss. The myelination pattern is appropriate for the age of the patient, indicating no evidence of dysmyelination or demyelination. **B**, Bone marrow aspirate from a patient with DADA2 at age 17 months, in whom the complete white blood cell count was 1.5×10^9/liter, with a neutrophil count of 0.0×10^9/liter and monocyte count of 0.0×10^9/liter, the hemoglobin level was 88 gm/liter (with hypochromia and microcytosis), and the platelet count was 901×10^9/liter. The aspirate is dilute and aparticulate, with a marked decrease in granulopoiesis.

on imaging and renal histopathologic findings showing necrotizing fibrinoid necrosis and pauci-immune glomerulonephritis with crescents.

Variation in the clinical treatment response. Disease severity at presentation ranged from mild and limited to the skin, to severe and lethal. All patients were initially treated with immune-suppressing medications and glucocorticoids (GCs). Initial treatments and the response to treatments are summarized in Table 4. Patients 1 and 2 had the mildest phenotype, with primarily skin disease. Patient 1 responded well to treatment with GCs, methotrexate, and colchicine. Patient 2 was treated with GCs, methotrexate, and intravenous (IV) immunoglobulin; IV cyclophosphamide was added at 6 months for ongoing disease activity. Five patients had moderate-to-severe disease: patient 3 had mild stroke, patient 4 had ulcerating skin disease and aneurysms of the vertebral and splanchnic arteries, patient 7 had stroke and cranial nerve involvement, patient 8 had renal disease, and patient 9 had stroke and cranial nerve involvement. Patient 3 was treated with GCs and 1 dose of rituximab, and then switched to etanercept 1 month later when DADA2 was diagnosed. He responded well to etanercept. Patient 4 and patient 7 were treated with GCs and IV cyclophosphamide. Patient 4 switched to mycophenolate mofetil after 6 months due to poor response. Patient 8 responded well to GCs and methotrexate. Patient 9 was treated with GCs and azathioprine but switched to IV cyclophosphamide due to lack of response. After several years of poorly controlled disease, the patient was started on etanercept, achieving an excellent response.

As previously described, sibling patients 5 and 6 had very severe disease. Patient 5 was initially treated successfully with GCs for the presumed Diamond-Blackfan anemia. At age 14 months, she developed fevers, severe neutropenia, anemia, elevated levels of inflammation markers, and transient rash. GCs and etanercept were initiated after the diagnosis of DADA2 was made; however, she died (due to bowel perforation) after receiving only 1 dose of etanercept. Pathologic examination of a section of the bowel revealed multifocal ischemia with severe bacterial overgrowth, suggesting neutropenic enterocolitis but no definitive evidence of vasculitis. A complete autopsy was

Table 4. Initial treatment and response*

	Initial treatment	Response to treatment
Patient 1	GC, methotrexate, colchicine	Improved on initial treatment; flared at 12 months and restarted on GCs
Patient 2	GC, methotrexate, IVIG	IV cyclophosphamide added 6 months after diagnosis due to ongoing disease activity; improved on cyclophosphamide
Patient 3	GC, rituximab (one dose)	Switched to etanercept 1 month after presentation (after diagnosis of DADA2 was made); inactive disease at 6 months without GC treatment
Patient 4	GC, IV cyclophosphamide	Switched to mycophenolate mofetil at 6 months due to ongoing disease activity and difficult IV access
Patient 5	GC	Etanercept started 1 year after presentation (when diagnosis of DADA2 was made); only received 1 dose of etanercept prior to death
Patient 6	GC, etanercept	Good response to initial treatment; relapse at 4 months; anakinra added to no benefit; underwent bone marrow transplantation
Patient 7	GC, IV cyclophosphamide	Improved on initial treatment; inactive disease at postinduction visit (6 months after diagnosis); switched to azathioprine for maintenance
Patient 8	GC, methotrexate	Improved on initial treatment
Patient 9	GC, azathioprine	Switched to IV cyclophosphamide 3 months after diagnosis, for ongoing disease activity; poor disease control for several years until etanercept started 5 years after diagnosis, with prompt and stable response to etanercept

* GC = glucocorticoid; IVIG = intravenous immumoglobulin; DADA2 = deficiency of adenosine deaminase 2.

declined by the family. Patient 6 was diagnosed as having DADA2 shortly after the death of her sibling and was promptly treated with GCs and etanercept, achieving a good response. After 4 months, the disease relapsed with a recurrence of the fevers and severe neutropenia. As she continued to deteriorate despite an increase in the GC dose and the addition of anakinra to the etanercept regimen, a hematopoietic stem cell transplantation was performed. Currently, at 6 months post-transplantation, she requires only low-dose GCs for treatment of mild graft-versus-host disease.

DISCUSSION

Herein we describe 9 patients with DADA2, of whom 8 had rare, biallelic variants in *ADA2* and 1 had abrogated ADA2 enzymatic activity but no rare variants in the splice-site or coding regions of *ADA2*. The identified variants included 2 variants showing a novel association with DADA2, Arg9Trp (c.25C>T) and Ala357Thr (c.1069G>A), and 1 novel variant, Leu351Gln (c.1052>A). An additional 2 patients carried a single, rare variant in *ADA2.* ADA2 enzyme activity assay and immunoblotting confirmed that patients with biallelic variants had circulating ADA2 protein with severely compromised enzyme activity as compared to that in patients with a single variant, in whom the ADA2 enzyme activity was similar to that in healthy children and children with other vasculitides. These data are consistent with the autosomal recessive mode of inheritance for DADA2 (1,2).

The clinical phenotype of the earliest described cohorts of DADA2 patients included young age at disease onset, early-onset stroke, and livedo reticularis/racemosa rash (1,2). Similarly, vasculitic skin rash (especially livedo reticularis and nodular lesions) and an initial diagnosis of PAN was characteristic of most of our cohort. In contrast, 4 of 9 patients were older than age 10 years at the time of symptom onset. In view of this onset of disease in adolescence and the high frequency of the disease (1 in 8) among the PAN cohort, DADA2 should be considered in the differential diagnosis of patients of all ages with a PAN phenotype. Our data also suggest that DADA2 should be considered in the differential diagnosis of patients outside of the PAN phenotype. The siblings were the youngest patients in our cohort and had the most severe disease, although there were no signs of overt clinical vasculitis (for example, persistent skin rash). Patient 6 had a history of early-onset stroke, while patient 5 had cerebral atrophy without evidence of stroke. Both patients had evidence of a systemic inflammatory response (fever, elevated levels of inflammation markers). Given the disease severity in these patients, DADA2 should be considered in the differential diagnosis of all patients with unexplained early-onset CNS disease (not limited to stroke) with systemic inflammatory features, even in the absence of "vasculitic" rash.

The earlier diagnosis of DADA2 in patient 5 may have been life-saving, and in patient 6, the earlier diagnosis may have enabled earlier treatment with etanercept or hematopoietic stem cell transplantation. Even patients in our cohort with predominantly skin disease required treatment with immune-suppressing medications and GCs—earlier diagnosis of DADA2 in such patients may have prompted alternative, more effective treatments and might have reduced the need for GCs. Several of the patients in our cohort required medication changes due to ongoing disease or flares of disease. Caorsi et al described a similar poor or partial response to conventional immunosuppression in their cohort of DADA2 patients, while patients who received anti-TNF therapy had high rates of remission (17). In our cohort, anti-TNF treatment was used in only 4 patients (patients 3, 5, 6, and 9), in whom disease onset, persisting disease activity, or flare occurred after the DADA2 entity was uncovered (Table 4). In 2 patients (patients 3 and 9), there was an excellent response. Patient 5 died but had only received 1 dose of anti-TNF medication, and patient 6 had an initial response followed by disease relapse. Both patient 5 and patient 6 had severe neutropenia and IgM immunodeficiency, and there is recent evidence that patients with DADA2 who have this phenotype may respond better to hematopoietic stem cell transplantation (18).

As reports of DADA2 cases accumulate, the clinical phenotype continues to evolve and expand. In our cohort, 3 patients were of South Asian descent and 1 was of East Asian descent. Few cohorts have described DADA2 patients of these ethnicities, and it is possible that they are underdiagnosed and underreported. The symptom of blurred vision in 4 patients is interesting. Other patients with PAN in our cohort did not have this reported symptom. The etiology of the blurred vision (either ocular, neurologic, or perhaps thrombotic) remains unclear, as no other ocular diagnoses were reported in any of these patients, no other neurologic abnormalities were identified in 2 of the patients, and none had thromboses at other sites. Additional retrospective elucidation in these patients is not feasible; however, prospective surveillance of this symptom in other patients may clarify its significance.

As the spectrum of DADA2 manifestations unfolds and the age range at the time of disease presentation is clarified, it will likely be increasingly included in the differential diagnosis for a spectrum of unexplained "vasculopathies" and systemic inflammatory diseases with CNS and PNS manifestations beyond the PAN phenotype. Our current inability to correlate genotype to phenotype and the absence of an identifiable genetic abnormality in 1 patient suggest that other factors are involved in the pathogenesis of DADA2, which may include modifying alleles, epigenetic modifications, or environmental exposures. For DADA2 patients with an autoimmune or bone marrow–deficient phenotype, hematopoietic stem cell transplantation may be an effective therapy (18,19). In addition, there is clinical evidence that patients with DADA2 elicit a positive response to biologic therapy, particularly anti-TNF therapy (2), but there is little mechanistic evidence for particular treatment choices. A deeper understanding of all of the factors contributing to the pathogenesis of DADA2 will be crucial to predict its disease course in individuals, inform clinical treat-

ment decisions, and improve the outcomes in patients with DADA2.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Drs. Cabral and Brown had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Gibson, Morishita, Dancey, Drögemöller, Graham, Hancock, Foell, Ross, Cabral, Brown.

Acquisition of data. Gibson, Dancey, Moorehead, Han, Benseler, Luqmani, Yeung, Shenoi, Bohm, Rosenberg, Cabral.

Analysis and interpretation of data. Gibson, Morishita, Dancey, Moorehead, Drögemöller, Ross, Cabral, Brown.

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