A Case Series of Adenosine Deaminase 2-deficient Patients Emphasizing Treatment and Genotype-phenotype Correlations

To the Editor:

Deficiency of adenosine deaminase 2 (DADA2) causes a vasculopathy with autoinflammatory features associated with mutations in *CECR1*[1]. The phenotype of DADA2 varies from only cutaneous lesions to full-blown systemic disease with central nervous system (CNS) involvement and aneurysms in visceral arteries that may overlap with the spectrum of polyarteritis nodosa (PAN)[1,2,3].

The Chapel Hill Consensus Conference (CHCC) 2012 defines PAN as a necrotizing vasculitis of medium or small arteries without glomerulonephritis or vasculitis in arterioles, venules, or capillaries and not associated with antineutrophil cytoplasmic antibodies[4]. Now, with the discovery of DADA2, we know that monogenic disorders may cause a PAN-like vasculopathy. Thus, DADA2 should be classified under the group of "vasculitis with a probable cause" in CHCC 2012[4].

We herein present the characteristics of 6 DADA2 patients and their response to various therapies.

Three of our patients had been initially screened at the U.S. National Institutes of Health (NIH) because they had suggestive features for the *CECR1* mutations. Subsequently, we screened 17 patients with suggestive features and identified 3 new cases. We have evaluated the course of these patients for a followup of median 8.5 years. All patients were Turkish and were followed in the departments of Rheumatology and Pediatric Rheumatology at Hacettepe University, Ankara, Turkey. Three (patients 2, 3, and 5) had been included in a previous paper[2].

Peripheral blood samples for DNA extraction were obtained. Sanger sequencing was performed to sequence 10 exons of *CECR1* in NIH (n = 3) and Hacettepe University (n = 3). Primer sequences are available in the Appendix. PCR products were directly sequenced using ABI Prism 3130 Automated Sequencer (Applied Biosystems).

We defined 6 DADA2 patients from 5 families. The characteristics and treatment of patients are summarized in Table 1 and Table 2. There was consanguinity in 3 families (patients 1, 5, and 6). Homozygosity for the p.G47R mutation in *CECR1* was detected in all patients.

All patients had a childhood onset of symptoms (median age 7.2 yrs). Five were initially diagnosed with systemic PAN fulfilling the 2008 Ankara or the American College of Rheumatology 1990 criteria[5,6]. One (patient 1) was diagnosed with cutaneous PAN.

The disease showed autoinflammatory features as abdominal pain and fever attacks in all patients. There were arterial aneurysms in 2 (patients 3 and 4) while the biopsies of all but 1 showed necrotizing arteritis. The patient without a skin biopsy (patient 4) had aneurysms in medium-sized arteries. Patient 3 had fever, a sudden decrease in thrombocyte count, and rising ferritin; thus, a probable diagnosis of macrophage activation syndrome was made. Because patient 5 had bicytopenia during admission, a bone marrow aspiration was performed and the diagnosis was myelofibrosis.

All patients were refractory to corticosteroids. The patient with a previous diagnosis of cutaneous PAN (patient 1) responded to mycophenolate mofetil (MMF). She was stable while receiving MMF for 6 months.

Patients 2 and 3 were siblings and had previously received various immunosuppressives with partial responses. The older sibling (patient 3) was diagnosed as having PAN and had been treated with cyclophosphamide and corticosteroids with no response. Hence, a trial of etanercept (ETN) was decided. After the first ETN dose, he had bowel perforation requiring resection and ileostomy. The pathological examination of the ileal resection material revealed necrotizing vasculitis in medium- and small-sized arteries. The bowel perforation after a single dose might have been because of insufficient disease control and late administration of ETN. We are not able to comment further on a possible association with ETN treatment itself. This patient went into a long period of low-grade activity after this period. The mild clinical inflammatory signs he had from time to time were managed with short-course corticosteroids. When he was diagnosed as having DADA2, he was treated with monthly fresh frozen plasma (FFP) at a dose of 10 ml/kg (his parents refused ETN treatment at that time). He remained stable for 8 months while receiving FFP; however, subsequently, he had constitutional symptoms and his acute-phase reactants increased. Anti-tumor necrosis factor (TNF) treatment was considered again in this patient.

When the younger sibling (patient 2) was diagnosed with DADA2 (her symptoms started about the same age), she had a higher disease activity. She continued to have high disease activity after FFP; thus, she was switched to ETN. She was stable at 4-month followup.

Patient 4 did not respond to FFP. He had resistant digital ulcers and focal and segmental glomerulosclerosis (FSGS; collapsing variant) while he was receiving immunosuppressives and iloprost. His disease was still active, but the final Birmingham vasculitis activity score decreased from 35 to 25 with ETN and plasmapheresis.

Patient 5 had myelofibrosis and secondary amyloidosis (renal and intestinal). He was resistant to the immunosuppressives and FFP, and died because of necrotizing pneumonia 2 months after DADA2 diagnosis. He

Table 1. The characteristics of patients with DADA2.

Characteristics	Patient 1	Patient 2	Patient 3, Sibling of Patient 2	Patient 4	Patient 5	Patient 6
Sex	Female	Female	Male	Male	Male	Female
Fever	Yes	Yes	Yes	Yes	Yes	Yes
Myalgia/arthralgia	Yes	Yes	Yes	Yes	Yes	Yes
CNS	No	Stroke	Stroke	Stroke	No	No
Skin	LR	LR, EN	LR, EN	LR, EN, necrotic ulcers	LR, EN, necrotic ulcers	LR
Eye	No	Strabismus	No	Optic neuritis	Strabismus	No
Hematological	No	No	MAS	No	Myelofibrosis	No
Immunological	Ig normal	Low IgM	Low IgM	Ig normal	Ig normal	Ig normal
Renal	No	No	No	FSGS, collapsing variant	AA amyloidosis	No
Gastrointestinal	Abdominal pain	Abdominal pain	Abdominal pain, bowel perforation	Abdominal pain, hypertransaminasemia	Abdominal pain, HSM, pancreatitis, amyloidosis	Abdominal pain
Autoantibodies				ANA 1/320	ANA 1/320, RF, LA-positive	

DADA2: deficiency of adenosine deaminase 2; CNS: central nervous system; LR: livedo racemosa; EN: erythema nodosum; MAS: macrophage activation syndrome; Ig: immunoglobulin; FSGS: focal and segmental glomerulosclerosis; HSM: hepatosplenomegaly; ANA: antinuclear antibody; RF: rheumatoid factor; LA: lupus anticoagulant; AA amyloidosis: secondary amyloidosis.

Table 2. Disease onset, diagnosis, and treatment of patients with DADA2.

Characteristics	Patient 1	Patient 2	Patient 3, Sibling of Patient 2	Patient 4	Patient 5	Patient 6
Age at onset, yrs	6.5	8	3.5	4	9	10
Age at diagnosis, yrs	13.5	10.5	15	22	22	21
Classification criteria for PAN*	Histopathology +2/5, cutaneous	Histopathology +3/5, systemic	Histopathology +angiography, +2/5, systemic	5/10, systemic	4/10, systemic	4/10, systemic
Previous treatment	NSAID, COL, MTX, CS	COL, MTX, CS, CYC, AZA, MMF, FFP	COL, CS, CYC, ETN	COL, CS, AZA, CYC, iloprost, FFP	CS, CYC, TCZ, IVIG, FFP	COL, CS, MTX, AZA
Current treatment	MMF	ETN	FFP	ETN, plasmapheresis	—	COL
Outcome	PVAS = 0	PVAS = 0	PVAS = 3	BVAS = 25	Died	BVAS = 0

* ACR 1990 criteria[5] for adults and Ankara 2008 criteria[6] for children. DADA2: deficiency of adenosine deaminase 2; PAN: polyarteritis nodosa; NSAID: nonsteroidal antiinflammatory drug; COL: colchicine; MTX: methotrexate; CS: corticosteroid; CYC: cyclophosphamide; AZA: azathioprine; MMF: mycophenolate mofetil; FFP: fresh frozen plasma; ETN: etanercept; TCZ: tocilizumab; IVIG: intravenous immunoglobulin; PVAS: pediatric vasculitis activity score; BVAS: Birmingham Vasculitis Activity Score; ACR: American College of Rheumatology.

did not have any known immunological defect besides DADA2. Cavities were observed in his thorax computerized tomography, and tuberculosis was ruled out; thus, he was diagnosed as having necrotizing pneumonia. An autopsy could not be performed.

Patient 6 had received corticosteroids, azathioprine, and methotrexate previously. She was stable in the last 4.5 years while being treated with colchicine only, which was started because she was a carrier for an *MEFV* mutation.

Currently, 3 of our living patients were stable (1 treated with colchicine, 1 with ETN, and 1 with MMF). Patient 3 had a mild activation while receiving FFP and patient 4 partially responded to ETN and plasmapheresis; however, his disease was still active.

There are a number of original features in this series. To our knowledge, this is the first report of DADA2 patients developing progressive and debilitating renal findings (FSGS, collapsing variant, and renal amyloidosis) and necrotizing pneumonia. In addition, response to FFP or MMF was not previously reported in DADA2.

There is no consensus on DADA2 treatment. Anti-TNF has proven useful in many patients including 2 in this series. In severe disease, hematopoietic stem cell transplantation and anti-interleukin 6 may be considered[7,8,9,10,11,12]. It was also speculated that FFP support may help with the improvement of disease because DADA2 presents in the plasma; however, there is no previous report of its use. In 1 of our patients, FFP improved the disease activity for 8 months. On the other hand, his sibling had no significant response and the treatment was changed to ETN. Thus, FFP may be beneficial in selected cases for a certain period of time; however, probably not at the time of severe clinical features. Further, another drawback of FFP is the fact that it is a human product. In the patient with mainly cutaneous features, interestingly, MMF was very effective. It is interesting that 1 of our patients enjoyed prolonged remission; we are unable to comment on whether colchicine has had any effect on her disease, though unlikely.

All our patients were homozygous for p.G47R mutation. Glycine encoded by codon 47 of *CECR1* is conserved in all sequenced species[1]. p.G47R was described as a pathogenic mutation in DADA2 patients, especially with high frequency in Georgian Jewish patients[1]. Whether these populations can be traced back to an origin in the eastern Mediterranean, which was a site of early human settlement, awaits further studies.

A total of 45 patients with DADA2 (including ours) have been described[1,2,3,8,9,10,11,12]. Fourteen of 19 patients with mutations other than p.G47R had CNS involvement, especially strokes, while only 5 of 23 p.G47R homozygous patients had strokes. On the other hand, in the Georgian Jews with the p.G47R mutations reported by Elkan, *et al*[1], PAN-like features dominated the clinical features similar to our patients. We suggest that a genotype-phenotype correlation can be considered with p.G47R mutations causing a predominantly PAN-like phenotype whereas the other mutations causing predominantly a skin vasculopathy with CNS disease (especially stroke). However, the identification of patients differed between studies; thus, an ascertainment bias may need to be considered. In addition, there is a limited number of patients representing mutations other than p.G47R, which makes it difficult to draw a firm conclusion. Further studies will enlighten the spectrum of phenotype and whether the phenotypic heterogeneity may be because of other genetic, epigenetic, or environmental factors.

It is interesting that the skin features and other clinical findings as well as the histopathological findings in DADA2 patients may mimic PAN. We suggest that this monogenic disease should be classified as a "vasculitis with probable cause" in reference to CHCC 2012[4], similar to hepatitis B-associated vasculopathy. DADA2 is also an autoinflammatory disease. However, the accompanying defects in B cells and the response to treatments such as MMF highlight the blurred border between innate and adaptive immune system in this autoinflammatory disease. Such diseases emphasize the need for revising our definition and taxonomy for these diseases. We suggest that *CECR1* mutations should be checked in patients with inflammation and livedoid vasculitis if they have neurological features, especially in the form of stroke; a PAN-like phenotype but a history suggestive for an inherited disease (affected siblings, consanguineous marriage); or resistance to conventional treatment. ETN may be preferred for treatment of DADA2; however, longer followup and prospective studies are needed to determine the optimum treatment.

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APPENDIX 1. Primer pairs used in PCR and sanger sequencing.

Primer Name	Primer Sequences, 5′–3′
CECR1 exon-1	F: CCA CAT GCA CAT AAA CCG AA
	R: GGT GCA GGT GGA TCA AGC CCA
CECR1 exon-2	F: AGA TCA GAG TTC CAC CCT TA
	R: CAG CCA CAA GCA CAG TAA TA
CECR1 exon-3	F: CCC TTT GTC TTC ACC ATT CTT
	R: TCT ATA GGT TTG TAC CAA GGG A
CECR1 exon-4	F: GGG ATA TGC AAG GTG GGT A
	R: CCT GAG TGG TCA ATT CAT GA
CECR1 exon-5	F: TCT CTC ACT GCT CAC CTG A
	R: CAG CCT AGT AGC TCT GCC T
CECR1 exon-6	F: CTC TCA GGG ATC ACA ATG G
	R: GGA TGT CAG GGT ACC AAC A
CECR1 exon-7	F: CTG CTC CTG GTC ATT CTT AA
	R: AGT GAG ATA GAG CAC AGG AA
CECR1 exon-8	F: AAT AAA GCC ATA TCA TAC CTC TC
	R: CCT CCT GAA TAA CTT TAC TAA CA
CECR1 exon-9–10	F: GCT CAA GGT CTC ACC TCA C
	R: CCA CAT GGA GCT GAT TCA AG

F: forward; R: reverse.