Extending the Clinical Phenotype of Adenosine Deaminase 2 Deficiency

Tal Ben-Ami, MD[1,*], Shoshana Revel-Vilk, MD, MSc[1*], Rebecca Brooks, MD[2], Avraham Shaag, PhD[3], Michael S. Hershfield, MD[4], Susan J. Kelly, PhD[5], Nancy J. Ganson, PhD[5], Shlomit Kfir-Erenfeld, PhD[6], Michael Weintraub, MD[1], Orly Elpeleg, MD[3], Yackov Berkun, MD[2], and Polina Stepensky, MD[1]

Adenosine deaminase 2 deficiency is an autoinflammatory disease, characterized by various forms of vasculitis. We describe 5 patients with adenosine deaminase 2 deficiency with various hematologic manifestations, including pure red cell aplasia, with no evidence for vasculitis. *(J Pediatr 2016;177:316-20).*

Autoinflammatory diseases is a relatively new term that describes a group of disorders characterized by recurrent or persistent inflammation, seemingly unprovoked, that appear in the absence of infectious or other apparent cause.[1-3] Monogenic defects of genes involved in innate immunity cause the majority of autoinflammatory diseases.[1-3]

Whole exome sequencing (WES) has become an important tool in deciphering genetic disorders and in improving our understanding of genotype-phenotype associations.[4] In 2014, 2 groups using WES discovered recessive loss-of-function mutations of the cat eye syndrome chromosome region candidate 1 (CECR1) gene, encoding adenosine deaminase (ADA) 2, associated with vasculopathy with a highly variable clinical expression.

Navon Elkan et al[5] described mutations in this gene in familial polyarteritis nodosa. Further cases of systemic vasculopathy, early onset lacunar strokes, hepatosplenomegaly, and livedo reticularis were reported by Zhou et al.[6] Since then, variable clinical manifestations of this disorder have been reported, all with vasculitic components.[7-13]

Herein we describe 5 patients with variable clinical presentations, mostly hematologic, who were diagnosed with ADA2 deficiency, but none with vasculitic manifestations.

Methods

The ethics committees of the Hadassah Medical Center and the Israeli Ministry of Health approved the study. Blood samples for DNA studies were obtained after parental consent. Genomic DNA was extracted from whole blood samples of affected children. The DNA samples of the patients were enriched with Agilent V.4 51 Mb Exome capture kit (Agilent, Santa Clara, California). Sequencing was carried out on HiSeq2500 (Illumina, San Diego, California) as 100-bp paired-end runs. Read alignment and variant calling were performed with DNAnexus software (Palo Alto, California) using the default parameters with the human genome assembly Hg19 as reference.

ADA	Adenosine deaminase
WES	Whole exome sequencing
CECR1	Cat eye syndrome chromosome region candidate 1
PRCA	Pure red cell aplasia
DBA	Diamond–Blackfan anemia

WES was performed on all patients. In cases where >1 child from the same family was affected, WES was performed on 1 of the affected children and the genetic findings were confirmed by site specific mutation analysis (Sanger sequencing) in the affected siblings and other family members (segregation analysis).

ADA2 Levels

Measurement of ADA2 enzymatic activity was performed on extracts of dried plasma spots on filter paper. Plasma separated by centrifugation from fresh EDTA whole blood samples was used to fill circles on filter paper cards (ie, similar to Guthrie cards, but using plasma instead of whole blood). After drying, the filter cards were mailed to Duke University. From 1-4 circles containing dried plasma were excised from the filter and extracted for 30 minutes at ambient temperature with 100 μL per spot of a solution consisting of 25 mmol/L Tris, 15 mmol/L KCl, 1 mmol/L EDTA, 1 mmol/L DTT, pH 7.4. After recovery of the extract by compression of the filter pulp, ADA2 activity in the extract (ie, the EHNA-resistant rate of deamination of 10 mmol/L adenosine) was then determined by the high-performance liquid chromatography method essentially as reported previously,[4] using 100 mmol/L Tris acetate, pH 7.0, instead of a phosphate assay buffer. ADA2 activity was normalized to the concentration of total protein in the extract, determined by the Lowry method, using bovine serum albumin as a standard.

Results

The cohort consisted of 5 Caucasian children from 4 unrelated Arab families, all born to consanguineous parents. The

From the [1]Department of Pediatric Hematology Oncology and Bone Marrow Transplantation, Hadassah Hebrew University Medical Center, Jerusalem, Israel; [2]Department of Pediatrics, Hadassah-Hebrew University Medical Center, Mount Scopus, Jerusalem, Israel; [3]Monique and Jacques Roboh Department of Genetic Research, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; [4]Department of Medicine and Biochemistry, Duke University School of Medicine, Durham, NC; [5]Department of Medicine, Duke University School of Medicine, Durham, NC; and [6]The Lautenberg Center for Immunology and Cancer Research, IMRIC, The Hebrew University-Hadassah Medical School, Jerusalem, Israel

*Contributed equally.

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median age at presentation was 2 years of age (range 1 month-8 years of age). These patients were referred to the Department of Pediatric Hematology Oncology and Bone Marrow Transplantation, Hadassah University Hospital, Jerusalem, Israel, for evaluation and treatment of various hematologic manifestations. All patients underwent thorough clinical and laboratory investigations in local hospitals, excluding genetic evaluation, before their referral. The median time from onset of symptoms to referral was 22 months (range, 3 months-6 years). Mean follow-up for all 4 living patients is 10 months (range, 7-15). WES was performed as part of the evaluation for a possible genetic cause in consanguineous families.

Patient characteristics are summarized in the **Table**. Two children (P1, P2) were referred for evaluation of severe macrocytic anemia and reticulocytopenia from early infancy, requiring regular blood transfusions. Bone marrow studies in both patients revealed paucity of erythroid precursors with maturation arrest, consistent with pure red cell aplasia (PRCA). Neither had congenital malformations as commonly seen in patients with Diamond–Blackfan anemia (DBA), nor signs of autoinflammatory phenomena. Neither showed improvement after treatment with corticosteroids. Immunologic evaluation, including lymphocyte phenotyping and function, and immunoglobulin levels was normal except for low IgA levels in one patient. Patient 3 (P3), an 8-year-old girl, presented with acute, severe Coombs positive hemolytic anemia (hemoglobin, 2.3 gr%) and splenomegaly. Her past medical history was unremarkable, and she had not been evaluated previously for other disorders. Extensive evaluation, including bone marrow studies, was normal. Owing to failure of corticosteroid therapy, she was treated with monoclonal anti-CD20 antibody (rituximab) with good response, but remained steroid dependent. Her younger brother (P4) presented with splenomegaly and a 2-year history of recurrent episodes of fever, pharyngitis, aphthous stomatitis, and oligoarthritis. An evaluation performed between episodes revealed mild normocytic anemia with no autoimmunity or inflammatory markers. Although the clinical presentation of these siblings was different, because they had consanguineous parents, a genetic disease was suspected and WES was performed.

Patient 5 was referred for evaluation at 8 years of age with severe pancytopenia, hepatosplenomegaly, and generalized lymphadenopathy for the previous 6 years. Her history included recurrent febrile events and generalized lymphadenopathy. Laboratory evaluation including lymphocyte phenotyping and function, immunoglobulin, and specific antibodies was normal. Bone marrow aspiration revealed mild dyserythropoiesis. Six months later, she was readmitted with a fatal pulmonary aspergillus infection. No previous immunosuppressive medications were given.

Exome analysis of patients P1, P2, P3, and P5 yielded, 45.5, 38.6, and 32.7 million reads, with a mean coverage of X110, X84, X62 and X53, respectively. After alignment and variant calling, we performed a series of filtering steps. These included removing variants called less than X8, were off-target, synonymous, or had a minor allele frequency of >1% at ExAC (Exome Aggregation Consortium, Cambridge, Massachusetts; http://exac.broadinstitute.org) or a minor allele frequency of >4% at the Hadassah in-house database (~800 ethnic matched exome analyses). There were 27, 26, 20, and 24 homozygous variants that survived this filtering, respectively. However, the only deleterious mutations in the exome analysis of patients 3 and 5 were at the CECR1 gene, Chr22: 17690424 InsG, c.143dupG and Chr22: 17672673 delCInsTATGG, NM_001282225 c.781delCInsTATGG, respectively. Given the rather similar phenotype in patients 1 and 2, we focused on the homozygous, rare CECR1 mutations in their exomes as well. These patients had homozygosity for Chr22: 17687970T>C, pF178S, and 17662799 C>A, p.L451F, respectively. Both mutations were not present in ExAC, segregated with the disease in the families and affected a highly conserved residue in the ADA2 protein. All 5 patients were found to have low levels of serum ADA2 activity (**Table**).

Discussion

Adenosine is a purine nucleoside that serves as a regulator molecule activing or silencing various intracellular pathways depending on its receptors.[14,15] ADA is the main regulator of adenosine concentration in the cell. There are 2 types of ADA. ADA1 is responsible for the intracellular degradation of adenosine, protecting the cell from apoptosis. Defects in ADA1 activity causes the accumulation of intracellular deoxyadenosine nucleotide, and results in severe combined immunodeficiency.[16] ADA2 plays an important role in the extracellular environment and has a lower affinity to adenosine compared with ADA1.[3,13,14] It is interesting to note that increased erythrocyte ADA levels are found in approximately 85% of patients with DBA, serving as an important diagnostic tool in the evaluation of suspected cases.[17] Although this association is well-known, the pathophysiology of this finding is not clear.[18] There is growing evidence that ADA2 is secreted by premonocytic cells, and serves as a growth factor for the monocyte lineage, including macrophages and dendritic cells.[3,13,14] Zebrafish models support a role for ADA2 in endothelial cells,[3,6] but there is still no proof for a similar role in human endothelium. These observations support the role of ADA2 in immune processes, but do not provide a pathophysiologic explanation for the diverse clinical manifestation of CECR1 mutations.

Since the first description of ADA2 deficiency by Zhou et al[6] and Navon Elkan et al,[5] >40 cases have been reported. The clinical presentations are variable and range from a vasculitic disease limited to the skin to a systemic and sometimes fatal form of vasculopathy.[5-12,19,20] There is increasing evidence that, even among patients with identical mutations, there are diverse clinical presentations.[21]

Our report describes 5 patients with ADA2 deficiency who presented with diverse hematologic manifestations without a vasculitic component. Other symptoms seen in our patients, such as recurrent fever, hepatosplenomegaly, mouth ulcers, and arthritis, are consistent with other reported cases.[6-9] The pro-