"Immune" Thrombocytopenia as Key Feature of a Novel ADA2 Deficiency Variant : Implication on Differential Diagnostics of ITP in Children

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Abstract: Thrombocytopenia presenting during early childhood is most commonly diagnosed as immune/idiopathic thrombocytopenic purpura (ITP), where the antibody-mediated destruction of thrombocytes is often transient. If treatment is indicated, the majority of patients respond to immune-modulation by intravenous immunoglobulin G infusion or systemic corticosteroids. Differential diagnoses to childhood ITP includes thrombocytopenia due to infections, drugs, rheumatologic conditions, immune dysregulation, and inherited bone marrow failures, for example, congenital amegakaryocytic thrombocytopenia. Isolated thrombocytopenia in an otherwise healthy appearing child that recurs after therapy and/or persists suggest a differential diagnosis rather than ITP. We present a case of symptomatic thrombocytopenia in a 2-year-old girl associated with adenosine deaminase deficiency.

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"Immune" Thrombocytopenia as Key Feature of a Novel ADA2 Deficiency Variant: Implication on Differential Diagnostics of ITP in Children

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Summary: Thrombocytopenia presenting during early childhood is most commonly diagnosed as immune/idiopathic thrombocytopenic purpura (ITP), where the antibody-mediated destruction of thrombocytes is often transient. If treatment is indicated, the majority of patients respond to immune-modulation by intravenous immunoglobulin G infusion or systemic corticosteroids. Differential diagnoses to childhood ITP includes thrombocytopenia due to infections, drugs, rheumatologic conditions, immune dysregulation, and inherited bone marrow failures, for example, congenital amegakaryocytic thrombocytopenia. Isolated thrombocytopenia in an otherwise healthy appearing child that recurs after therapy and/or persists suggest a differential diagnosis rather than ITP. We present a case of symptomatic thrombocytopenia in a 2-year-old girl associated with adenosine deaminase deficiency.

Key Words: thrombocytopenia, ADA2, splenomegaly, DADA2

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CASE REPORT

A 2-year-old girl presented to the emergency room with a 3-day history of widespread petechiae (ie, face, abdomen, extremities) and cutaneous hematomas (mainly lower extremities) that developed without significant trauma. A few petechial bleedings were seen in the oral mucosa, but no other signs of bleeding could be found. Beside the cutaneous bleedings, the recent medical history was unremarkable (eg, no recent infections, no other complaints, and no recent new medications). Vital signs were stable and physical examination was unremarkable, apart from the presence of a slightly enlarged spleen. Laboratories on presentation were significant for a white blood cell (4.4×10^9/L), neutrophils (1.2×10^9/L), lymphocytes (2.5×10^9/L), thrombocytes ($<5\times10^9$/L), hemoglobin (Hb) (113 g/L), erythrocytes (4.7×10^{12}/L), and reticulocytes (97×10^9/L). A peripheral blood smear displayed lymphocytopenia and neutropenia as the only deviations.

The patient was the first child of nonconsanguineous parents of northern European decent. Family history was unremarkable with no reports of hematologic/immunologic conditions nor early-onset stroke. The medical history included an eventful pregnancy with delivery at term by vacuum extraction due to fetal distress and need for ventilation briefly. Fetal anemia (Hb, 80 g/L) was noted at the neonatal ward. Investigations could not identify any cause (ie, no signs of fetal-maternal transfusion, immunization, etc.). The baby was prescribed iron substitution that was discontinued after 4 months upon normalization of the Hb level (113 g/L). Neonatal cranial sonography identified a small parietal area with increased echogenicity where a magnetic resonance imaging follow-up could not confirm any pathology. After discharge from the neonatal ward, the girl had normal growth and psychomotor development and only a few medical consultations, mainly due to common viral infections.

An abdominal ultrasound, to investigate the splenomegaly, showed a slightly enlarged spleen with multiple small irregular hypoechogenic areas. Primary differential diagnoses included immune/idiopathic thrombocytopenic purpura (ITP) or hematologic malignancy and further workup included a bone marrow examination. A thrombocyte transfusion was given before the invasive diagnostic procedure and resulted in a transient discrete increase in platelet count (7×10^9/L). The results of the bone marrow examination were unremarkable with normal megakaryocytopoiesis. Intravenous immunoglobulin G (IVIg) was infused leading to rapidly increasing thrombocyte counts on the following day (58×10^9/L) that was sustained 2 days later (73×10^9/L). Hence, the patient was discharged with a diagnosis of suspected ITP and further outpatient follow-up was scheduled, including additional diagnostic investigations on the enlarged spleen. During the following 2 months the patients symptomatic thrombocytopenia reoccurred on multiple occasions and repeated single dose infusions of IVIg 1 g/kg were only able to elevate the thrombocyte count $>5\times10^9$/L for 7 to 10 days. The patient also had a constantly elevated erythrocyte sedimentation rate (ESR, 55 mm/h) that was not affected by the IVIg therapy. Treatment with oral corticosteroids was initiated and the peripheral thrombocyte count (208×10^9/L) and ESR were normalized after 5 months of 10 mg prednisone daily. After 6 months, a further tapering of prednisone to a dose of 5 mg per day led to a rapid decline of peripheral thrombocytes and the previous dose had to be reinstated.

In light of the intriguing clinical picture, that is, not fully consistent with ITP, extensive immunologic workup and whole genome sequencing were performed. Immunologic workup was remarkable for a decreased amount of peripheral B cells (0.28×10^9/L), a slightly increased proportion of double negative (CD4−, CD8−) T cells (3.7%) and serum interleukin-10 was also elevated at 25.1 ng/L. The remainder of the workup was unremarkable including normal levels of serum immunoglobulins (assessed >6 mo after the last dose of IVIg) and normal activation of T cells and B cells to stimulation with mitogens, as assessed by our clinically operational FASCIA analysis.[1]

Whole genome sequencing showed the presence of 2 missense mutations in *CECR1* (c.506G>A and c.932T>G), leading to 2 amino acid substitutions in adenosine deaminase (ADA) 2 (p.Arg169Gln and p.Leu311Arg). The c.506G>A variant has been previously associated with ADA2 deficiency as a compound heterozygous variant in the context of early-onset stroke and vasculopathy, as well as with a variable clinical phenotype in a case series of individuals who were all homozygous for the variant.[2,3] The c.932T>G variant is however novel, but several variant evaluation metrics predicted it to be deleterious (SIFT, Mutation Taster, and FATHMM) and the variant had a deleterious annotation of genetic variants using neural networks score of 0.9967.[4] Both of the variants were confirmed by Sanger-sequencing and the patient's parents were identified to be heterozygous carriers (Fig. 1). To evaluate the effect of the *CECR1* variants on protein function, the activity of ADA2 from the patient was tested. Samples from the patient showed severely impaired activity of ADA2 as compared with healthy controls (Fig. 2), thus confirming the predicted deleterious effect of the 2 compound heterozygous missense variants.

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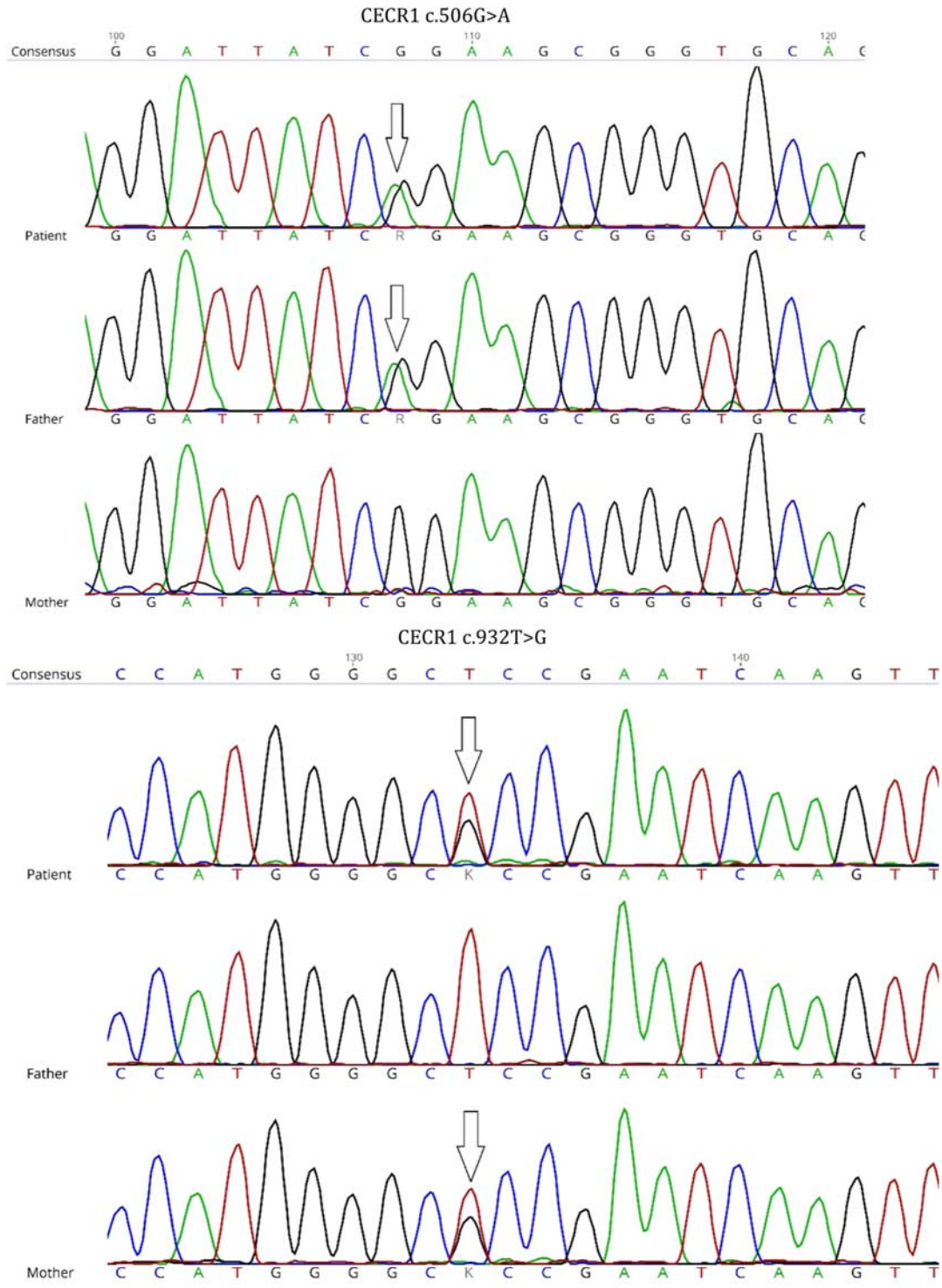

FIGURE 1. Sanger-sequencing of the patient and her parents. Arrows indicate the heterozygous variants. full color online

In light of the previously published association between *CECR1* variants, impaired ADA2 activity, and the development of early-onset stroke and vasculopathy, in combination with the patients thrombocytopenia, stem cell transplantation (SCT) was considered, as it has previously been shown to be effective.[3,5] The patients general good health, normal development, and absence of overt signs of vasculopathy, however, prompted us to initiate therapy with the tumour necrosis factor inhibitor adalimumab, as it has been associated in case reports with improved outcomes in patients with deficiency of ADA2.[3,6,7] The patient has now been treated for 6 months with adalimumab monotherapy and appears clinically well with slightly decreased thrombocyte counts (122×10^9/L) and mild lymphocytopenia (2.0×10^9/L). The ESR is

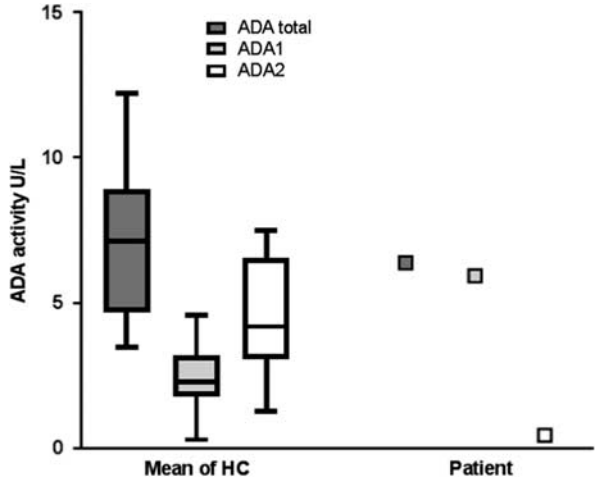

FIGURE 2. The ADA activity assay (Diazyme, Poway, CA) was slightly modified by adding erythro-9-(2-hydroxy-3-nonyl)adenine (Sigma-Aldrich, Zwijndrecht, the Netherlands) for ADA1 inhibition to allow discrimination between ADA1 and ADA2. Box plots represent mean ± SD of 21 HCs. Patient samples were measured in duplicates with identical measurements. ADA indicates adenosine deaminase; HC, healthy control.

normalized and the splenomegaly has resolved, whereas interleukin-10 remains elevated at a lower level (13.6 ng/mL). Oral corticosteroid therapy has been stopped and clinical evaluation as well as magnetic resonance imaging of the brain has shown no clinically significant evidence of progressive vasculopathy.

DISCUSSION

Although deficiency of ADA1 is associated with early developing severe combined immunodeficiency, deficiency of ADA2 (DADA2) has been linked to a highly variable clinical syndrome in previously published cases.[2,3,6,8] It is tempting to speculate that this variability is caused by differences in the described missense variants, leading to differential effects on ADA2 functional activity. A recent study by Van Montfrans et al[3] describing the variable clinical picture in a case series of children who were all homozygous for the c.506G > A variant, however, argues for a more complex regulation of the ADA2 activity.

ADA2 expression seems to be particularly high in the spleen and while the presence of increased levels of adenosine have been linked to both proinflammatory and immunoregulatory effects on immunologic cells, several aspects of our case argues for a regulatory role of functioning ADA2 in the current clinical situation.[7,9–11] First, the slight splenomegaly coupled with the normal appearing bone marrow and the rapid increase in peripheral thrombocytes after initiated therapy argues for an increased consumption of thrombocytes as the main cause of thrombocytopenia. Second, the absence of overt signs of disseminated intravascular coagulation as well as the absence of large volume blood loss argues for phagocytosis-driven consumption as the main mechanism of consumption. Third, the rapid response to therapies with immunosuppressive properties (IVIg, corticosteroids, and tumour necrosis factor-α inhibition) as well as a normalization of a previously elevated ESR also argues for a primarily regulatory function of ADA2 in the present context. Whether functional ADA2 exerts its regulatory effects through conversion of adenosine to inosine, through binding to cell surface receptors on lymphocytes or through some yet unknown mechanism, however remains to be elucidated.

In light of the current clinical literature on DADA2, decisions on treatment strategy remain challenging. The correlation of the degree of diminished ADA2 in vitro activity with the level of increased risk of early-onset stroke, coupled to the probable devastating clinical consequences of such an event makes SCT a valid treatment option.[5–7] SCT however, is naturally also associated with several short and long-term adverse effects, which makes it a challenging treatment option for patients, especially if other less hazardous treatments are available. We decided to treat the current patient with, the for DADA2 investigational drug, adalimumab, mainly based on earlier reports of clinical success coupled to the absence of clinical or radiologic signs of overt vasculopathy.[3,6,7]

In conclusion, we present a case of symptomatic thrombocytopenia presenting in early childhood that was associated with DADA2 and we suggest that DADA2 should be incorporated into the differential diagnosis of thrombocytopenic children. Our case also noticeably illustrates the importance of the publication of treatment outcomes in rare diseases, of genetically well-characterized patients; to assist in the further development of personalized sequencing-based clinical medicine.

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