Journal of Clinical Immunology (2019) 39:842–845
https://doi.org/10.1007/s10875-019-00697-2

LETTER TO EDITOR

Homozygous Splice *ADA2* Gene Mutation Causing ADA-2 Deficiency

Herberto Jose Chong-Neto[1] · Gesmar Rodrigues Silva Segundo[2,4] · Márcia Bandeira[1] · Débora Carla Chong-Silva[1] · Cristine Secco Rosário[1] · Carlos A. Riedi[1] · Michael S. Hershfield[3] · Hans Ochs[4] · Troy Torgerson[4] · Nelson Augusto Rosário[1]

Received: 26 June 2019 / Accepted: 25 September 2019 / Published online: 15 October 2019

To the Editor:

One recently discovered immune dysregulation syndromes is deficiency of adenosine deaminase 2 (DADA 2) caused by biallelic loss of function mutations in *ADA2*, previously known as *CECR1* [1–3]. The clinical phenotype of DADA2 was initially described as intermittent fevers, early-onset ischemic or hemorrhagic strokes and other neurovascular manifestations, livedo reticularis, polyarteritis nodosa hepatosplenomegaly, systemic vasculopathy, and hypogammaglobulinemia [1–3]. Subsequently, case reports suggested that DADA2 patients may present with a highly variable clinical phenotype and that many symptoms are responsive to therapy with anti-tumor necrosis factor agents, including cytopenia and bone marrow failure [4–7].

Here, we present a Brazilian girl with recurrent ischemic and hemorrhagic strokes starting at age 18 months who at 6 years of age was diagnosed with a homozygous splice mutation in the *CECR1* gene.

The patient was born to healthy non-consanguineous parents; her older sister was asymptomatic and the family history was negative for autoimmune or autoinflammatory disorders or recurrent infections.

At 9 months of age, she presented with recurrent fever episodes and at age 18 months, she was admitted to the intensive care unit (ICU) with acute onset ocular deviation, seizures, and flaccid tetraparesis, with a diagnosis of ischemic and hemorrhagic stroke by CNS imaging (Fig. 1). At that time, livedo reticularis was noticed (Fig. 2). She was hospitalized four more times with episodes of ischemic/hemorrhagic strokes, at 28 months and at 4, 5, and 6 years of age. Following the last episode, she was admitted to the ICU and referred to the Immunology Division.

At that time, she had mild hypogammaglobulinemia (IgG, 467 mg/dL (665–1465 mg/dL); IgA, 21 mg/dL (47–267 mg/dL); IgM, 14 mg/dL (49–218 mg/dL), IgE < 25 kU/L (< 25 kU/L)). Lymphocyte subsets: CD3 = 853 cells/μL (1280–2413 cells/μL); CD4+, 499.2 cells/μL (618–1348 cells/μL); CD8+, 353.8 cells/μL (390–1024cells/μL); CD56+, 80.9 cells/μL (217–515 cells/μL); and CD19, 113/μL (471–1031 cells/μL).

Based on medical history and physical findings, we suspected DADA2 and analyzed the *CECR1* gene by Sanger sequencing. A homozygous mutation located at position − 2 in the acceptor splice site of intron 6 (c.973-2A>G (genomic location Chr22: 17669339 on Assembly GRCh37) was identified, and predicted to be pathogenic (Figs. 3 and 4). To confirm the pathogenicity, we performed two additional analyses, mRNA expression and the enzyme activity of ADA2. The cDNA analysis showed lack of *CECR1* mRNA expression and ADA2 enzyme activity, performed by dried plasma spot, showing 0.0 mU/g of protein, confirming a loss of function mutation.

Based on these findings, she was started on the anti-TNF monoclonal antibody, etanercept, with excellent results, being symptom free for the last 2 years.

ADA1 converts adenosine to inosine and 2′-deoxyadenosine to 2′-deoxyinosine and its deficiency causes lymphopenia and severe combined immunodeficiency disease (SCID) due to the accumulation of toxic deoxyadenosine nucleotides in the cytoplasm [1]. While human ADA1 and ADA2 show partial structural homology, they are structurally and probably functionally distinct, since the accumulation of deoxyadenosine nucleotides is absent in erythrocytes from patients with ADA2 deficiency [2, 3].

✉ Herberto Jose Chong-Neto

[1] Hospital de Clínicas, Federal University of Paraná, Curitiba, Brazil

[2] Hospital de Clínicas, Federal University of Uberlândia, Uberlândia, Brazil

[3] Department of Rheumatology and Immunology, Duke University Medical Center, Durham, NC, USA

[4] Seattle Children's Hospital, Seattle, WA, USA

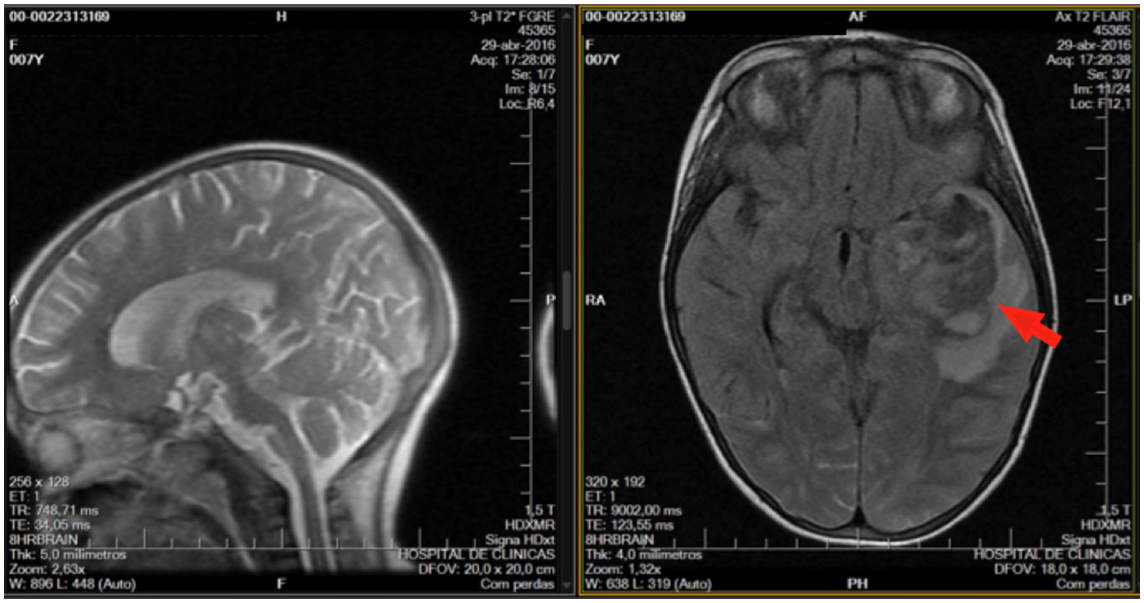

Fig. 1 CNS MR demonstrating intraparenchymal hematoma at the hyperacute/acute stage in the left temporal lobe, associated with signs of perilesional ischemia and hemoventricle signs

ADA2 is predominantly expressed in myeloid cells and plays a role in the differentiation of macrophages; however, its overall function is poorly understood. Biopsies and blood samples of DADA2 patients have increased production of proinflammatory cytokines suggesting a monocyte-macrophage polarization to the M1 subset, known to promote inflammation and tissue damage. The pathogenic vascular effect of DADA2 could be directly demonstrated in man and zebrafish, causing disruption of the cell junctions in cocultured monolayers of human microvascular endothelial cells and increased intracranial bleeding in zebrafish [3].

The clinical and laboratorial presentation in patients with DADA2 is very heterogeneous including vasculopathies, immunodeficiencies, and hematological diseases. Mucocutaneous, neurological, and immunological alterations are more frequent described in the patients with DADA2, including livedo reticularis (50%), polyarteritis nodosa (PAN) (34%), ischemic strokes (27%), splenomegaly (29%), hypogammaglobulinemia (22%), recurrent infections (20%), and low IgM (18%); but several additional manifestations have been described [(3)]. The patient's phenotype reported here presented the core symptoms previously found in DADA2 patients.

Mutations in the exon-intron junctions are accountable for approximately 10% of pathogenic mutations. Around 90% of splicing mutations happen between the last 5 nucleotides of the exon and the first 15 nucleotides of the intronic region. Variants on the donor splicing region are more frequently pathogenic than variant on the acceptor region [8]. Previously, other authors have described a DADA2 patients with the same *ADA2* mutation found in our case; however, as a part of compound heterozygous mutations and in silico analysis, the mutation described in these case reports indicated that the nucleotide exchange could impair splicing [9, 10]. cDNA analysis of our patient confirmed that the c.973-2A>G results in absence of mRNA indicating this splice site mutation results in missplicing and unstable mRNA and the absence of protein, indirectly reconfirmed with the absence of ADA2 enzyme activity.

Different immunosuppressive therapies were used to control autoimmune manifestation present in ADA2 patients. The consensus is present to use anti-TNF agents that achieve control of fever episodes and vasculopathy, and prevention of strokes as seen in our patient [1–8]. However, other investigators have reported occasional failures in the control of cytopenia and immunodeficiency in DADA2 patients treated with anti-TNF agents [6, 9]. Patients presenting with recurrent or life-threatening

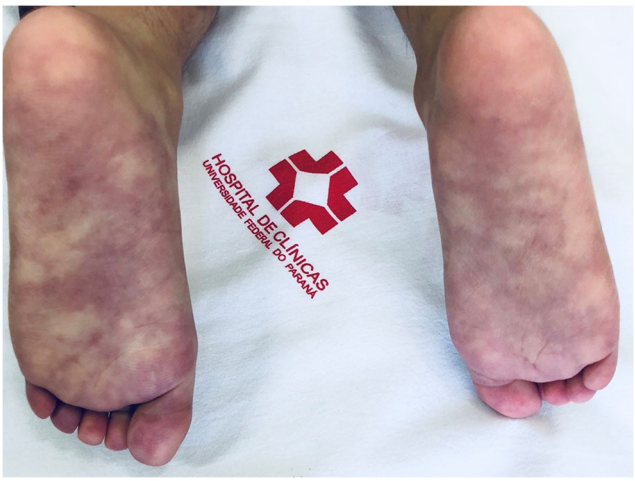

Fig. 2 Livedo reticularis on plantar feet

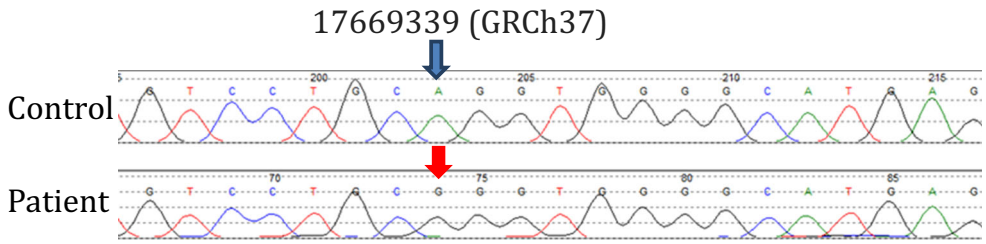

Fig. 3 ADA2 gDNA Sanger chromatogram showing a homozygous mutation located at genomic position chr22:17669339 leading to a splicing mutation at position c.973-2A>G

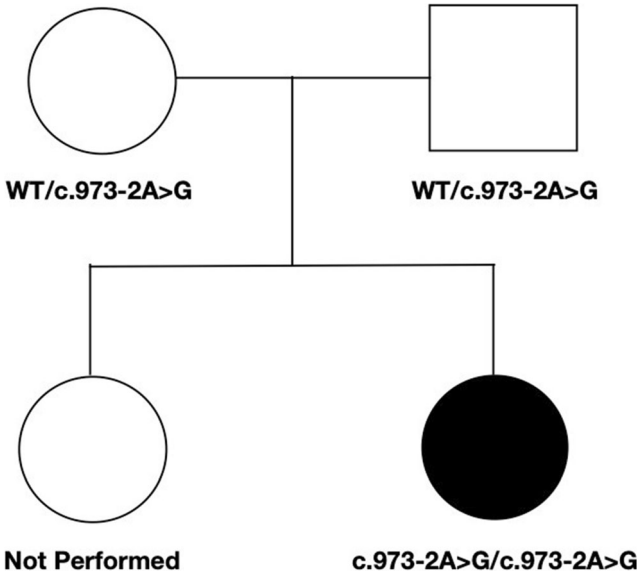

Fig. 4 A family pedigree with the segregation of the ADA2 mutation

infections associated with hypogammaglobulinemia should receive long-term immunoglobulin replacement, antibiotic, and in some cases, antiviral treatment [9, 10]. Hematopoietic stem cell transplantation (HSCT) has been shown to be successful with excellent survival and resolution of symptoms. The decision to proceed to HSCT depends on response to treatment with TNF-inhibitors, the availability of a donor, and the patient general health condition [6, 11, 12].

In conclusion, we report here a patient with ADA2 deficiency carrying a homozygous splicing mutation in the *CECR1* gene, previously described in compound heterozygous patients and classified as likely pathogenic, allowing the mRNA and protein studies to confirm the mutation pathogenicity. The awareness of clinical spectrum of disease is very important for physicians to think, diagnose, and adequately treat the DADA2 patients.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Zhou Q, Yang D, Ombrello AK, Zavialov AV, Toro C, Zavialov AV, et al. Early-onset stroke and vasculopathy associated with mutations in ADA2. N Engl J Med. 2014;370:911–20. https://doi.org/10.1056/NEJMoa1307361.
2. Navon Elkan P, Pierce SB, Segel R, Walsh T, Barash J, Padeh S, et al. Mutant adenosine deaminase 2 in a polyarteritis nodosa vasculopathy. N Engl J Med. 2014;370:921–31. https://doi.org/10.1056/NEJMoa1307362.
3. Meyts I, Aksentijevich I. Deficiency of adenosine deaminase 2 (DADA2): updates on the phenotype, genetics, pathogenesis, and treatment. J Clin Immunol. 2018;38(5):569–78. https://doi.org/10.1007/s10875-018-0525-8.
4. Batu ED, Karadag O, Taskiran EZ, Kalyoncu U, Aksentijevich I, Alikasifoglu M, et al. A case series of adenosine deaminase 2-deficient patients emphasizing treatment and genotype-phenotype correlations. J Rheumatol. 2015;42(8):1532–4. https://doi.org/10.3899/jrheum.150024.
5. Michniacki TF, Hannibal M, Ross CW, Frame DG, DuVall AS, Khoriaty R, et al. Hematologic manifestations of deficiency of adenosine deaminase 2 (DADA2) and response to tumor necrosis factor inhibition in DADA2-associated bone marrow failure. J Clin Immunol. 2018;38:166–73. https://doi.org/10.1007/s10875-018-0480-4.
6. Cipe FE, Aydogmus C, Serwas NK, Keskindemirci G, Boztuğ K. Novel mutation in CECR1 leads to deficiency of ADA2 with associated neutropenia. J Clin Immunol. 2018;38(3):273–7. https://doi.org/10.1007/s10875-018-0487-x.
7. Fayand A, Sarrabay G, Belot A, Hentgen V, Kone-Paut I, Grateau G, et al. Multiple facets of ADA2 deficiency: vasculitis, autoinflammatory disease and immunodeficiency: a literature review of 135 cases from literature. Rev Med Interne. 2018;39(4):297–306. https://doi.org/10.1016/j.revmed.2017.11.006.
8. Krawczak M, Thomas NS, Hundrieser B, Mort M, Wittig M, Hampe J, et al. Single base-pair substitutions in exon-intron junctions of human genes: nature, distribution, and consequences for mRNA splicing. Hum Mutat. 2007;28:150–8. https://doi.org/10.1002/humu.20400.
9. Schepp J, Proietti M, Frede N, Buchta M, Hubscher K, Rojas Restrepo J, et al. Screening of 181 patients with antibody deficiency for deficiency of adenosine deaminase 2 sheds new light on the disease in adulthood. Arthritis Rheum. 2017;69(8):1689–700. https://doi.org/10.1002/art.40147.
10. Arts K, Bergerson JRE, Ombrello AK, Similuk M, Oler AJ, Agharahimi A, et al. Warts and DADA2: a mere coincidence? J Clin Immunol. 2018;38:836–43. https://doi.org/10.1007/s10875-018-0565-0.
11. Hashem H, Kumar AR, Muller I, Babor F, Bredius R, Dalal J, et al. Hematopoietic stem cell transplantation rescues the hematological, immunological, and vascular phenotype in DADA2. Blood.

2017;130(24):2682–8. https://doi.org/10.1182/blood-2017-07-798660.

12. Van Eyck L Jr, Hershfield MS, Pombal D, Kelly SJ, Ganson NJ, Moens L, et al. Hematopoietic stem cell transplantation rescues the immunologic phenotype and prevents vasculopathy in patients with adenosine deaminase 2 deficiency. J Allergy Clin Immunol. 2015;135(1):283–7 e5. https://doi.org/10.1016/j.jaci.2014.10.010.