Deficiency of adenosine deaminase 2 (DADA2) is a rare autoinflammatory disease that was firstly described in patients with early-onset strokes, livedo reticularis, and periodic fever resembling polyarteritis nodosa. In reported case series, researchers described highly variable manifestations, including autoimmunity, immunodeficiency, hepatosplenomegaly, pancytopenia, ichthyosiform rash, and arthritis, in patients with DADA2. A thirteen-year-old female patient who was born to consanguineous parents was admitted to our hospital with generalized edema and leg pain. A physical examination revealed splenomegaly and left knee arthritis. Nephrotic-range proteinuria and hypoalbuminemia were present, and a renal biopsy revealed amyloidosis. Despite the absence of periodic fever and livedo reticularis, our patient had suggestive features of DADA2, including low serum immunoglobulin G and immunoglobulin M levels, hepatosplenomegaly, and renal amyloidosis. We found a heterozygote Met694Val mutation in the Mediterranean fever gene and a novel homozygote Thr317Argfs*25 (c.950-950delCys) mutation in the cat eye chromosome region 1 gene. A functional analysis revealed absent plasma adenosine deaminase 2 activity. Canakinumab was administered because of unresponsive proteinuria despite 2 months of treatment with colchicine and methylprednisolone. Proteinuria improved after 7 doses of canakinumab. In conclusion, DADA2 should be considered in the differential diagnosis of renal amyloidosis, particularly in the absence of homozygote Mediterranean fever mutations. Although anti–tumor necrosis factor agents are widely offered in DADA2 treatment, we speculate that canakinumab may be an appropriate treatment of renal amyloidosis in DADA2.

abstract

Deficiency of adenosine deaminase 2 (DADA2) is a rare autoinflammatory disease that was firstly described in patients with early-onset strokes, livedo reticularis, and periodic fever, which are compatible with polyarteritis nodosa. The cat eye chromosome region 1 (CECR1) (NM_001282225.1) gene encodes adenosine deaminase 2 (ADA2), a 511–amino acid protein that is essential for leukocyte development. CECR1 gene mutations cause reduced or absent ADA2 activity, possibly leading to monocyte differentiation toward M1-type proinflammatory macrophages and endothelial dysfunction. Patients with decreased levels of serum immunoglobulin G (IgG) and immunoglobulin M (IgM), low switched memory B cells, and lymphopenia highlighted the presence of mild immunodeficiency in DADA2. Furthermore, in the reports in which the patients with DADA2 who had only hematologic or dermatological findings were described, researchers described highly variable manifestations, including autoimmunity, immunodeficiency, hepatosplenomegaly, pancytopenia, ichthyosiform rash, and arthritis. In conclusion, DADA2 should be considered in the differential diagnosis of renal amyloidosis, particularly in the absence of homozygote Mediterranean fever mutations. Although anti–tumor necrosis factor agents are widely offered in DADA2 treatment, we speculate that canakinumab may be an appropriate treatment of renal amyloidosis in DADA2.

Reference:
1) The cat eye chromosome region 1 (CECR1) (NM_001282225.1) gene encodes adenosine deaminase 2 (ADA2), a 511–amino acid protein that is essential for leukocyte development. CECR1 gene mutations cause reduced or absent ADA2 activity, possibly leading to monocyte differentiation toward M1-type proinflammatory macrophages and endothelial dysfunction. Patients with decreased levels of serum immunoglobulin G (IgG) and immunoglobulin M (IgM), low switched memory B cells, and lymphopenia highlighted the presence of mild immunodeficiency in DADA2.

were evaluated, attention was drawn to the clinical heterogeneity.6,7 Here, we present the first patient with DADA2 in the literature in whom renal amyloidosis was successfully treated with canakinumab, a human anti–interleukin 1β (IL-1β) monoclonal antibody.

**CASE REPORT**

A 13-year-old female patient, previously healthy, who was born to consanguineous parents was admitted to our hospital with generalized edema, left knee swelling, and pain. She was diagnosed with nephrotic syndrome and had been treated with prednisolone and cyclosporine for the last 3 months in another medical center. A physical examination revealed cushingoid appearance, hepatosplenomegaly, and left knee arthritis. Skin appearance was normal, revealing no livedoid changes or subcutaneous hyperemic nodules. A neurologic examination was also normal. In laboratory findings, complete blood count, serum complement levels, and liver and renal function tests were in normal range. Massive proteinuria (9.1 mg of protein per milligram of creatinine in random urine; normal: <0.2 mg of protein per milligram of creatinine), hypoalbuminemia (albumin levels of 2.2 g/dL; normal: 3.5–5.5 g/dL), and slightly elevated C-reactive protein levels (3.7 mg/dL; normal: <0.5 mg/dL) were found. Despite the absence of recurrent fever or infections, the patient had decreased serum IgG levels (496 mg/dL; normal: 698–1194 mg/dL). Serum IgM and immunoglobulin A levels were normal. Autoantibody test results, including tests for antinuclear antibody and anti-DNA, were negative. A renal Doppler ultrasound did not reveal any signs of vasculitis, such as stenosis, occlusion, or aneurysms. A renal biopsy revealed secondary AA-type amyloidosis; therefore, colchicine (1.5 mg/day) was added to an oral methylprednisolone treatment of 16 mg/day, and administration of cyclosporine was ceased.

Molecular studies revealed a heterozygote Met694Val mutation in the Mediterranean fever (MEFV) gene and a novel homozygote Thr317Argfs*25 (c.950-950delCys) mutation in the CECR1 gene (Fig 1). Thus, we administered canakinumab (150 mg monthly) because of unresponsive proteinuria despite 2 months of previous treatment. Moreover, the tumor necrosis factor receptor superfamily member 1A (TNFRSF1A) gene was sequenced to determine if it was related to amyloidosis, but sequencing revealed no mutations.

To identify and clarify the effect of the novel mutation found by a targeted next-generation sequencing (NGS) gene panel in the CECR1 gene, parental tests were performed and revealed the heterozygosity of both parents. More than that, as a functional study, dried plasma samples of the patient were studied for ADA2 activity, and analysis revealed the complete absence of ADA2 activity (Fig 2).

To sum up, the diagnosis was proven to be DADA2 with renal amyloidosis in our patient. Her arthritis and hepatosplenomegaly findings were improved, and she had a decreased protein/creatinine level ratio (0.3 mg of protein per milligram of creatinine) after 7 doses of canakinumab. Also, immunodeficiency was indicated by low serum IgG (616 mg/dL) and IgM levels (26 mg/dL) at the end of a 1-year follow-up without any related symptoms.

**DISCUSSION**

DADA2 was first described in 2 studies, which revealed that CECR1 gene mutations that have the loss-of-function effect may lead to vasculopathy resembling polyarteritis nodosa. The disease is characterized by recurrent fever and an elevation of acute-phase reactants usually associated with livedo reticularis and neurologic manifestations.1-2 In case series, researchers described highly variable manifestations, including autoimmunity, immunodeficiency, hepatosplenomegaly, pancytopenia, ichthyosiform rash, and arthritis, in DADA2.7-9 Despite the absence of recurrent fever and livedo reticularis, our patient had hypogammaglobulinemia, hepatosplenomegaly, and renal amyloidosis possibly relevant to DADA2, with a novel mutation in CECR1 and functional confirmation. The most common reported mutation among Turkish and Georgian populations was Gly47Arg (c.139Gly>Ala), which was previously suggested to lead to a vasculopathy phenotype. Systemic steroids and immunosuppressive drugs, including azathioprine, methotrexate, calcineurin inhibitors, and cyclophosphamide, were reported to have a partial benefit in the treatment of DADA2. With introducing the use of biological drugs, particularly anti–tumor necrosis factor (anti-TNF) agents, more favorable responses to etanercept have been reported in the literature.10 Because little is still known on the pathogenesis of DADA2, Zhou et al showed increased staining for IL-1β, tumor necrosis factor–α, and inducible nitric oxide, indicating the presence of inflammation in the skin biopsy of the experimental models of DADA2.11 Most recently, it was also reported that serum IL-1β and tumor necrosis factor–α levels were higher in the active phase of a patient with homozygote deletion in both interleukin 17 receptor A and CECR1 regions of chromosome 22.12 Because anti-TNF agents are the most preferred treatment modality against vasculitis in DADA2, anti–interleukin 1 treatment and its outcomes are much less well known than anti-TNF.
agents. Up to date, Anakinra, an anti–interleukin 1 receptor antagonist treatment, was reported in 8 patients with DADA2, and only 1 of them had a good response.\textsuperscript{10,13} Canakinumab was introduced only in 1 patient with vasculitis, and the disease relapsed with neurologic symptoms even after the initial response.\textsuperscript{11} On the other hand, the only patient in the literature with DADA2 and amyloidosis was treated with corticosteroids, cyclophosphamide, intravenous immunoglobulin, fresh-frozen plasma, and tocilizumab.\textsuperscript{14} Thus, we report the first case of DADA2 in which renal amyloidosis was treated with canakinumab and resulted in a successful outcome.

Despite improvements in genetics, the diagnosis of familial Mediterranean fever (FMF) is still made clinically. Previously, FMF was known as a recessive inherited disease; however, carrying a single heterozygous MEFV mutation has also been linked with typical FMF manifestations.\textsuperscript{15} Even when the intronic mutations were revealed by the most advances in molecular medicine, polymorphisms by NGS techniques, and deletion and/or duplication analysis by multiplex ligation-dependent probe amplification, a proportion of patients with FMF remained heterozygous. Patients with heterozygote MEFV mutations usually present with mild disease.\textsuperscript{15,16} Heterozygote MEFV mutations lead to a 6.3 to 8.1 times elevated risk of FMF symptoms compared with nonmutations. Therefore, it was suggested that heterozygosity may

\begin{figure}
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\caption{Electropherogram of a targeted NGS gene panel in the \textit{CECR1} gene revealing a novel homozygote Thr317Argfs\textsuperscript{*}25 (c.950-950delCys) mutation in the patient with DADA2.}
\end{figure}
be a susceptibility factor for FMF. However, it is still unknown whether any other genetic, epigenetic, or environmental factors are present in patients with FMF with heterozygote MEFV mutations.\(^{15-17}\)

Our patient did not fulfill the Yalcinkaya et al\(^{18}\) and Tel Hashomer\(^{19}\) diagnostic criteria for FMF. Additionally, another autoinflammatory disease related to amyloidosis, tumor necrosis factor receptor–associated periodic syndrome, was excluded because of the absence of a mutation in the tumor necrosis factor receptor superfamily member 1A gene. The presence of hypogammaglobulinemia, a homozygote mutation in the CECR1 gene, and absent plasma ADA2 activity confirmed a DADA2 diagnosis. However, it is still not clear whether renal amyloidosis is due to DADA2 or FMF in our patient.

Amyloidosis is the most devastating complication of FMF. The appropriate colchicine treatment was suggested to decrease the rate of amyloidosis to 8.6% in a large cohort from Turkey.\(^{20}\) In the pediatric population, the rate of amyloidosis was as low as 0.3% in patients with FMF.\(^{21}\) A recent study revealed that male sex, Met69Val homozygosity, a family history of amyloidosis, and the presence of arthritis were related to a higher risk of amyloidosis in patients with FMF.\(^{20}\) Another study from Armenia revealed similar results in adults with typical FMF symptoms, and only 9% of patients with amyloidosis were found to be homozygous for the Met69Val mutation in MEFV.\(^{22}\)

Renal amyloidosis can also be an initial presentation in \(~7%\) to 25% of patients with FMF, without typical symptoms. This condition is named phenotype II, which is extremely rare in childhood.\(^{23,24}\)

On the basis of the lack of typical FMF symptoms in the pediatric patient, with the presence of hypogammaglobulinemia, we were led to consider that amyloidosis in our patient may not be due to FMF. We speculate that the occurrence of phenotype II FMF in patients with heterozygote mutations or no mutations in MEFV (and particularly pediatric onset) may be due to additional mutations in genes related to other autoinflammatory diseases, as in our case.

CONCLUSIONS

This is the first patient with DADA2 who was proven by a novel mutation in the CECR1 gene and by functional study to have DADA2 and then be successfully treated for renal amyloidosis with canakinumab. DADA2 should be considered in the differential diagnosis of renal amyloidosis, after excluding other reasons, even in the absence of recurrent fever and livedo reticularis. Although anti-TNF agents are widely offered as the optimal treatment in DADA2 currently, we suggest that canakinumab may be an appropriate treatment of renal amyloidosis in DADA2.

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ABBREVIATIONS

ADA2: adenosine deaminase 2
anti-TNF: anti–tumor necrosis factor
CECR1: cat eye chromosome region 1
DADA2: deficiency of adenosine deaminase 2
FMF: familial Mediterranean fever
IgG: immunoglobulin G
IgM: immunoglobulin M
IL-1β: interleukin 1β
MEFV: Mediterranean fever
NGS: next-generation sequencing
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