Abstract

Three prime repair exonuclease 1 (TREX1) degrades single- and double-stranded DNA with 3'-5' exonuclease activity. TREX1 mutations are related to type 1 interferon-mediated autoinflammation owing to accumulated intracellular nucleic acids. Several cases of systemic lupus erythematosus, Aicardi–Goutieres syndrome (AGS), familial chilblain lupus (FCL), and retinal vasculopathy-cerebral leukodystrophy caused by TREX1 mutations have been reported, so far. In this report, we described five patients with TREX1 mutations from three families with three different disorders, which include AGS, FCL, and FCL with central nervous system vasculitis.

Keywords: Children, clinical heterogeneity, interferonopathies, three prime repair exonuclease 1, mutations

INTRODUCTION

Encoding gene three prime repair exonuclease 1 (TREX1) is located on the chromosome 3p21.31 and its mutations are related to type 1 interferon (IFN)-mediated autoinflammation owing to accumulated intracellular nucleic acids.[1] TREX1 degrades single-stranded DNA (ssDNA) and double-stranded DNA in mammalian cells with its 3'-5' exonuclease activity. Mutations in the human TREX1 gene have been linked to a broad spectrum of autoimmune diseases including Aicardi–Goutieres syndrome (AGS), monogenic form of cutaneous lupus erythematosus named “familial chilblain lupus” (FCL), systemic lupus erythematosus (SLE), and retinal vasculopathy-cerebral leukodystrophy (RVLC).[2-4]

AGS is the prototype of type I interferonopathies which were characterized by systemic inflammatory disorder with onset in early infancy. In most cases, AGS presents as a leukoencephalopathy with basal ganglia calcifications and progressive cerebral atrophy and its clinical phenotype resembles congenital viral infection.[1] FCL is a monogenic form of cutaneous lupus erythematosus characterized by cold-induced, bluish-red skin lesions in acral locations such as fingertips, heels, nose, and auricles, with onset in early childhood.[1]

Central nervous system (CNS) vasculitis, which is a prominent cause of childhood strokes, occurs secondary to systemic vasculitis, metabolic diseases, malignancies, drugs, and radiotherapy exposures or might be limited to CNS vessels named primary CNS (pCNS) vasculitis. pCNS is classified into two groups: large-medium-vessel and small-vessel pCNS.[5]

Aforementioned diseases are now classified within type 1 interferonopathies. Type 1 interferonopathies comprise a genetically and phenotypically heterogeneous group of autoinflammatory and autoimmune disorders which were characterized by constitutive activation of the antiviral type I IFN axis. The term type I interferonopathies was given due to the recognition of an abnormal upregulation of the type I IFN as a unifying phenotype of this novel group of diseases.[6]

Recently, our understanding of the molecular pathology underlying the type 1 interferonopathies was greatly influenced...
by advances in the field of innate immunity and human genetics. To date, several cases with alterations in distinct genes that lead to type I interferonopathies have been described, of which one of them is TREX1.\(^1\)

In this report, we described three different phenotypes in five patients with TREX1 mutations.

### Case Reports

#### Case 1

Case 1 was an 8-year-old boy who was admitted to the hospital for seizures and developmental delay. He had severe mental retardation and was unable to walk. He was born prematurely at 36 weeks through spontaneous vaginal delivery to consanguineous parents as a first child. There was no history of maternal diabetes or exposure to any teratogenic agents of the mother during the pregnancy. There were no information on his birth weight, length, and head circumference. Although the neonatal period was normal, a global delay of development was prominent at 4 months of age. At that time, the child was often irritable and had feeding difficulties. At 5 months of age, he had suffered from frequent tonic seizures requiring antiepileptic drug (AED) therapy. Moreover, cerebral magnetic resonance imaging (MRI) revealed white matter lesions. He was diagnosed with spastic cerebral palsy in another hospital, due to the history of prematurity and MRI findings. At the time of admission to our department, his neurological examination revealed microcephaly, mental retardation, and spasticity, but the cranial nerves were normal. There were increased tones in the upper and lower limbs and hyperreflexia with bilateral positive Babinski sign. Ophthalmologic examination was normal. He was unable to sit, walk, and speak. There was no hepatosplenomegaly. Laboratory parameters revealed normal complete blood count, serum electrolytes, liver and renal function, and thyroid function tests. The serology for toxoplasmosis, rubella, cytomegalovirus, and herpes simplex was all negative. Urinary amino and organic acids and metabolic screen were all normal. The cranial computed tomography (CT) scan showed calcifications in the basal ganglia and periventricular white matter [Figure 1]. The brain MRI revealed brain atrophy and demyelination of the white matter [Figure 2]. He was found to have a cardiomyopathy by echocardiography.

Clinical and neuroimaging findings have suggested AGS, and the mutation analysis of the TREX1 gene revealed a homozygous novel mutation, p.R169H (c.506G>A).

#### Case 2

The patient was the sister of case 1. She was born full term through spontaneous vaginal delivery. Her medical history revealed that she had tonic seizures and was treated with AED at the age of 3 months. She was diagnosed at the age of 5 years. At the time of the diagnosis, she had severe mental retardation and spastic tetraplegia. The deep tendon reflexes were brisk in all the extremities. She was unable to sit, walk, and speak. Her CT and MRI showed very similar features with case 1. Besides family history, her clinical and neuroimaging findings suggested AGS, and the mutation analysis of the TREX1 gene revealed a homozygous novel mutation, p.R169H (c.506G>A) same mutation as case 1 had.

#### Case 3

A 3-year-old boy presented with developmental delay, seizures, and microcephaly. He was a first child of consanguineous parents. He was born prematurely at 35 weeks through spontaneous vaginal delivery after an uneventful pregnancy. Her development progress was extremely poor. He had poor visual attention and made no progress in his motor skills. Later on, he developed progressive microcephaly. At the age of 5 months, she developed tonic seizures and was treated with AED. At the age of 24 months, cerebral CT and MRI were performed in another hospital. Due to clinical and imaging findings, she was diagnosed with intrauterine infection. At the time of admission to our department, her neurological examination revealed microcephaly, mental retardation, and spasticity. In addition, the deep tendon reflexes were brisk in all the four extremities. He was unable to sit, walk, and speak. However, there were no signs of any cutaneous lesion. CT imaging showed multiple areas of calcification in the basal ganglia and in the white matter of both cerebral hemispheres. MRI revealed prominent frontal and perisylvian atrophy, and severe delay in myelination. Therefore, clinical and neuroimaging findings suggested AGS. Genetic analyzes revealed a homozygote novel mutation, p.R169H (c.506G>A) in TREX1 gene.

#### Cases 4 and 5

Cases 4 and 5 were reported previously elsewhere.\(^7\) They were brothers, born to consanguineous parents. The older brother (case 4) had muscle weakness and diminished deep tendon reflexes in his left arm. Moreover, his physical examination revealed cold-induced chilblains on the skin of his auricles, feet soles, and toes [Figure 3]. Laboratory investigations were all normal except increased acute-phase reactants. MRI angiography revealed reduced calibration in bilateral internal carotid arteries and nonvisualization of the middle and posterior cerebral arteries and their branches, suggesting large-vessel pCNS vasculitis. Case 5 had recurrent ulcers on the toes and auricles, particularly prominent in winter. His physical examination was normal except for skin findings such as crusty wounds and hyperemic ulcers on acral surfaces. Moreover, laboratory parameters and imaging findings were all normal. Therefore, he was diagnosed as having FCL without any systemic signs. Both patients had the same homozygous c. 340C>T p.Arg114Cys (R114C) mutation in TREX1.

### Discussion

The type I interferonopathies comprise an expanding number of genetically defined disorders that are caused by alterations of the immune system. Although type I interferonopathies contain phenotypically heterogeneous disorders, they are commonly characterized by systemic autoinflammation and
varying degrees of autoimmunity or immunodeficiency. *TREX1* gene, which is the major 3′-5′ exonuclease in mammalian cells, is one of the mutations that lead to type I interferonopathies.[11] Loss-of-function mutations of *TREX1* cause accumulation of ssDNA metabolites derived from diverse biological processes. Those unmetabolized ssDNA species are recognized as danger signals and trigger a type I IFN response. *TREX1* mutations lead to various diseases such as SLE, AGS, FCL, and RVLC.[1,3,4]

AGS is a systemic inflammatory disorder with onset in early infancy. The clinical diagnosis of AGS is based on features first described in 1984, including a severe progressive encephalopathy, acquired microcephaly, cerebral atrophy, basal ganglia calcifications, and white matter abnormalities in neuroimaging as well as lymphocytosis in cerebrospinal fluid.[8]

Furthermore, several extra neurological features including vascular necrotic cutaneous lesions of the toes, fingers, earlobes looking like chilblains, erythematous periungual skin, puffy hands, and cold feet had been reported in AGS. It is a genetic disease due to mutations in various genes including *TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1,* and *IFIH1.* In the majority of cases, patients with AGS have biallelic loss-of-function mutations of *TREX1.* However, several cases with heterozygous de novo *TREX1* mutations have also been reported.[1] Two clinical manifestations of AGS can be delineated: neonatal and later-onset form. A neonatal form, which is highly reminiscent of congenital infections, was observed particularly in participants with *TREX1* mutations. However, patients with apparently static or slowly progressive disease, sometimes presenting after several months of normal development, have also been reported.[8,9]

As we know, AGS is a rare disorder with variable clinical manifestations. Recently, clinical and molecular spectrum of 24 AGS patients have been reported by Al Mutairi *et al.*[10] They have found developmental delay (100%), spasticity (100%), speech delay (95.8%), profound intellectual disability (87.5%), truncal hypotonia (87.5%), seizures (75%), and epileptic encephalopathy (62.5%) in their patients. All three patients included in our report showed developmental delay, spasticity, speech delay, seizures, epileptic encephalopathy, and microcephaly. They all presented with neurologic symptoms in the 1st year of life. Although some features of SLE particularly FCL may occur in people with AGS, we did not find any signs or symptoms of autoimmunity in our patients.

Spasticity and dystonia have been recognized as key features of the AGS ever since the first description.[8] However, epilepsy is one of the most frequent and severe manifestations of AGS as well, and it is mainly characterized by early onset, predominantly tonic semiology, and a refractory course. In previous two studies which included 10 and 21 patients diagnosed with AGS on clinical grounds alone, the presence of epilepsy was reported between 10% and 30% of cases. The characteristics of epilepsy in these cohorts were either focal tonic or generalized seizures.[11,12]

In two cohorts that include 123 and 20 mutation-positive AGS patients, 53% and 55% of patients suffered from epilepsy, respectively.[9,13] Similarly, in another study, epilepsy was reported 24% of 21 AGS patients.[11] Despite the fact that epilepsy is a major problem of AGS patients, comprehensive data on seizure types and electroencephalography findings are scarce. A study was conducted to analyze the clinical features of epilepsy in AGS, including age at seizure onset and seizure types, in 2013. In that report, the proportion of epilepsy was 75% in AGS. They concluded that epilepsy is a cardinal feature of the disease and outlined its natural history often with occurrence in the 1st year of life and manifestation with predominantly tonic semiology including infantile spasms.[14] In this study, we reported three AGS patients of whom they all had epilepsy. Due to the small number of our patients, we could not compare our findings to the results of other studies.

Neuroradiologic findings, particularly punctuate calcifications,
especially in the cerebral basal nuclei, white matter, and/or dentate nuclei, have been considered essential for diagnosing AGS since the first descriptions of the syndrome. According to the studies, the neuroradiologic presentation is characterized by three features: cerebral calcifications, white matter abnormalities, and cerebral atrophy. In a very recent study, neuroimaging showed white matter abnormalities in 91.7%, cerebral atrophy in 75%, and small, multifocal calcifications in the lentiform nuclei and deep cerebral white matter in 54.2% of AGS patients. In the current study, neuroimaging revealed calcification, cerebral atrophy, and demyelination of the white matter in three AGS patients.

FCL is a monogenic form of cutaneous lupus erythematosus characterized by cold-induced, bluish-red skin lesions in acral with onset in early childhood. FCL was first described by Lee-Kirsch et al. in 2006. Later on, they have showed that a defect in a single TREX1 allele causes FCL and patients with AGS, who have defects in both TREX1 alleles, also sometimes develop FCL. Heterozygous loss-of-function mutations in TREX1 and SAMHD1 and heterozygous gain-of-function mutations in STING have been reported in patients with FCL, so far.

Moreover, Yamashiro et al., for the first time, have described a family with FCL accompanying cerebral vasculitis, in 2013. After a while, in 2017, we have reported a homozygote TREX1 mutation in two siblings of whom one had only FCL but the other FCL accompanying cerebral vasculitis, patients 4 and 5 of this report, respectively. This was the second reported patient of FCL with cerebral vasculitis.

Consequently, we have previously reported the cases 4 and 5, two siblings with the same homozygote mutations of TREX1 gene, presenting distinctive courses. In this paper, we also reported three more patients with the same novel homozygote mutations of TREX1 gene, presenting with AGS.

In conclusion, we highlighted that patients with biallelic or nonallelic TREX1 mutations may be associated with various phenotypes including AGS, FCL, SLE, RVLC, and cerebral vasculitis.

Informed consent
Informed consent was obtained from the parents of the children included in the study.

Declaration of patient consent
The authors certify that they have obtained all appropriate patient consent forms. In the form, the parents of the children have given their consent for their children’s images and other clinical information to be reported in the journal. The parents of the children understand that their children’s names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest
There are no conflicts of interest.

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