Aicardi-Goutières Syndrome Type 1: A Novel Missense Variant and Review of the Mutational Spectrum

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ABSTRACT

Objectives

Mutations in the TREX1 gene cause Aicardi-Goutières syndrome (AGS) 1, associated with a spectrum of autoimmune and neurodegenerative manifestations. AGS 1, the most severe neonatal type of AGS, is characterized by abnormal neurologic findings, visual inattention, hepatosplenomegaly, thrombocytopenia, skin rash, restlessness, and fever.

Materials & Methods

The present study described two affected siblings from an Iranian family whose phenotypes overlap with intrauterine infections. They had almost similar presentations, including developmental delay, microcephaly, no fix and follow epileptic seizures and the same pattern of brain CT scan involvements. Following clinical and paraclinical assessments, whole-exome sequencing was employed to determine the disease-causing variant, and subsequently, PCR-Sanger sequencing was performed to indicate the segregation pattern of the candidate variant in family members.

Results

Genetic analysis revealed a novel homozygous missense variant (c.461A>C; p.D154A) in the TREX1 gene in affected family members. Sanger sequencing of other family members showed the expected zygosities.

Conclusion

This study identifies a novel mutation in the TREX1 gene in this family and highlights the efficiency of next-generation sequencing-based techniques for obtaining a definite diagnosis in patients with early-onset encephalopathy.

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Introduction

Aicardi-Goutières syndrome (AGS) is a rare heterogeneous disorder with both autosomal dominant and recessive modes of inheritance manifesting as an early-onset encephalopathy that mainly affects the brain, the immune system, and the skin (1). It usually results in severe intellectual and physical disability but, in some cases, may not cause these complications. Jean Aicardi and Françoise Goutières first described the disease as a progressive disorder of the central nervous system with bilateral spasticity and dystonia, acquired microcephaly in association with radiologic evidence of intracranial calcifications, profound white matter abnormalities, and cerebral atrophy (2, 3). The genetic cause of the disease is linked to mutations in nine genes, including DNA Three-Prime Repair Exonuclease 1 (TREX1) (4), and the three subunits of the ribonuclease H2 (RNase H2) endonuclease complex (RNase H2A, RNase H2B, and RNase H2C) (5). Other genes involved are deoxynucleoside triphosphate triphosphohydrolase and ribonuclease SAM domain and HD domain 1 (SAMHD1) (6), adenosine deaminase acting on RNA (ADAR; also known as DRADA) (7), the double-stranded RNA (dsRNA) cytosolic sensor IFN-induced helicase C domain-containing protein 1 (IFIH1; also known as MDA5) (8), U7 small nuclear RNA-associated protein (LSM11), and U7 small nuclear RNA 1 (RNU7-1) (9, 10). Monoallelic pathogenic variants in *TREX1* cause familial chilblain lupus (FCL) [OMIM 610448] (11), retinal vasculopathy with cerebral leukodystrophy (RVCL) [OMIM 192315] (12), associating with susceptibility to systemic lupus erythematosus (SLE) [OMIM 152700]. Furthermore, its monoallelic or biallelic mutations lead to AGS type 1 (AGS1) [OMIM 225750].

No definitive cure exists for AGS1. However, treatments like chest physiotherapy, managing respiratory complications, focusing on diet and feeding methods to ensure proper caloric intake, and using standard protocols and medications to control seizures can help manage the disease. *TREX1,* located on the 3p21.31 chromosome, contains just one coding exon. It encodes a protein with 3 exonuclease activity, which plays a role in DNA repair and has the function of proofreading for DNA polymerase. Moreover, it acts as a blocker of cGAS (cyclic GMP–AMP synthase) activation by degrading micronuclear DNA arising from genome instability and prevents subsequent inflammation via type I interferon production (13, 14, 15).

TREX1 has 46 pathogenic and 24 likely pathogenic registered variants in ClinVar, of which the most prevalent is a missense change (p.Arg114His), particularly common in people from northern Europe (16).

The present study performed Whole Exome Sequencing (WES) to identify the genetic cause of the disease in a patient with typical features of AGS who had a sister who died with the same presentation.

Materials & Methods

Subjects

Informed consent was obtained from all participating subjects, and blood samples were then collected from family members. This study was performed in line with the principles of the Declaration of Helsinki. The Ethics Committee of the Tehran University of Medical Sciences granted approval. Two sisters, born to consanguineous parents, died exhibiting the same signs and symptoms. Their family history revealed similar presentations in the pedigree (Figure 1).

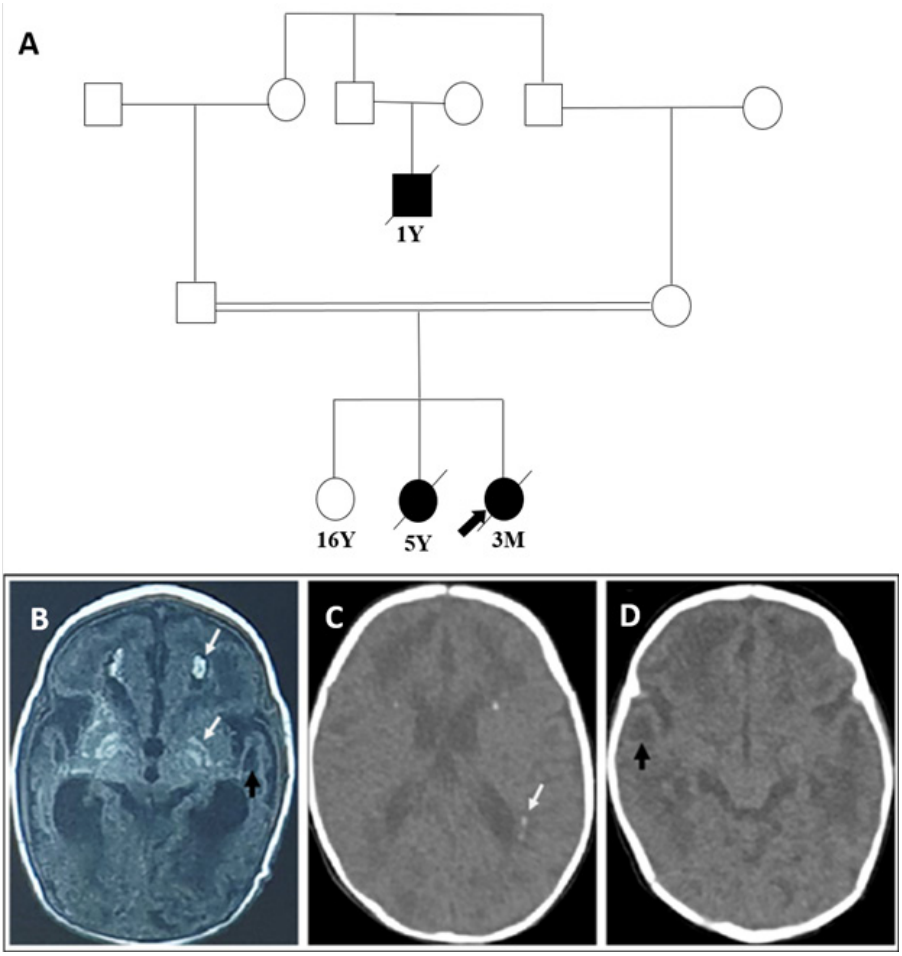

Fig 1. *The AGS1-affected family pedigree and brain imaging. A) The affected proband is indicated by black arrow. Circles indicate female, and squares indicate male. Consanguinity is represented by parallel lines. B) Brain MRI of proband's affected sister demonstrated bilateral high signal intensity in Globus pallidus and periventricular areas (white arrow in a) and anterior temporal subcortical cyst (black arrow in B) on FLAIR sequence. C & D) Proband's brain CT demonstrated hyperdensity of periventricular areas (white arrow in C) and anterior temporal subcortical cyst (black arrow in D).*

The affected family member, a common cousin of the proband's parents, was a 12-month-old boy. He experienced intellectual disability, developmental delays, restlessness, fever, and frequent respiratory infections before he passed away. Although his parents were from the same small town, they were not closely related by blood. Sadly, no genetic samples from him were available for testing. The proband was a 3-month-old infant with intrauterine growth retardation (IUGR), severe developmental delay and microcephaly. She was born at term and her birth weight was 2400 g. Since her parents decided to conceive again, she was referred to Watson Genetic Laboratory (Tehran, Iran) for genetic counseling and testing. Her manifestations started at birth with frequent epileptic spastic seizures, excessive crying, restlessness, and diarrhea. At the age of two months, she was admitted to the hospital due to fever and then a skin rash appeared. Abdominal ultrasound findings included the normal size of the liver and spleen. She had no fix and follow and brain CT scan displayed white matter involvement, calcification, and ventriculomegaly (Figure 1). In detail, relatively symmetric hypodensity of subcortical and periventricular white matter, in addition to mild dilation of lateral ventricles in favor of ventriculomegaly associated

with periventricular calcified foci, was found in the brain CT scan (Figure 1). Furthermore, opacification of bilateral mastoid air cells and middle ear cavities in favor of otomastoiditis was noted. As she died two weeks after the visit, it was not possible to obtain a brain MRI for her. At first, she was suspected of CMV infection but antibody testing indicated no evidence of it.

The older sister, who died at the age of five years, had the same signs and symptoms as the proband but somewhat more severe. She was born an IUGR fetus with low birth weight (1440g) and preterm (34 weeks). She was hospitalized at birth due to meconium aspiration and respiratory distress. Neurological examination revealed global developmental delay, microcephaly, and spasticity. She was prescribed Phenytoin, Topiramate, Phenobarbital, and Sabril because of a history of seizures at birth that occurred frequently. She also had excessive crying, restlessness, visual inattention, thrombocytopenia, constipation, extensive tooth decay, swallowing difficulty, and poor feeding. In the paraclinical evaluations, general biochemical evaluations revealed that aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated. However, the levels of creatinine, sodium (Na), potassium (K), and chloride (Cl) were normal. Hepatosplenomegaly was observed in sonographic findings, Electroencephalography (EEG) showed mildly abnormal results, and T3 and T4 were upper than normal limits so levothyroxine was prescribed. Sonography of the brain revealed a widening of bilateral extra-axial spaces, dilation of the lateral ventricles, calcifications in the basal ganglia and periventricular areas, atrophy of the cerebral cortex, and tiny cystic foci, particularly in the bilateral parietal regions, indicative of cystic encephalomalacia. Brain MRI demonstrated bilateral high signal intensity in globus pallidus and periventricular areas in addition to anterior temporal subcortical cyst (Figure 1). The same as her sister, the diagnosis of CMV infection was considered primarily but the TORCH study was normal.

Whole Exome Sequencing

Genomic DNA was extracted from the peripheral blood sample of the proband using the Exgene™ Blood SV DNA purification kit (GeneAll®, Korea) according to the manufacturer's instructions. DNA concentrations were determined on a Thermo Scientific™ Nanodrop 2000. Exome sequencing was done for the proband by CeGaT GmbH (Germany). Exons were enriched using Twist Human Core Exome Kit (Twist Bioscience, USA), and paired-end sequencing was performed by Illumina NovaSeq 6000 platform (Twist Bioscience, USA) following the Illumina protocols (Illumina Inc., San Diego, CA, U.S.A.).

Bioinformatics Analysis

After the base calling and quality control of the produced data, Burrows-Wheeler Aligner (BWA) was used to align the reads to the human reference genome 19 (GRCh37/hg19), and Assembly Based ReAligner (ABRA) [version 2.18] were applied for indel realignment and base recalibration. Variants to the reference were called using the Genome Analysis Tool Kit (GATK) HaplotypeCaller program and subsequently annotated using the ANNOVAR program. WES data was analyzed using a stepwise filtering approach to prioritize variants. Variants filtered based on MAF < 0.01 in dbSNP147 (https://www.ncbi.nlm.nih.gov/SNP/), 1000 Genomes Project (https://www.internationalgenome.org/), Exome Aggregation Consortium (ExAC) (http://www.Exac.broadinstitute.org/), Genome Aggregation Database (gnomAD) (https://

gnomad.broadinstitute.org/), the Iranome catalog of genomic variants in the Iranian population with 800 healthy individuals, and in-house database of Iranian exomes (Pishgam Biotech Company, Iran). Only variants (SNPs and indels) located in exonic regions or canonical splicing sites were included for analysis. In order to prioritize genes responsible for AGS and other neurological diseases, OMIM (Online Mendelian Inheritance in Man) and PubMed were used. HGMD (https://www.hgmd.cf.ac.uk/ac/index.php), ClinVar (https://www.ncbi.nlm.nih.gov/clinv ar/), and literature reviewed to evaluate the pathogenicity of candidate variants. Multiple in-silico prediction algorithms including Sorting Intolerant from Tolerant (SIFT), Polymorphism Phenotyping version 2 (PolyPhen-2), MutationTaster (http://www.mutationtaster.org), Protein Variation Effect Analyzer (PROVEAN), Functional Analysis through Hidden Markov Models (FATHMM), Combined Annotation Dependent Depletion (CADD) and DANN applied to obtain a functional prediction. Finally, the candidate variants associated with the patient's phenotype were interpreted based on the American College of Medical Genetics and Genomics (ACMG) (17).

Segregation Snalysis and Variant Confirmation

A specific set of primers was designed for the amplification of the *TREX1* gene, including the variants of interest (NM_033629), and Primer sequences are as follows:

Forward: 5′- CCAGACTAAGGGGGCACTAGG -3′.

Reverse:5′-GTCTTGGCTTGGTCCTAGCAGAGG -3′.

PCR-Sanger sequencing (ABI 3500 Genetic Analyzer) was then done to validate the causative variant in the proband and her affected sister and to perform segregation analysis in the other family members (including the healthy sister and their parents). The sequencing results were aligned to the reference sequence using the Chromas software (version 2.6.2).

Analysis of Three-Prime Repair Exonuclease 1 Structural Stability and Pathogenicity of Determined Variant

Calculating Gibbs free energy changes (ΔΔG) is an appropriate method to evaluate the effect of a missense variant on protein stability, so this study used FoldX plugin (version 4) on YASARA program (version 22.8.21) (18). First, the structure of human Three-Prime Repair Exonuclease 1 was obtained from Protein Data Bank (https://www.rcsb.org/) with PDB code 7TQQ. Then, energy optimization was performed with FoldX. The stability of this structure was assessed by calculating its free energy (ΔG_{wt}). The missense variant in *TREX1,* obtained from NGS data analysis of proband, was introduced to the structure of Three-Prime Repair Exonuclease 1. Energy optimization and calculation of the free energy (ΔG_{mt}) was performed. Finally, ΔG change was calculated with the following formula:

$\Delta\Delta G = \Delta G_{mt} - \Delta G_{wt}$

Moreover, in-silico prediction algorithms (SIFT, PolyPhen2, Mutation Taster, and CADD Phred score) were used to evaluate the variant's deleteriousness and disease-causing potential.

Results

Exome and Sanger Sequencing Results

After quality control processing of raw FASTQ files, it generated 74,358,328 uniquely mapped reads, whereas 68.2% of reads mapped to 36.5 Mb target regions. The Mean on-target coverage of 93 × was achieved, while 97.6% of target bases covered at > 20x. WES led to the identification

Table 1. Features of the variant found in the TREX1 gene

Gene	Variant	Transcript	Zygosity	Type and classification
TREX1	c.461A>C p.D154A	ENST00000436480.2 NM_033629	Homozygous	Missense, Likely Pathogenic

of a novel homozygous variant [NM_033629: c.461A>C; (p.D154A)]. The variant is absent in population databases (ExAC, 1000G, dbSNP 147, HGMD, ClinVar, and our local database), and no case has been reported in the literature (Table 1 shows other variant features). Finally, the candidate variant was classified as Likely Pathogenic based on the American College of Medical Genetics and Genomics (ACMG) guidelines.

Sanger sequencing results indicated the detected variant was in homozygous status in the proband, and her affected sister, her healthy sister and her parents were heterozygote carriers for this variant (Figure 2).

Investigation of Three-Prime Repair Exonuclease 1 Thermal Stability and the Pathogenicity of c.461A>C Variant

The free energy change (ΔΔG) of the activated Three-Prime Repair Exonuclease 1 was calculated with FoldX plug-in on YASARA for the causative variant found in this study (Table 2). The ΔΔG for the p.D154A variant was +6.924, preferring protein destabilization.

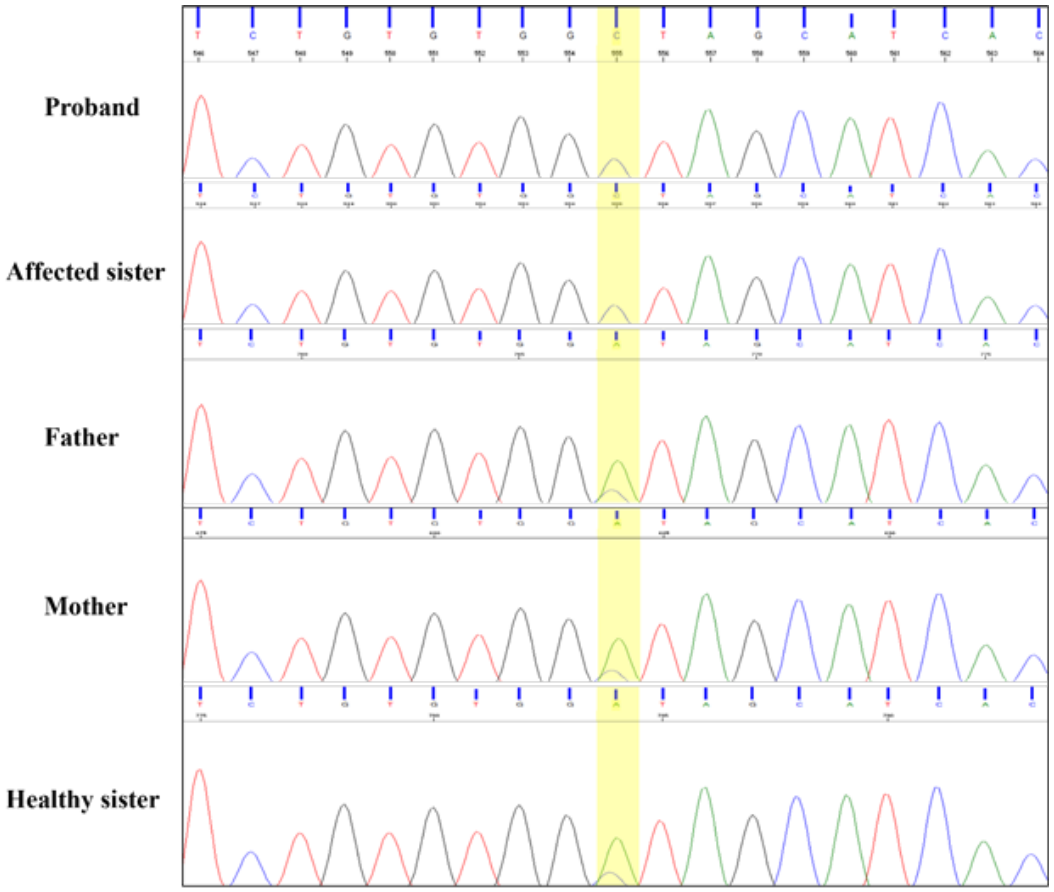

Fig 2. *Sanger sequencing chromatogram. The proband and her affected sister are homozygous for the c.461A>C variant in TREX1 gene. Her healthy sister and parents are carriers for this variant*

Table 2. The pathogenicity of c.461A>C variant

Amino acid change	Thermal stability changes (Kcal/mol)	SIFT & PolyPhen2	Mutation Taster	CADD PHRED score
D154A	+6.924	Deleterious &Probably Damaging	Disease causing	27.1

SIFT and PolyPhen2, with relatively high scores in the CADD tool, predict that the variant of interest is deleterious and disease-causing. Table 2 summarizes the results of these prediction tools, and Figure 3 illustrates the structures of wild-type and mutant Three-Prime Repair Exonuclease 1.

Discussion

This study found a novel likely pathogenic missense variant in patients with typical signs and symptoms of AGS1 such as developmental delay, encephalopathy, microcephaly, fever, and restlessness. The structural stability of Three-Prime Repair Exonuclease 1, a product of *TREX1*, deteriorated with the destabilizing ($\Delta\Delta G>0$) p.Asp154Ala mutation. In silico predictions for this substitution showed a deleteriousness effect on the product, as seen in SIFT analysis. PolyPhen2 analysis also indicated this variant as "probably damaging," the mutation taster program predicted it as a "disease-causing" variant. In addition, the CADD Phred score was relatively high (27.1).

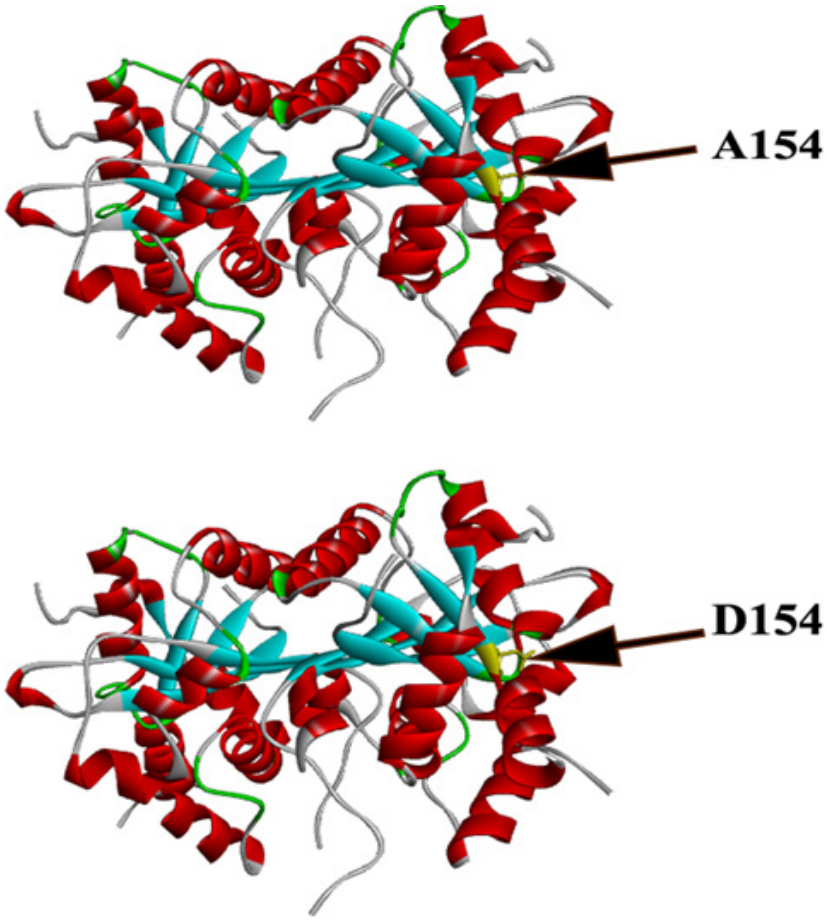

Fig 3. *The structures of Three-Prime Repair Exonuclease 1 in wild type and mutant type. The position of amino acid changed in our patients is indicated by arrow*

These bioinformatic analyses followed the causality of the variant in our patients.

AGS, a severe neurodegenerative disorder, typically has onset in the first year of life (19). It is characterized by leukoencephalopathy, global developmental delay, progressive microcephaly, hepatosplenomegaly,thrombocytopenia, and specific neuroimaging findings such as calcifications and white matter abnormalities. Visual function varies from normal to cortical blindness, and in some cases, skin lesions occur (20, 21). AGS has a variable phenotypic range among affected patients, showing inter- and intra-familial variability (22). In this study, although the affected members belonged to the same family with an identical variant, they had somewhat different presentations. Undeniably, both were similar in neurological manifestations, but the severity of the disease was higher in the older sister, and some signs and symptoms like thrombocytopenia, respiratory distress, hepatosplenomegaly, swallowing difficulty, and poor feeding were exclusively related to her.

To date, nine genes related to AGS have been reported, of which *TREX1* is responsible for one of the most severe types called AGS1. The *RNASEH2A*, *RNASEH2B*, and *RNASEH2C* genes produce nucleases to break down extra DNA and RNA molecules, which may be mistaken for viral invaders, causing immune system reactions in multiple body systems that lead to encephalopathy, skin lesions, and other signs and symptoms of AGS. Since the protein product of *SAMHD1*, *IFIH1*, and *ADAR* genes are involved in the immune system, pathogenic and likely pathogenic mutations result in inappropriate activation of the body's immune response and , consequently, inflammatory damage to the brain and skin which occurs in AGS (2, 9).

TREX1 mutations have been frequently reported in association with a neonatal presentation, suggesting in-utero onset of AGS, and among other diseases associated with AGS, AGS1 leads to the highest number of deaths (23). All cases in this study experienced a very early onset of disease and died before the age of one year. *TREX1* encodes a cytosolic protein that is key role in clearing degraded DNA to prevent aberrant inflammation and immune activation. Mutations in *TREX1* cause accumulation of the micronuclear DNA, stimulating the production of type I interferon via cGAS activation. Thus, *TREX1* deficiency in patients with AGS1 leads to an interferonopathy that mimics congenital infection (pseudo-TORCH) (14, 24). Unquestionably, the main differential diagnosis for AGS1 is a TORCH infection, which can be excluded by negative antibody testing. In both, calcification patterns typically involve the periventricular area and basal ganglia in a punctate distribution, consistent with our study's patients. Both cases in this study were initially suspected of placental-acquired TORCH infection, but further serology investigations ruled it out. The molecular basis of the disease was identified by the whole exome sequencing technology, a powerful method that significantly accelerates the detection of disease-causing genetic changes. WES has emerged as a robust and cost-effective procedure among next-generation sequencing (NGS) techniques to detect the causative variants in diseases with locus heterogeneity (25). Especially in the case of AGS, which mimics an acquired disease caused by an infection, WES ultimately ends the debate about the actual cause of the disease.

The total number of pathogenic and likely pathogenic missense variants classified in UniProt, ClinVar, VarSome, and PubMed databases for

the TREX1 gene is 18 and 9, respectively. As shown in Table 3, the local database, including 3400 exome sequencing data, only contains one pathogenic and two likely pathogenic missense mutations. However, the number of benign and likely benign variants is higher than that reported in other databases. This significant difference is due to the underreporting of the benign and likely benign variants in the studies. Nevertheless, the exact number of these types of mutations is essential for better interpreting the variants. Of the total 3400 patients sequenced by the NGS method in our center, 1168 variants were detected, of which 1150 are benign and likely benign.

Many TREX1 mutations specifically concentrate in the C-terminus region, crucial in anchoring the protein to the endoplasmic reticulum. As a result, these mutations often lead to mislocalization of the protein within the cell rather than directly causing a loss of enzyme activity (11, 15). In a study by Rice et al., sequencing of the TREX1 gene was performed in three patients with typical AGS; a common c.375dupT mutation was found in all of them. They explained that this mutation produces a truncated protein lacking 188 amino acids (26).

Ramantani et al. reported a patient with symptoms of both AGS and SLE, caused by a heterozygous de novo TREX1 mutation (p.D200H). This mutation affects one of the four magnesium-coordinating residues crucial for catalytic function (27). Another heterozygous de novo TREX1 mutation affecting the same residue (p.D200N) had been previously reported in an AGS patient (26). They suggested a vital role for this residue in recognizing, binding, or processing nucleic acids and implied the possible dominant-negative mechanism of these mutations.

Crow et al. examined families with AGS-affected members and identified the c.341G>A missense mutation in seven pedigrees in homozygous and compound heterozygous states. They stated that this residue is responsible for protein dimerization. They also found another mutation (c.600_601insGAT) duplicating an aspartate residue critical for divalent cation binding. Additionally, they identified a homozygous missense mutation (c.602T>A) in the same exonuclease (Exo) III motif, emphasizing the significance of this domain. The study also demonstrated the absence of detectable TREX1 exonuclease activity in cell lines derived from patients with homozygous p.R114H substitution, as well as in the case of the c.602T>A mutation occurring at a different catalytic site (4).

Importantly, variable expression of the same mutation was observed within and between families: Kisla Ekinci et al. declared different phenotypes, including chilblains and cerebral vasculitis, for the same TREX1 mutation (p.R114C) in two siblings, while Yang et

Table 3. The list of variants reported in public databases (the first number in each cell) vs. our local database (the second number in each cell) for the TREX1 gene

Classification	Pathogenic	Likely Pathogenic	Uncertain Significance	Likely Benign	Benign
Missense	18-1	9-2	201-13	8-16	1-2
Synonymous	0-0	1-0	10-0	79-39	5-1093
Frameshift	31-2	13-0	11-0	0-0	0-0

al. previously reported a patient with AGS manifestations associated with this mutation (28). Barizzone et al. conducted a mutational analysis of the TREX1 gene and identified two new nonsynonymous variants - p.Met232Val and p.Glu198Lys. These variants were found in a patient with systemic sclerosis (SSc) and a patient with Sjogren's syndrome (SS), respectively. The p.Met232Val variant is located within a protein segment between the exonucleasic domain and the membrane binding domain TMH but is not considered part of a functional region. Mutations in this protein region have been reported in patients with SLE, AGS, and RVCL. The second variant (p.Glu198Lys) affects the functional domain Exo3, and several other mutations affecting this domain have been reported in AGS patients (12). The functional analysis conducted in vitro for specific mutations, such as p.Asp200Asn, p.Asp201ins, and p.Val201Asp, demonstrated a substantial reduction in exonuclease activity. The decrease in activity ranged from 4-fold for p.Val201Asp to a remarkable 35,000-fold for p.Asp201ins (15).

The variant found in this study (p.Asp154Ala) is related to the domain between exonuclease 2 and 3. A number of mutations in this domain have been reported to be associated with AGS patients, highlighting its importance. Some of these mutations are indicated in pink in Figure 4.

In summary, the examination of TREX1 mutations emphasizes the importance of specific amino acid residues, their impact on protein function, and the broad spectrum of clinical manifestations observed in different mutation carriers.

In Conclusion

AGS is a devastating neurodegenerative disorder with a range of symptoms that can vary from patient to patient. The condition has been linked to mutations in a number of genes, including TREX1, responsible for one of the most severe forms of the disease. This study used WES to identify a novel likely pathogenic missense variant in the TREX1 gene in two siblings who presented with typical signs of AGS1. Overall, this study highlights the power of next-generation sequencing techniques in identifying disease-causing genetic changes, ultimately leading to

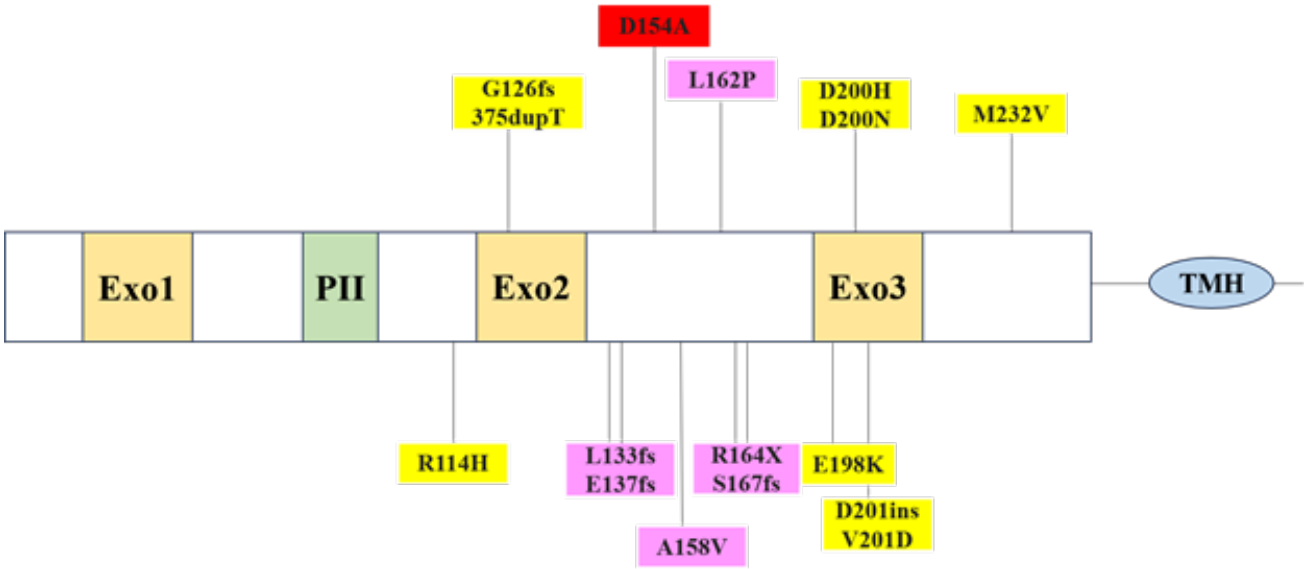

Fig 4. *TREX1 mutations. The mutation of our patients is highlighted in red. Yellow highlighted mutations are discussed in the text. Pink highlighted mutations are from the same domain as our patients. Exo 1, 2, 3 domains; PII: polyproline II domain; TMH: transmembrane domain*

better diagnosis and treatment of AGS.

Acknowledgment

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of Watson Genetic Laboratory, Tehran, Iran (Watsonlab-112-210116) and with the 1964 Helsinki declaration and its later amendments.

Author's Contribution

Behnoosh Tasharrofi wrote the main manuscript text. Mostafa Asadollahi and Sepideh Hasani performed bioinformatic analysis. Parvaneh Karimzadeh**,** Morteza Heidari, and Mohammad Keramatipour performed clinical and paraclinical evaluations. Mohammad Keramatipour designed the study and critically reviewed the primary manuscript. All authors commented on previous versions of the manuscript and approved its final version

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

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