

Heterozygous Mutations in *TREX1* Cause Familial Chilblain Lupus and Dominant Aicardi-Goutières Syndrome

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TREX1 constitutes the major 3'→5' DNA exonuclease activity measured in mammalian cells. Recently, biallelic mutations in *TREX1* have been shown to cause Aicardi-Goutières syndrome at the *AGS1* locus. Interestingly, Aicardi-Goutières syndrome shows overlap with systemic lupus erythematosus at both clinical and pathological levels. Here, we report a heterozygous *TREX1* mutation causing familial chilblain lupus. Additionally, we describe a de novo heterozygous mutation, affecting a critical catalytic residue in *TREX1*, that results in typical Aicardi-Goutières syndrome.

TREX1 (GenBank accession numbers AAK07616 and NM_033627) represents the major DNA-specific 3'→5' exonuclease activity measured in mammalian cells.¹ The non-processive autonomous mode of action of *TREX1* suggested a possible proofreading role during lagging-strand DNA synthesis or a gap-filling role during DNA repair.² However, *Trex1*-null mice show no increase in spontaneous mutation frequency or cancer incidence.³ Rather, they develop an inflammatory myocarditis with progressive dilated cardiomyopathy, indicating a previously unrecognized cellular role for the enzyme. Of note, although there was a dramatically increased morbidity of *Trex1*-null mice postweaning, these mice did not exhibit any obvious neurological defect.

Aicardi-Goutières syndrome (AGS [MIM 225750]) is a genetically determined encephalopathy characterized by calcification of the basal ganglia and white matter, demyelination, and raised levels of lymphocytes in the cerebrospinal fluid.⁴ Neurological dysfunction becomes clinically apparent in infancy and manifests as progressive microcephaly, spasticity, dystonia, and psychomotor retardation. We identified the *AGS1* locus elsewhere,⁵ and we recently demonstrated that biallelic mutations in *TREX1* result in AGS at the *AGS1* locus⁶ and that mutations in genes encoding the three nonallelic components of the RNASEH2 protein complex also cause AGS.⁷

We and others have drawn attention to features suggesting immune dysfunction in AGS, and a number of reports have specifically highlighted the phenotypic overlap of AGS with the autoimmune syndrome systemic lupus erythematosus (SLE).^{8–12} Recently, Lee-Kirsch et al.¹³

described a family segregating a monogenic form of cutaneous lupus in which affected individuals presented with ulcerating lesions of the skin in acral locations, features highly reminiscent of the chilblains seen in some children with AGS.¹⁴ They mapped this disease, termed "familial chilblain lupus" (FCL [MIM 610448]), to the *AGS1* critical interval and drew attention to the phenotypic overlap with AGS.

Here, we describe a family with FCL that segregates a heterozygous pathogenic mutation in *TREX1* as a dominant trait. Additionally, we report a child with typical AGS whose condition results from a de novo heterozygous mutation in *TREX1*. These novel findings show that certain rare mutations in the *TREX1* gene can result in a dominant phenotype with symptoms of FCL and/or AGS.

We ascertained a nonconsanguineous Bangladeshi family in which two brothers and a sister demonstrate features of FCL (fig. 1 and table 1). Their father had been similarly affected throughout his life but was not available for study. There are three siblings without symptoms, one of whom was also studied, and their mother is healthy, with no features of disease in her 8th decade. The onset of the disease was in early childhood. Affected individuals presented with painful bluish-red swelling of the skin affecting mainly the fingers, toes, ears, helices, and, occasionally, the nose. The lesions were induced by cold temperatures and were significantly worse in the winter months. These lesions could ulcerate; in the two affected males, the ulcerations led to a loss of ear cartilage and destruction of the proximal interphalangeal (PIP) joints and distal toes (fig. 2). Ulcerative lesions healed but left areas of atrophic

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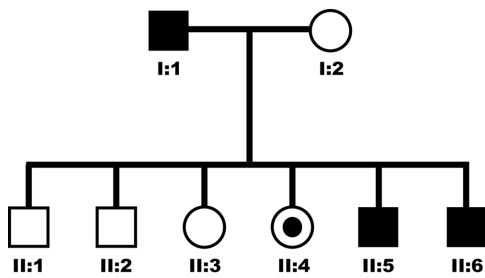


Figure 1. Pedigree of the nonconsanguineous family showing vertical transmission of FCL (affected individuals are indicated by blackened symbols). Note that individual II:4 is clinically asymptomatic but carries the familial disease-causing mutation.

and hypopigmented skin. There was no history of photosensitivity, fever, weight loss, immune deficiency, or malignancy. There was no associated Raynaud phenomenon, and there was no response to nifedipine or to a 3-d infusion of the prostacyclin analog iloprost. Treatment with prednisolone, sulfasalazine, and hydroxychloroquine was also unhelpful. One patient experienced swelling of the knee on two occasions, and joint aspiration demonstrated a sterile effusion consistent with a seronegative inflammatory arthropathy. Skin biopsy was never performed. There was no evidence of cryoglobulinemia, cryofibrinogenemia, cold agglutinins, or hepatitis C. Tests for rheumatoid factor, anticardiolipin antibodies, and extractable nuclear antigens were consistently negative. However, antinuclear antibody titres were intermittently raised, and one patient exhibited persistently elevated erythrocyte-sedimentation rate (ESR).

Sequencing of the single-exon *TREX1* gene revealed that all three affected individuals carried a c.375dupT and a c.50T→C transition that resulted in the replacement of a

phenylalanine with a serine at position 17 (p.F17S). Cloning into a pGEM vector system demonstrated that these variants were present on different alleles. The mother is heterozygous for the c.50T→C change only. Sequencing of the *RNASEH2A*, *-B*, and *-C* genes was normal. The *TREX1* c.375dupT is predicted to result in a truncated protein missing the last 188 aa. Despite its position within the EXO I domain, one of three sequence motifs containing four acidic residues participating in the coordination of divalent metal ions necessary for catalysis—the phenylalanine at position 17—is not conserved in mouse *Trex1* or the related human *TREX2*.^{2,15} Moreover, the purified recombinant TREX1 F17S/F17S homodimer exhibited normal 3'→5' exonuclease activity, indicating that this amino acid change does not adversely affect the catalytic activity of the recombinant TREX1 when measured in vitro (table 2).¹⁶ Thus, although the F17S change was not seen in a panel of 50 Asian control individuals, these data—together with the history of chilblains in the father, who must harbor the c.375dupT—led us to conclude that c.50TC is a rare polymorphism. Interestingly, one sister (II:4) was unaffected on clinical examination but carried the same molecular changes seen in her affected siblings. Further testing in this asymptomatic woman revealed a significant lymphopenia (1.16×10^9 /liter; normal $1.5\text{--}4.0 \times 10^9$ /liter) and raised ESR (23 mm/1st hour; normal 0–7 mm/1st hour), suggesting subclinical penetrance. Results of serum interferon alpha (IFN- α) testing in one symptomatic patient were normal.

Lymphoblastoid cell lines were established from affected individuals, and TREX1 exonuclease activity in cell extracts was measured, as described elsewhere, with use of a polydeoxynucleotide substrate.³ Extracts of control lymphoblastoid cell lines exhibited readily detected TREX1 activity. In contrast, lymphoblastoid cell lines derived from all three patients with FCL showed exonuclease activity

Table 1. Summary of Clinical Features in the FCL-Affected Family

Subject and Clinical Description	Laboratory Findings	Comments
<p>II:3:</p> <p>Presentation as an infant. Lesions affecting hands and feet, especially over PIP joints, sides of fingers, and ends of toes. Active disease associated with extension of swelling and redness along the digit, resulting in functional loss with pain for the duration of the swelling. No involvement of ears or nose. Large joint arthralgias, mainly of the knees, without swelling. In the quiescent periods, she is left with scarred, atrophic, and hypopigmented lesions.</p>	<p>Variable antinuclear antibodies, ranging from normal to 1:1,000 (homogeneous). Persistently raised ESR (20–40; normal <3). Negative extractable nuclear antigens (Sm, RNP, Scl70, Jo1, PM1, Ro, and La), anti-double-stranded DNA (anti-dsDNA), pANCA/cANCA, cardiolipin, rheumatoid factor, hepatitis C, cryoglobulins, and cryofibrinogen. Normal C3 and C4.</p>	<p>Treatment with hydroxychloroquine and sulfasalazine not tolerated. No response to prednisolone or iloprost.</p>
<p>II:5:</p> <p>Initial presentation at age 4 years, with recurrent cold fingers and toes. Digits swelled in cold weather, becoming red and “peeled.” Received diagnosis of chilblains. Continued episodes associated with pain and tissue infarcts and ulceration involving the digits, tip of the nose, and pinnae. Eventual “autoamputation” of the 4th right toe. Two episodes of frank knee swelling and effusion. He is left with marked tapering of the digits, with tightening of the skin and prominence of the PIP and distal interphalangeal joints.</p>	<p>Antinuclear antibodies 1:100 (homogeneous) on one occasion. Left synovial knee aspirate showed 13,000 white cells/mm³. Negative extractable nuclear antigens, anti-dsDNA, pANCA/cANCA, cardiolipin, rheumatoid factor, cryoglobulins, cryofibrinogen, C3, and C4.</p>	<p>X-ray of hands demonstrated resorption of terminal phalanges of all toes, left little and ring fingers, and right little finger, with subluxation of the PIP joint of the left little finger. No response to nifedipine or epoprostenol.</p>
<p>II:6:</p> <p>Presentation at age 4 years. Features similar to those of subject II:5, although he has not experienced episodes of large joint swelling.</p>	<p>No data available.</p>	

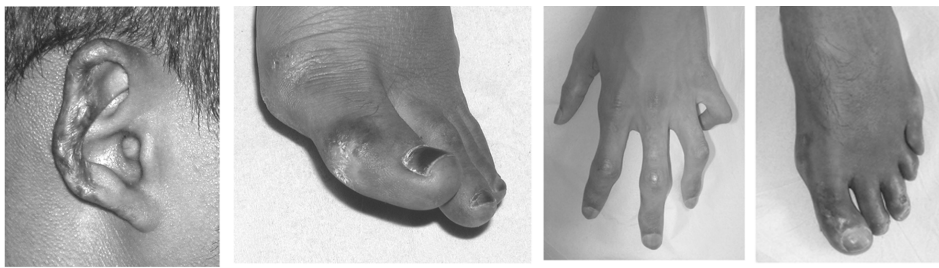


Figure 2. Skin features observed in FCL-affected family. The lesions are quiescent at present, but there are residual areas of atrophic and hypopigmented skin. Previous ulcerations have led to a loss of ear cartilage and destruction of the PIP joints and distal toes. Note the tapering of the fingers, with tight, shiny skin.

with a consistently marked reduction, to ~15%–35% that of a normal lymphoblastoid control, but clearly more than seen in an AGS cell line⁶ with biallelic inactivating *TREX1* mutations (fig. 3).

We also ascertained a child with a classic history of AGS born to nonconsanguineous Scottish parents. He presented at age 4 mo with developmental delay. Cerebrospinal fluid examination at age 3 years demonstrated 4 white cells/mm³ and a raised titre of IFN- α , 6 IU/liter (normal <2 IU/liter). Magnetic resonance imaging showed demyelination, and calcification of the basal ganglia was seen on CT scan. At age 7 years, he was profoundly delayed, with no meaningful communication, and was fed by gastrostomy tube. He demonstrated severe spasticity with dystonic posturing and was microcephalic. He had never experienced seizures. He had several chilblainlike lesions on his toes and hands and a more generalized patchy mottling of the skin on all four limbs and over his trunk (fig. 4). These lesions first developed at age ~12 mo and, although present throughout the year, were significantly worse in the winter.

Sequencing of *TREX1* revealed a heterozygous c.598G→A mutation resulting in a D→N substitution at amino acid residue 200 (fig. 5). Both parents had a homozygous wild-type genotype at this position. Differentiation of the maternal and paternal alleles was possible because of a frequently observed C→T SNP at position 531 and subsequent sequencing of single clones, which allowed us to demonstrate that the mutation had arisen on the maternal allele. Genotyping at several loci was consistent with maternity and paternity. This mutation was not present on 210 European control alleles, and sequencing of the *RNASEH2A*, *-B*, and *-C* genes was normal. Using the same method as

above, we measured *TREX1* activity in a lymphoblastoid cell line from the affected child. Interestingly, and in contrast to AGS-affected patients with disease resulting from biallelic *TREX1* mutations, we found that *TREX1* exonuclease activity was within 50%–100% of the activity of a normal lymphoblastoid control (fig. 3), comparable to the level seen in cells from unaffected heterozygous parents of subjects with recessive AGS.⁶

Our previous analyses of AGS-affected patients with biallelic *TREX1* mutations indicated that AGS at the *AGS1* locus results from an abrogation of *TREX1* enzyme activity.⁶ The *TREX1* protein functions as a dimer.¹⁷ We documented an ~75% reduction of *TREX1* activity in FCL-affected heterozygotes, which is consistent with the heterozygous inactivating c.375dupT mutation interfering with the function of a dimeric molecule. Importantly, our data show that certain heterozygous mutations lead to strongly diminished levels of *TREX1* activity and that FCL

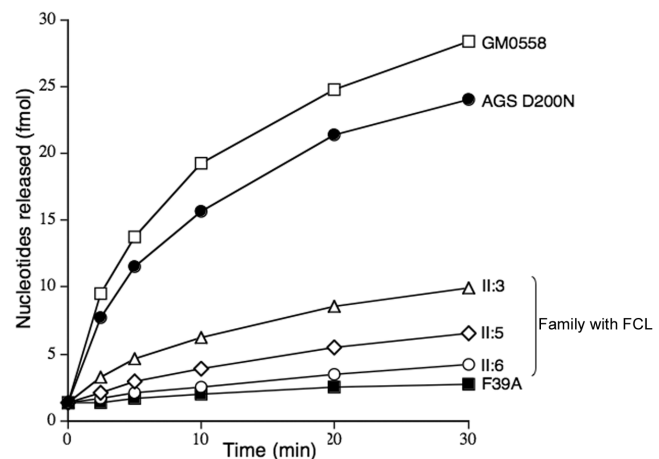


Figure 3. *TREX1* exonuclease activity. Cell-free protein extracts were assayed for 3' DNA exonuclease activity with a 3' labeled poly(dA) substrate after partial purification by column chromatography on single-stranded DNA cellulose. GM0558 is a normal lymphoblastoid control. F39A is a lymphoblastoid cell line from a child with biallelic mutations in *TREX1*.

Table 2. *TREX1* Enzyme Activity of Wild-Type and Recombinant F17S/F17S Homodimers

Dimer	Activity ^a	Relative Activity
Wild-type/wild-type	2.8	1
F17S/F17S	3.5	1.2

^a Measured as fmol nucleotides released per s.

can be due to *AGS1* mutations. In contrast, enzymatic analysis of the AGS-affected patient we describe here demonstrated close-to-normal activity in a standard exonuclease assay. On the basis of homology with TREX2, the aspartic acid at position 200 in TREX1 represents one of four residues essential for coordinating two magnesium ions involved in DNA binding and catalysis.¹⁸ It seems likely that D200N represents a gain-of-function mutation conferring altered substrate specificity, DNA binding, or protein-protein interactions; these would not be detected in the standard TREX1 exonuclease assay.

The observation of heterozygous *TREX1* mutations producing an enzymatic and clinical phenotype is novel and relevant. AGS has been considered an autosomal recessive disorder. Our ongoing genetic studies confirm that this is true in most cases, but we show here that AGS can also occur as a result of a de novo mutation in the *TREX1* gene, a finding with obvious implications for genetics counseling. No major reduction of enzyme activity was observed in the few *TREX1* heterozygotes tested elsewhere,⁶ and it has been considered that the parents of patients with AGS show no overt phenotype. However, a recent survey of AGS-affected families (Y.J.C., unpublished data) revealed that 4–40 parents experienced intermittent chilblains following exposure to cold temperatures, although none reported the severe and persistent skin lesions described here. The explanation for the severity of the lesions seen in the FCL-affected family we present remains uncertain, but the observation of nonpenetrance in one sibling is consistent with the lack of symptoms experienced by most subjects heterozygous for an AGS mutation. It is of interest that we know one parent of a patient with AGS who has



Figure 4. Skin features seen in the child with AGS due to a de novo heterozygous D200N *TREX1* mutation. Ulcerative lesions are seen at the ends of the toes and fingers, and there is a more generalized patchy mottling of the skin, seen here on the legs.

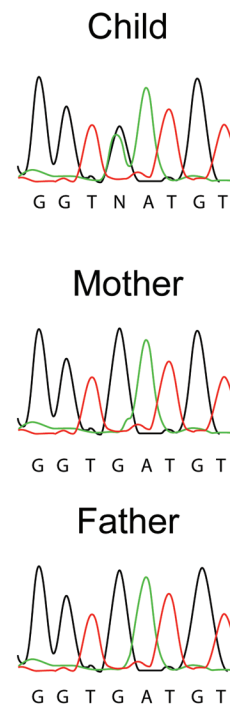


Figure 5. Sequence electropherograms illustrating the de novo c.598G→A *TREX1* mutation in the child affected with AGS.

SLE, and we suggest that a systematic study of such parents for biomarkers of SLE is warranted.

In view of the consistent association with elevated levels of IFN- α ,⁴ we predicted elsewhere that elucidation of the molecular basis of AGS would provide insights into the pathogenesis of SLE.⁸ Our recent finding in AGS-affected patients of mutations in genes encoding the three non-allelic components of the RNASEH2 endonuclease protein complex⁷ and in the 3'→5' exonuclease TREX1,⁶ together with the observation that TREX1 may be involved in caspase-independent apoptosis,¹⁹ adds weight to this prediction, since abnormalities of IFN- α metabolism²⁰ and apoptosis²¹ represent two central themes in the causation of lupus and other autoimmune diseases. We have now identified heterozygous *TREX1* mutations in a pedigree segregating a monogenic form of FCL. Of note in the context of AGS, mutations in DNase I are associated with a lupus phenotype,²² and mice deficient in DNase II accumulate undigested DNA in macrophages, with consequent IFN- β up-regulation, and recapitulate the clinical and immunological phenotype of rheumatoid arthritis.²³ We have suggested⁶ that AGS results from a failure of nuclease activities, with consequent accumulation of anomalous nucleic acid species triggering an IFN- α -mediated innate immune response. For these reasons, further evaluation of the specific biochemical defect(s) in TREX1 function underlying dominant AGS and/or FCL should give general insights into the etiology of inherited lupus.

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Web Resources

Accession numbers and URLs for data presented herein are as follows:

GenBank, <http://www.ncbi.nlm.nih.gov/GenBank/> (for TREX1 protein [accession numbers AAK07616 and NM_033627, with the A at 2986 as the first base of the initiating ATG codon])

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for AGS and FCL)

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