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Expanding the Phenotypic Spectrum of Lupus Erythematosus in Aicardi-Goutières Syndrome

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Objective. **Aicardi-Goutières syndrome (AGS) is an early-onset encephalopathy resembling congenital viral infection that is characterized by basal ganglia calcifications, loss of white matter, cerebrospinal fluid (CSF) lymphocytosis, and elevated interferon-α levels in the CSF. Studies have shown that AGS is an autosomal-recessive disease linked to mutations in 5 genes, encoding the 3′-repair DNA exonuclease 1 (*TREX1*), the 3 subunits of ribonuclease H2 (*RNASEH2A–C*), and sterile alpha motif domain and HD domain–containing protein 1 (*SAMHD1*). In this study we further characterized the phenotypic spectrum of this disease.**

Methods. **Clinical and laboratory data were obtained from 26 patients fulfilling the clinical diagnostic criteria for AGS. Genomic DNA was screened for mutations in all 5 AGS genes by direct sequencing, and sera were analyzed for autoantibodies.**

Results. **In 20 patients with AGS, 20 mutations, 12 of which were novel, were identified in all 5 AGS genes. Clinical and laboratory investigations revealed a high prevalence of features (some not previously described in patients with AGS) that are commonly seen in patients with systemic lupus erythematosus (SLE), such as thrombocytopenia, leukocytopenia, antinuclear antibodies, erythematous lesions, oral ulcers, and arthritis, which were observed in 12 (60%) of 20 patients with AGS. Moreover, the coexistence of AGS and SLE, was for the first time, demonstrated in 2 patients with molecularly proven AGS.**

Conclusion. **These findings expand the phenotypic spectrum of lupus erythematosus in AGS and provide further insight into its disease mechanisms by**

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showing that activation of the innate immune system as a result of inherited defects in nucleic acid metabolism could lead to systemic autoimmunity.

Aicardi-Goutières syndrome (AGS) was first described in 1984 in 8 children who presented with an early-onset progressive encephalopathy, characterized by basal ganglia calcifications, white matter abnormalities, and chronic cerebrospinal fluid (CSF) lymphocytosis (1). Familial occurrence of the disease suggested that it has an autosomal-recessive inheritance. Furthermore, the finding of raised levels of the antiviral cytokine interferon-α (IFNα) in the CSF of affected children implies that there are similarities between AGS and congenital viral infection, in spite of a lack of evidence to support common prenatal infections (2,3).

Affected children typically present with irritability, inconsolable crying, dystonia, hypotonia, and seizures. Symptoms occur in an episodic manner starting as early as the first day of life, leading to progressive microcephaly and spastic quadriplegia, with severe developmental delay. Extraneurologic manifestations include hepatosplenomegaly, thrombocytopenia, and chilblain lesions, which are inflammatory cutaneous lesions at acral locations (4). On the basis of symptoms described in 3 case reports, it was first suggested that there is clinical overlap of AGS with the autoimmune disease lupus erythematosus, as observed in patients who presented with lupus-like rash and antinuclear antibodies, in addition to progressive encephalopathy with intracranial calcifications (5–7). However, in a recent large study that included 123 patients with AGS, no further lupus-associated symptoms were observed, and only 6 patients were reported to have an abnormal antibody profile (8).

AGS is a genetically heterogeneous disorder, and to date, biallelic mutations have been identified in 5 genes, encoding the 3′-repair DNA exonuclease 1 (*TREX1*; in locus AGS1), the 3 subunits of the ribonuclease H2 complex (*RNASEH2B*, *RNASEH2C*, and *RNASEH2A* in loci AGS2, AGS3, and AGS4, respectively), and sterile alpha motif domain and HD domain–containing protein 1 (*SAMHD1*; in locus AGS5), which is a putative regulator of the innate immune system (9–11). Homodimeric *TREX1* functions as an intracellular DNA exonuclease with high specificity for single-stranded DNA. The *RNASEH2* complex constitutes an endonuclease that cleaves RNA with RNA:DNA hybrids or single ribonucleotides embedded within DNA duplexes. These findings suggest that an inappropriate activation of the innate immune response that occurs as a result of defects in nucleic acid metabolism may underlie the pathogenesis of AGS.

Heterozygous mutations in *TREX1* have also been described in patients with familial chilblain lupus, an autosomal-dominant form of cutaneous lupus erythematosus manifesting in early childhood (12–14), and in patients with autosomal-dominant retinal vasculopathy with cerebral leukodystrophy, an adult-onset disorder characterized by central nervous system degeneration, retinal vasculopathy, and nephropathy (15). Furthermore, we identified heterozygous mutations in *TREX1* in patients with sporadic systemic lupus erythematosus (SLE), further expanding the phenotypic spectrum of *TREX1* mutations (16). This is an intriguing finding, in view of the fact that both diseases, AGS and SLE, are characterized by the activation of interferon pathways (2,3,17,18).

TREX1 deficiency has been shown to impair DNA damage during granzyme A–mediated apoptosis (13). Thus, improper clearance of altered DNA may induce, through as yet unidentified intracellular sensors and signaling pathways, an immune-mediated inflammatory response. Moreover, recent evidence suggests that intracellular accumulation of single-stranded DNA in *TREX1*-deficient cells may be attributable to defects in the degradation of nucleic acids derived from chronic cell-cycle checkpoint activation or from endogenous retroviruses (19,20). Collectively, these findings underpin the importance of defects in intracellular nucleic acid metabolism as a possible pathogenic mechanism of systemic autoimmunity.

In this report, we present clinical and molecular data from 20 patients with AGS who were found to carry mutations in all 5 of the AGS genes known to date. Our results provide evidence that phenotypic features of lupus erythematosus are more prevalent in AGS than have hitherto been appreciated.

PATIENTS AND METHODS

Patients. Patients with a clinical presentation suggestive of AGS underwent molecular analysis of all 5 AGS genes. Patients were included in this study based on the following diagnostic criteria: 1) neurologic symptoms of an encephalopathy, 2) intracranial calcification, 3) absence of findings of common prenatal infections, and/or 4) a CSF white cell count of $\geq$5 white cells/mm^3 or raised levels of IFNα (>2 IU/liter) in the CSF. IFNα levels were measured using a cytopathic effect inhibition assay with vesicular stomatis virus. All mutation-negative patients and patients in whom only 1 mutation could be identified also fulfilled the diagnostic criteria outlined above.

A total of 26 patients of German, Turkish, Lebanese,

Table 1. Mutations in patients with AGS*

Family/ancestry	Consanguinity	Locus/gene	Nucleotide change	Protein change
1/Turkish	No	AGS1/*TREX1*	**c.150_151delTC** + c.907A>C	**Q51fsX100** + T303P
2/Turkish	Yes	AGS1/*TREX1*	c.341G>A, hom	R114H
3/European American	No	AGS1/*TREX1*	c.341G>A + c.500delG	R114H + S166fsX179
4/German	No	AGS1/*TREX1*	c.341G>A + **c.635delC**	R114H + **P212fsX276**
5/Turkish	Yes	AGS1/*TREX1*	**c.592G>A,** hom	**E198K**
6/German	No	AGS1/*TREX1*	**c.598G>C,** het, de novo†	**D200H**
7/Lebanese	Yes	AGS1/*TREX1*	**c.859_876del18,** hom	**L287_G292del**
8/Portuguese	Yes	AGS2/*RNASEH2B*	c.529G>A, hom	A177T
9/Sinti	Yes	AGS2/*RNASEH2B*	c.529G>A, hom	A177T
10/German	No	AGS2/*RNASEH2B*	c.529G>A, het‡	A177T
11/European American	No	AGS2/*RNASEH2B*	c.529G>A, het‡	A177T
12/European Canadian	No	AGS2/*RNASEH2B*	c.529G>A + **c.685T>C**	A177T + **S229P**
13/German	No	AGS3/*RNASEH2C*	**c.115G>T** + **c.343_344delGA**	**D39Y** + **D115fsX169**
14/Indian	Yes	AGS3/*RNASEH2C*	c.205C>T, hom	R69W
15/European Canadian	No	AGS4/*RNASEH2A*	**c.4G>T;8T>C** + c.716_717dupGC	**D2Y;L3P** + T240fsX316
16/Moroccan	Yes	AGS4/*RNASEH2A*	c.556C>T, hom	R186W
17/Turkish	Yes	AGS5/*SAMHD1*	**c.499C>T**, hom	**H167Y**
18/German	No	AGS5/*SAMHD1*	**c.869G>A** + c.1642C>T	**R290H** + Q548X

* Novel mutations in patients with Aicardi-Goutières syndrome (AGS) are indicated in boldface. For complementary DNA numbering, +1 corresponds to the A of the ATG initiation codon. *TREX1* = 3′-repair DNA exonuclease 1; hom = homozygous; het = heterozygous; *RNASEH2B* = ribonuclease H2 subunit B; *SAMHD1* = sterile alpha motif domain and HD domain–containing protein 1.
† Mutation was confirmed to be a de novo change according to genetic analyses.
‡ Only a single mutation was identified in AGS1–5.

Portuguese, Sinti, Hindi, or Moroccan origin were screened. All patients from Canada or the US were of European descent. After the patients provided their informed written consent, blood samples were obtained from all patients and, if available, also from their parents and siblings. Patients and their families were seen by the primary investigators or were referred to us by the pediatric neurologist responsible for the care of the patient. Clinical, neuroimaging, and laboratory data were obtained from the medical records, magnetic resonance imaging (MRI) scans, and computed tomography scans. All patients for whom serum was available were tested for autoantibodies (antinuclear antibodies [ANAs], anti–extractable nuclear antigen, anti–double-stranded DNA, anti–single-stranded DNA, anti-C1q, and anticardiolipin) and for their levels of complement (C3 and C4). Autoantibodies and complement were determined at the diagnostic laboratory of the Institute of Immunology, Technical University Dresden, according to standard procedures.

Mutation analysis. Genomic DNA and RNA were isolated from peripheral blood leukocytes using the QIAamp Blood Mini Kit and the RNeasy mini kit, respectively (Qiagen). Polymerase chain reaction (PCR) amplification of all coding exons and flanking intronic regions of *TREX1*/AGS1 (314 amino acids), *RNASEH2A*/AGS4 (299 amino acids), *RNASEH2B*/AGS2 (308 amino acids), *RNASEH2C*/AGS3 (164 amino acids), and *SAMHD1*/AGS5 (626 amino acids) was performed using gene-specific oligonucleotide primers (sequences available from the corresponding author upon request). Following verification of the PCR product size by agarose gel electrophoresis, amplicons were column purified and sequenced in both directions using fluorescently labeled dideoxy nucleotides on ABI 3100 or 3730 genetic analyzers (Applied Biosystems). Sequencing data were analyzed with Vector NTI software (Invitrogen) or Seqpilot software (JSI Medical Systems). Peripheral blood leukocytes from at least 100 unrelated healthy individuals were also sequenced, to confirm the absence of newly identified missense changes in healthy individuals.

RESULTS

Molecular findings. Of the 26 patients fulfilling the clinical diagnostic criteria for AGS, 20 patients from 18 families were found to harbor mutations in 1 of the 5 AGS genes known to date. Altogether, we identified 20 mutations in AGS1–5, 12 of which were novel mutations (Table 1). Seven families (39%) carried mutations in *TREX1*/AGS1, and 3 of these were either homozygous or compound heterozygous for a common substitution (R114H) (9). One patient carried a novel heterozygous missense mutation, D200H, that was confirmed to be de novo based on parental genotypes. In this patient, the presence of a second wild-type allele could be confirmed based on the finding of heterozygosity for a common single-nucleotide polymorphism (SNP) at c.531 in *TREX1*.

Five families (28%), of various ethnic origins, carried mutations in *RNASEH2B*/AGS2, including a novel missense mutation, S229P, and a recurrent substitution, A177T (9). Two families (11%) harbored mutations in *RNASEH2C*/AGS3. One patient, of Indian origin, was homozygous for an R69W substitution, which was previously reported to occur on a common haplo-

Table 2. Phenotypic characteristics of the patients with AGS*

Family/age/sex	Presentation	Laboratory findings	Additional features
1/4 years/M	Dystonia, hypotonia, MC, SZ, death at 4 years	Thrombocytopenia	Septicemia
2/1 year/M	Neonatal SZ, HSM, dystonia, MC	ALFT, thrombocytopenia	None
3/1 year/M	Multiple purpuric lesions at birth, HSM, MC, dystonia, newborn SZ, death at 14 months	Thrombocytopenia, leukocytopenia, normal levels of IFNα and PT in CSF	ASD, hearing loss, retinal hemorrhages, corneal clouding
4/3 months/F	Dystonia, hypotonia, MC, SZ	Elevated levels of IFNα, protein, and PT in CSF, ALFT, thrombocytopenia	Nystagmus, hearing loss
5/19 years/M	Marked startle reaction, dystonia, MC, refractory SZ, SE	Elevated lactate levels, protein levels, and cell count in CSF, ANA 1:1,280, anti-ENA, decreased C3 levels	Nystagmus, duplicated kidney, contractures, neuropathic scoliosis, chilblain lesions on feet, oral ulcers
6/2 years/F	Dystonia, hypotonia, MC	ALFT, ANA 1:1,280, anti-dsDNA, anti-C1q, anticardiolipin IgG	Chilblain lesions on fingers/toes/nose, oral ulcers
7/14 years/M	Dystonia, severe DD	NA	None
8/26 years/F†	Dystonia, severe DD, MC	NA	None
8/20 years/F†	Dystonia, moderate DD, MC	NA	None
9/3 years/F	Recurrent fever, dystonia, MC, SZ, SE, moderate DD (evident after vaccination)	CSF lymphocytosis, normal IFNα and PT levels in CSF, leukocytopenia, anticardiolipin IgM	None
10/4 years/M	Dystonia, severe DD, MC	NA	None
10/12 years/F	Dystonia, severe DD, MC	NA	None
11/3 years/M	Dystonia, severe DD	NA	None
12/1 year/M	SZ, spastic quadriparesis	NA	None
13/1 year/M	MC, dystonia, marked startle reaction (evident after vaccination)	Elevated IFNα levels, lactate levels, cell count, and PT levels in CSF, anti-ssDNA	None
14/6 months/M	Newborn SZ, hypotonia, dystonia, MC	Thrombocytopenia	Blindness, nystagmus, hearing loss, arrhythmia
15/19 years/M†	Hypotonia, refractory SZ, spastic quadriparesis, MC	CSF lymphocytosis	None
15/20 years/M†	Moderate DD, no MC	CSF lymphocytosis	Chilblain lesion on toe
16/6 months/F	HSM, newborn SZ, dystonia, MC	Elevated IFNα levels and lymphocytosis in CSF, ALFT	Intestinal atresia, VSD
17/8 months/F	Preterm, intrauterine brain calcification, HSM, hypotonia, dystonia, MC	Thrombocytopenia, elevated cell count, elevated IFNα and PT levels in CSF	None
18/11 years/M	Newborn SZ, refractory SZ, SE, dystonia, MC, marked startle reaction, recurrent fever, spastic quadriparesis	Thrombocytopenia, leukocytopenia, elevated IFNα levels and lymphocytosis in CSF, normal PT levels in CSF, ANA 1:160	Cortical blindness, hearing loss, arthritis in knees/finger, chilblain lesions on finger/toes, oral ulcers
19/1 year/F	Hypotonia, dystonia, MC	Elevated IFNα levels and lymphocytosis in CSF, decreased C4 levels	None
20/8 months/F	Hypotonia, dystonia, severe DD, SZ, MC	CSF lymphocytosis, thrombocytopenia, leukocytopenia	None
21/3 years/F	Hypotonia, dystonia, moderate DD, MC	Elevated IFNα levels in CSF, ALFT, thrombocytopenia, ANA, 1:1,250, anti-ENA, anti-dsDNA	Arthritis
22/2 months/M	Hypotonia, dystonia, MC	Elevated IFNα levels in CSF, thrombocytopenia, leukocytopenia	None
23/3 months/M	HSM, hypotonia, dystonia, death at 3 months	CSF lymphocytosis, ALFT, thrombocytopenia, leukocytopenia	Septicemia
24/1 year/F	Hypotonia, dystonia, SZ, MC	Unremarkable	None

* All patients with Aicardi-Goutières syndrome (AGS) presented with basal ganglia calcifications and various degrees of white matter defects and brain atrophy. In patients from families 19–24, no mutations in any of the 5 AGS genes were identified. MC = microcephaly; SZ = seizures; HSM = hepatosplenomegaly; ALFT = abnormal liver function test (results); IFNα = interferon-α; PT = pterins; CSF = cerebrospinal fluid; ASD = atrial septal defect; SE = status epilepticus; ANA = antinuclear antibodies; anti-ENA = autoantibodies against extractable nuclear antigen; anti-dsDNA = autoantibodies against double-stranded DNA; DD = developmental delay; NA = not available; anti-ssDNA = autoantibodies against single-stranded DNA; VSD = ventricular septal defect.
† Members of same sibship.

type in Pakistani patients (9). A further patient, of German origin, was compound heterozygous for 2 novel mutations, including the first frameshift change (D39Y + D115fs) observed in *RNASEH2C*. Mutations in *RNASEH2A*/AGS4 were detected in 2 families (11%). In 1 family, 2 novel missense changes on the same allele were found, along with a previously described 2-basepair duplication on the other allele (D2Y;L3P + T240fsX316). One patient carried a homozygous missense change, R186W, which has previously been reported as a single mutation in AGS4 in a patient with AGS (8). Two patients (11%) harbored mutations in *SAMHD1*/AGS5, including a Turkish patient with a novel homozygous missense change, H167Y, and 1 German patient who was compound heterozygous for a novel missense mutation, R290H, and a previously reported nonsense mutation in *SAMHD1* (11). In the 3 families in which only 1 mutation could be detected, mutations in the other known AGS genes were excluded.

None of the newly identified missense changes was annotated as an SNP in the public databases, and all of the mutations were absent in at least 200 control alleles from healthy individuals. With the exception of the 1 case in which a heterozygous de novo *TREX1* mutation was identified, all parents for whom there were available data were heterozygous for a single mutation. In 3 of the 6 patients in whom no mutations could be identified on a genomic level (families 20, 22, and 23 in Table 2), sequencing of the complementary DNA of all 5 AGS genes did not provide any evidence of aberrant messenger RNA as a result of undetected intronic variants affecting splicing.

Clinical findings. With 1 exception, all patients carrying mutations in AGS1–5 were born at term. One child with intrauterine growth retardation and brain calcification was born at 35 weeks of gestation. In most cases, the birth weight and head circumference were within normal limits. Five children (2 with *TREX1* mutations and 1 each with mutations in *RNASEH2C*, *RNASEH2A*, or *SAMHD1*) presented, within the first days of life, with neonatal seizures, hepatosplenomegaly, thrombocytopenia, or feeding problems (Table 2). All other patients experienced, within the first weeks to 6 months of life, a sudden-to-subacute onset of severe encephalopathy, characterized by irritability, inconsolable crying with insomnia, and a loss of motor skills during the acute episode. These episodes usually lasted several weeks and were followed by a more stable phase, without further deterioration. Prior to the onset of symptoms, all patients thrived and developed normally. In 2 cases, symptoms became evident within 1–2 weeks after the first vaccination with a combination vaccine against diphtheria, pertussis, *Haemophilus influenzae* type b, tetanus, polio, and hepatitis B, occurring at the age of 3 months in both patients.

Neurologic signs were characterized by peripheral spasticity with paroxysmal dystonic movements, truncal hypotonia, and lack of head control. Some patients developed severe contractures secondary to spastic quadriparesis. Seizures were reported in 11 (55%) of 20 patients with molecularly proven AGS, and these comprised either neonatal seizures, febrile seizures, refractory epilepsy, or status epilepticus. Two children demonstrated a marked startle reaction to sudden acoustic stimuli, and 2 presented with intermittent sterile pyrexias. Severe developmental delay was seen in almost all of the patients and was associated with progressive microcephaly.

Apart from 2 children with biallelic *TREX1* mutations who died at 14 months and 4 years of age, respectively, all patients with molecularly proven AGS are currently alive, 8 of whom are older than 10 years of age, with the oldest being 26 years. Teenaged or adult patients were relatively more common among those with *RNASEH2B*/AGS2 or *RNASEH2A*/AGS4 mutations. These patients also tended to be older at the time of symptom manifestation. Although developmental delay was an invariable finding, it appeared less severe in these patients, and at least 2 children with *RNASEH2B* mutations and 1 with *RNASEH2A* mutations were able to walk and talk. In addition, a significant phenotypic variability was noted in the 3 families with 2 affected children; one of these families had 1 child with severe impairment and the other child with only moderate developmental delay or no microcephaly.

Hearing loss was reported in 4 cases, and ophthalmologic findings, varying from retinal hemorrhages with corneal clouding to cortical blindness, were noted in 3 patients. Chilblain lesions were observed in 4 patients (20%), of whom 2 carried mutations in *TREX1* (Figures 1C–E), 1 carried mutations in *RNASEH2A*, and 1 carried mutations in *SAMHD1*. Three patients presented with intermittent oral ulcers. These lesions were mainly located in the buccal mucosa but also extended into the pharyngeal region in 1 patient. A lesional biopsy was performed in 1 patient, with the findings revealing nonspecific signs of inflammation. Additional cutaneous findings included purpuric lesions due to severe thrombocytopenia, occurring at birth in 1 patient. One patient with *SAMHD1* mutations developed, at the age of 6 years, recurrent rheumatoid factor–negative arthritis of the knees and proximal and middle phalangeal joints,

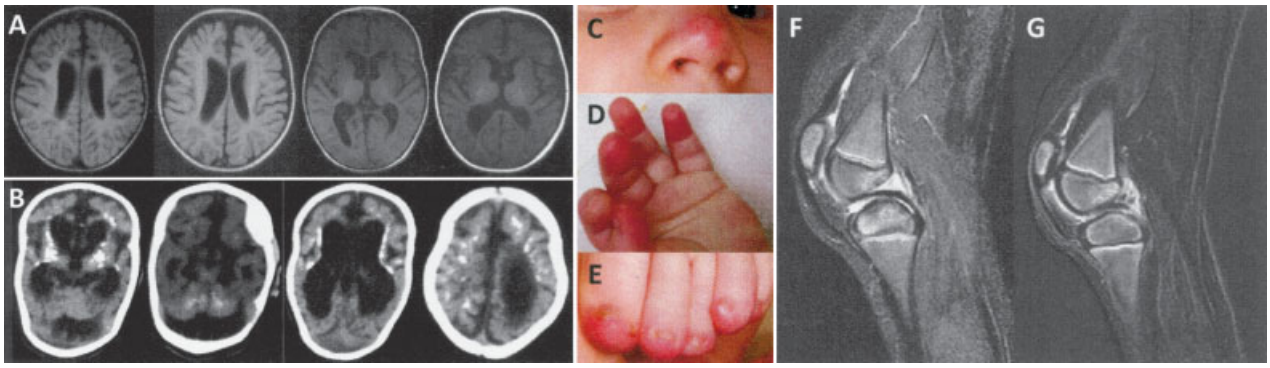

Figure 1. Imaging findings and cutaneous signs in patients with Aicardi-Goutières syndrome (AGS). **A,** Magnetic resonance imaging of an AGS patient with mutations in ribonuclease H2 subunit C (*RNASEH2C*)/AGS3 (patient 13), showing extensive white matter abnormalities with an anteroposterior gradient, in addition to cortical atrophy. **B,** Computed tomography imaging of an AGS patient with *RNASEH2A*/AGS4 (patient 16), demonstrating marked brain atrophy with prominent cerebellar atrophy, as well as symmetric calcifications of the basal ganglia and periventricular region extending throughout the cerebrum. **C–E,** Typical chilblain lesions on the nose, fingertips, and toes of an AGS patient with a heterozygous de novo 3′-repair DNA exonuclease 1 (*TREX1*) mutation (patient 6), presenting as painful erythematous swellings. **F,** Sagittal STIR sequence through the left knee of an AGS patient with mutations in sterile alpha motif domain and HD domain–containing protein 1 (*SAMHD1*)/AGS5 (patient 18), showing effusion and thickened synovia without surrounding soft tissue edema or bone erosion. **G,** Subtracted T1-weighted scan, after contrast media application, of the left knee of an AGS patient with *SAMHD1*/AGS5 (same patient as in **F**), showing marked contrast enhancement of the synovia, especially adjacent to the posterior cruciform ligament, indicating features compatible with a diagnosis of nonerosive arthritis.

which responded to therapy with prednisone and azathioprine. Infrequent features included congenital heart defects, renal malformation, and intestinal atresia. The clinical features of the 6 mutation-negative patients are also presented in Table 2.

Imaging findings. Intracranial calcifications involving the basal ganglia and loss of white matter, as well as atrophic changes, were observed in all patients, albeit with great variability (Figures 1A and B). In some cases, calcifications were evident in the newborn period, presenting as a few calcified spots or confluent symmetric lesions extending into the deep white matter. White matter abnormalities ranged from discrete hyperintensities on T2-weighted MRI to severe frontotemporal leukodystrophy. In 1 patient with *SAMHD1* mutations, bitemporovacuolar lesions were also observed. While cortical atrophy was a common feature, several children demonstrated marked brain atrophy with brainstem and cerebellar atrophy, dilatation of ventricles, and thinning of the corpus callosum. In 1 patient with *SAMHD1* mutations and recurrent arthritis, MRI of the knee demonstrated effusion and thickening of the synovia, consistent with the characteristics of nonerosive arthritis (Figures 1F and G).

Laboratory findings. All patients whose CSF was analyzed underwent lumbar puncture within 3 months of initial presentation of the leukencephalopathy and/or seizures. An elevated white cell count in the CSF was found in 8 (80%) of the 10 patients tested (Table 2), and when mutation-negative patients were included, 11 (73%) of 15 showed an elevated white cell count in the CSF. Levels of intrathecal IFNα were raised in 5 (71%) of the 7 patients tested; with inclusion of mutation-negative patients, 8 (80%) of 10 had elevated IFNα levels in the CSF. In 1 severely affected child with biallelic *TREX1* mutations, findings in the CSF were unremarkable. Four children were found to have lymphocytosis in the absence of elevated IFNα levels, or vice versa. Elevated pterin levels were recorded in 3 (50%) of 6 patients. Thrombocytopenia ($<$100,000/μl) was observed in 7 (35%) of 20 patients, and leukocytopenia ($<$4,000/μl or counts lower than the age-specific third percentile on 2 or more occasions) was observed in 3 (15%) of 20 patients. With mutation-negative patients included in these analyses, 11 (42%) of 26 patients showed thrombocytopenia, and 6 (23%) of 26 presented with leukocytopenia.

Five (71%) of 7 patients who were tested for serologic signs of systemic autoimmunity were found to have ANAs or autoantibodies against extractable nuclear antigens, double-stranded or single-stranded DNA, C1q, and cardiolipin or to have reduced complement levels (Table 2). When mutation-negative patients were included, 7 (78%) of 9 patients were autoantibody positive or showed reduced complement levels (Table 2). In all patients, titers of autoantibodies were determined beyond the first acute neurologic phase, and the

oldest patient was 19 years of age at the time of the examinations.

DISCUSSION

In this report, we present clinical and molecular data from a cohort of patients with AGS that further define the phenotypic and genotypic spectrum of AGS and establish a firm link between AGS and lupus erythematosus at the clinical, molecular, and biochemical levels. The most common AGS subtype in our series was *TREX1*/AGS1, followed by *RNASEH2B*/AGS2. The remaining subtypes, *RNASEH2C*/AGS3, *RNASEH2A*/AGS4, and *SAMHD1*/AGS5, were observed with equal frequencies. Although a potential role of pathogenic mutations in regulatory regions or larger deletions affecting gene expression or splicing could not be fully excluded, the lack of detection of mutations in any of the known AGS genes in the 6 patients with a clinical presentation highly suggestive of AGS may point to the existence of at least 1 additional AGS gene.

Consistent with previous reports, the neurologic phenotype was characterized by the subacute onset of severe encephalopathy with irritability, dystonia, hypotonia, and seizures, resulting mostly in severe developmental delay and microcephaly (1). All children in our cohort presented with this phenotype within the first year of life. In most cases, symptoms occurred after a period of apparently normal development, without evidence of an environmental trigger, while for some, the onset of symptoms was associated with a vaccination. Although no further disease progression beyond the encephalopathic period was observed in most patients, several children were reported to experience continued episodes of irritability, inconsolable crying, and insomnia during later years. Overall, the clinical features among patients with AGS1–5 were indistinguishable, although neonatal onset or more severe abnormal neurologic findings were more frequent among patients with *TREX1*/AGS1 and *SAMHD1*/AGS5, whereas patients with *RNASEH2B*/AGS2 tended to be less severely affected.

On the basis of 3 case reports, it was first suggested that there is clinical overlap of AGS with lupus erythematosus, as observed in patients who presented with lupus-like rash and ANAs, in addition to progressive encephalopathy with intracranial calcifications (5–7). However, in the largest case series on AGS to date, which involved 123 patients, thrombocytopenia was observed in only 15 patients (12%), and only 6 patients (<5%) were reported to have an abnormal antibody profile, without further details as to the antigens involved or the number of patients examined for the presence of autoantibodies (8). In this study, apart from chilblain lesions, no further symptoms associated with lupus erythematosus were described (8). Likewise, in 3 previous studies of patients with AGS (involving 21 patients, 11 patients, and 10 patients, respectively), no further lupus-associated symptoms were noted, and in 2 of these studies, the results of testing for ANA or complement deficiency were reported to be negative (21–23). This is in contrast to the results reported here, in which 60% of the patients (12 of 20) presented with clinical findings (lupus rash, arthritis, oral ulcers) or laboratory findings (ANAs, autoantibodies to extractable nuclear antigens, reduced complement levels, thrombocytopenia, leukocytopenia) commonly seen in patients with lupus erythematosus.

If seizures are taken into account as the phenotypic expression of cerebral lupus, the proportion of patients showing signs of lupus erythematosus in this study would rise to 75% (15 of 20 patients). In fact, the neurologic phenotypes of AGS and SLE show intriguing similarities. Thus, seizures, which are commonly observed in patients with AGS, constitute a diagnostic criterion for SLE. Moreover, neuroimaging findings in patients with SLE include calcifications, white matter changes, and atrophy, which are typically observed in patients with AGS (24–26). One may therefore speculate about common pathogenic mechanisms underlying the neurologic phenotype of AGS and that of cerebral lupus, although this notion requires further investigation.

Novel lupus-associated manifestations that were observed, but previously not described in patients with AGS, included leukocytopenia, oral ulcers, and arthritis, which constitute some of the diagnostic criteria for SLE. In fact, 2 patients, 1 of whom was carrying a heterozygous de novo *TREX1* mutation and 1 of whom was carrying biallelic *SAMHD1* mutations, presented with at least 4 of the 11 diagnostic criteria formally required to establish the diagnosis of SLE, demonstrating, for the first time, the coexistence of AGS and SLE in patients with molecularly proven AGS (27). Of note, nonerosive arthritis was observed in a patient with biallelic *SAMHD1* mutations. However, determining whether arthritis represents a manifestation specific for the AGS5 subtype must await further studies.

It is of interest, in this context, that familial chilblain lupus, a monogenic form of cutaneous lupus erythematosus manifesting in early childhood, is also caused by heterozygous mutations in *TREX1* (12–14).

Chilblain lupus is characterized by erythematous lesions at acral locations, which are precipitated by cold exposure and resemble the chilblain lesions seen in AGS (4,12). However, unlike patients with AGS, patients with familial chilblain lupus do not show any neurologic phenotype, even at old age (12,28). Heterozygous mutations in *TREX1* have also been described in patients with autosomal-dominant retinal vasculopathy with cerebral leukodystrophy, an adult-onset disorder characterized by central nervous system degeneration, retinal vasculopathy, and nephropathy (15). Furthermore, we identified rare variants in *TREX1* conferring a high relative risk for developing SLE in patients with sporadic SLE, further expanding the phenotypic spectrum of systemic autoimmunity due to *TREX1* mutations (16).

In 1 patient, the symptoms of AGS and SLE were attributed to a heterozygous de novo *TREX1* mutation (D200H), which affects 1 of 4 magnesium-coordinating residues required for catalytic function (29). Interestingly, the only other heterozygous de novo *TREX1* mutation reported so far in a patient with AGS affects the same amino acid residue (D200N) (14). These findings may suggest a distinct role for this residue, presumably during recognition, binding, or processing of nucleic acid, and may indicate that these de novo mutations act in a dominant-negative manner.

Our findings suggest that serologic parameters indicative of systemic autoimmunity, such as ANAs, may represent valuable diagnostic markers for AGS. This is of particular relevance during the initial diagnostic evaluation of an infant who presents with an encephalopathy suggestive of AGS, because early diagnosis of AGS may potentially have a significant impact on clinical management with immune-modulating agents. With all due caution regarding the neurotoxicity of immunosuppressive agents, one may speculate that early immune-modulating intervention during the encephalopathic phase may be helpful in reducing permanent brain damage.

The nucleases *TREX1* and *RNASEH2*, as well as *SAMHD1*, constitute putative components of the innate immune system. Although their exact molecular functions remain not fully understood, current data suggest that they are involved in the removal of nucleic acid species produced during apoptosis, chronic cell-cycle checkpoint activation, or propagation of endogenous retroviruses, and that a failure of these processes results in an inappropriate activation of the innate immune system (11,13,19,20). Thus, the elucidation of the genetic causes of AGS has revealed a novel functional relationship between intracellular nucleic acid metabolism, nucleic acid recognition, and the activation of an innate immune response, underscoring the importance of nucleic acid metabolism for mechanisms of antiviral immune defense and tolerance. Elevated levels of the antiviral cytokine IFNα in the CSF are a hallmark of AGS, and emerging molecular and genetic evidence suggests an important role of interferon pathways in lupus pathogenesis (17,18,30). The coexistence of AGS and SLE in the same patient strongly supports common pathogenic mechanisms for these 2 diseases. Thus, the phenotypic spectrum of lupus erythematosus in AGS defines this monogenic disorder as a novel facet of the immunological disease continuum (31), which paradigmatically highlights the interplay between the innate and the adaptive immune systems in the pathogenesis of systemic autoimmunity.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Lee-Kirsch had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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