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Genetic, Phenotypic, and Interferon Biomarker Status in ADAR1-Related Neurological Disease

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Abstract

We investigated the genetic, phenotypic, and interferon status of 46 patients from 37 families with neurological disease due to mutations in *ADAR1*. The clinicoradiological phenotype encompassed a spectrum of Aicardi–Goutières syndrome, isolated bilateral striatal necrosis, spastic paraparesis with normal neuroimaging, a progressive spastic dystonic motor disorder, and adult-onset psychological difficulties with intracranial calcification. Homozygous missense mutations were recorded in five families. We observed a p.Pro193Ala variant in the heterozygous state in 22 of 23 families with compound heterozygous mutations. We also ascertained 11 cases from nine families with a p.Gly1007Arg dominant-negative mutation, which occurred de novo in four patients, and was inherited in three families in association with marked phenotypic variability. In 50 of 52 samples from 34 patients, we identified a marked upregulation of type I interferon-stimulated gene transcripts in peripheral blood, with a median interferon score of 16.99 (interquartile range [IQR]: 10.64–25.71) compared with controls (median: 0.93, IQR: 0.57–1.30). Thus, mutations in *ADAR1* are associated with a variety of clinically distinct neurological phenotypes presenting from early infancy to adulthood, inherited either as an autosomal recessive or dominant trait. Testing for an interferon signature in blood represents a useful biomarker in this context.

Keywords

Aicardi–Goutières syndrome; bilateral striatal necrosis; spastic paraparesis; dystonia; idiopathic basal ganglia calcification

Introduction

Adenosine deaminases acting on RNA (ADARs) catalyze the hydrolytic deamination of adenosine to inosine in double-stranded RNA, and thereby potentially alter the information content and structure of cellular RNAs.[1] ADAR1 is encoded by a single-copy gene that

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Authors' Contributions

J.H.L. and Y.J.C. collated and reviewed all clinical and radiological data. G.I.R. performed quantitative PCR analysis, with assistance from N.K., M.B., T.A.B., A.C.E.B., M.L.C., A. M.C., C.C.,R.C.D., F.R.D.,N.D., B.De A., V.De G., C.G.E.L. De G.,I. D., C De L., A.E., M.C.F., P.F., A.F., E.F., M.P.G., N.R.G., M.H., M.A. K., N.L., J.-P.S.-M.L., M.A.L., S.S.M., R.M., L.M.-S., G.M., M.M., V. N., S.O., J.D.O.-E., B.P.-D., F.P., K.M.R., M.R., F.R., P.R.-P., A.R., T.I. S., M.B.T., A.T., F.U., N.U., A.V., and A.W. provided clinical samples and critically reviewed clinical and immunological patient data. Y.J.C. conceived the study and wrote the initial draft with the assistance of G.I.R. All authors critically reviewed the article and agreed to its publication.

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maps to human chromosome 1q21 and is present in two main isoforms in mammalian cells. In mice, a loss of ADAR1 activity leads to a dramatic upregulation of interferon-stimulated gene (ISG) expression, which is dependent on the editing activity of ADAR1 and specific to the interferon-inducible full-length p150 isoform of the protein.[2–4]

In 2012, we reported mutations in *ADAR1* to cause a phenotype consistent with the infantile encephalopathy Aicardi–Goutières syndrome (AGS), and demonstrated that, similar to the *ADAR1* null mouse, the mutant genotype was associated with an upregulation of type I interferon signaling.[5] Further to this, in 2014, we described both bilateral striatal necrosis (BSN), sometimes occurring after a trivial childhood infection, and otherwise nonsyndromic, slowly progressive spastic paraparesis associated with normal intellect occur due to ADAR1 dysfunction, again in association with the enhanced expression of type I interferon-induced gene transcripts.[6–8] These data indicate that neurological disease can occur through inappropriate induction of the innate immune system by self-derived nucleic acids.

Here, we present an update of our experience of screening for *ADAR1* mutations, describing the clinical, radiological, molecular, and interferon biomarker characteristics of a cohort of 46 patients from 37 families with neurological dysfunction due to mutations in *ADAR1*.

Materials and Methods

Patients and Methods

We ascertained clinical and molecular data through direct contact and/or via collaborating physicians. The study was approved by the Leeds (East) Research Ethics Committee (reference number 10/H1307/132), and the Comité de Protection des Personnes (ID-RCB/EUDRACT: 2014-A01017-40).

A diagnosis of AGS was suggested by characteristic clinical and neuroimaging features including cerebral atrophy, white matter disease, and intracranial calcification.[9] BSN was diagnosed in the context of an acute or subacute onset of a dystonic/rigid motor disorder associated with magnetic resonance imaging features of bilateral striatal signal change with or without swelling. Spastic paraparesis/tetraparesis and spastic dystonia were diagnosed according to clinical signs, in the presence of either normal neuroimaging or mild nonspecific changes sometimes including calcification of the basal ganglia. Assessment of the motor and communication status of patients over the age of 1 year was made using the Gross Motor Function Classification System (GMFCS),[10] the Manual Ability Classification System (MACS),[11] and the Communication Function Classification System (CFCS).[12]

Mutational Analysis

Primers were designed to amplify the coding exons of *ADAR1* (Supplementary Table S1, online-only). Purified polymerase chain reaction (PCR) amplification products were sequenced using BigDye terminator chemistry and an ABI 3130 DNA sequencer. Mutation description is based on the reference cDNA sequence NM_001111.4, with nucleotide numbering beginning from the first A in the initiating ATG codon. Variants were assessed using the in silico programs SIFT (http://sift.jcvi.org) and Polyphen2 (http://

genetics.bwh.harvard.edu/pph2/), and population allele frequencies obtained from the ExAC (http://exac.broadinstitute.org) and gnomAD (http://gnomad.broadinstitute.org) databases.

Interferon Score

Whole blood was collected into PAXgene tubes, total RNA extracted using a PreAnalytix RNA isolation kit and RNA concentration assessed using a spectrophotometer (FLUOstar Omega, Labtech). Quantitative reverse transcription PCR (qPCR) analysis was performed using the TaqMan Universal PCR Master Mix (Applied Biosystems), and cDNA derived from 40 ng total RNA. Using TaqMan probes for *IFI27* (Hs01086370_m1), *IFI44L* (Hs00199115_m1), *IFIT1* (Hs00356631_g1), *ISG15* (Hs00192713_m1), *RSAD2* (Hs01057264_m1), and *SIGLEC1* (Hs00988063_m1), the relative abundance of each target transcript was normalized to the expression level of *HPRT1* (Hs03929096_g1) and *18S* (Hs999999001_s1), and assessed with the Applied Biosystems StepOne Software v2.1 and DataAssist Software v.3.01. For each of the six probes, individual data were expressed relative to a single calibrator. Relative quantification is equal to $2^{-\Delta\Delta Ct}$ that is, the normalized fold change relative to the control data. The median fold change of the 6 genes compared with the median of 29 previously collected healthy controls is used to create an interferon score for each individual, with an abnormal interferon score being defined as greater than +2 standard deviations above the mean of the control group, that is, 2.466.

Results

Molecular Data

We collected data on 46 patients from 37 families of pan-ethnic origin with either biallelic mutations in *ADAR1* (28 families) or the single known dominant-negative mutation p. Gly1007Arg (nine families) (Table 1; Fig. 1). In four families, the p.Gly1007A mutation was considered to have occurred de novo, while in three families, inheritance was confirmed or inferred (two paternal half-siblings born to an unaffected father unavailable for testing), with somatic mosaicism recorded in one case. In two families, inheritance could not be determined because DNA from both parents was not available. We observed three distinct homozygous mutations in five families (two families each sharing the same mutation), in four of which the parents were knowingly related. All of these mutations were missense. Of 23 families with compound heterozygous mutations, 22 carried the p. Pro193Ala mutation on one allele. In 13 of 22 families segregating this p.Pro193Ala substitution, the second molecular lesion was a null or splicing variant.

Clinical Radiological Phenotype

Clinical radiological characteristics of all patients are summarized in Table 2, and characteristic radiological appearances are summarized in Fig. 2. Median age of disease onset was 14 months (range: birth–30 years). We observed 21 and 25 affected females and males, respectively. Although spasticity and dystonia were common features present in the majority of patients, clinically and radiologically distinct phenotypes could be defined, including classical AGS (15 patients), BSN (16 patients), apparently isolated spastic paraparesis (1 patient)/tetraparesis (2 patients), and a progressive spastic dystonic motor disorder (7 patients). In two of these latter cases, the initial presentation was of isolated

lower limb spasticity, with a dystonic component and involvement of the upper limbs only becoming evident several years later. Four patients demonstrated radiological features of both AGS and BSN. The mother of a child with an AGS presentation was diagnosed at the age of 30 years with subtle psychological features and marked intracranial calcification. We identified three patients with significant neurological disease (a spastic/dystonic phenotype) in the absence of changes on brain imaging at presentation.

A total of 25 patients were considered to have demonstrated normal development prior to disease onset, in 18 of whom there was a history of either vaccination (4 patients) or a notable infectious episode (14 patients) in the period shortly preceding the development of clinical signs (Fig. 3A). Several patients experienced a rapid onset of dystonia/spasticity and loss of skills, with two patients being admitted to intensive care due to severe dystonic crisis. Others exhibited a more slowly progressive onset over weeks or months. Definite clinical progression beyond the initial presentation was recorded in 16 cases. Nine patients are deceased, between the ages of 10 months and 19 years, six of whom had early-onset disease consistent with AGS.

An assessment of gross motor function, manual ability, and communication status at last contact was made in 45 patients, of whom 27 were recorded to have none of any purposeful gross motor, hand and communication function (score of 5 on all three scales) (Fig. 3B). Five patients were able to walk with no or some support (GMFCS I–III). Eleven patients were capable of effective sender and receiver communication (CFCS I–III). Although formal testing was not undertaken, seven patients were considered to have normal intellectual function.

Five patients were reported to demonstrate hypo/hyper-pigmentation consistent with dyschromatosis symmetrica hereditaria (DSH) 1, and two patients were described with chilblain-like vasculitic lesions. Four patients were documented with autoimmune hemolytic anemia. Glaucoma was not recorded in any patient.

Interferon Status

We derived 52 interferon scores from 34 patients, 50 of which were abnormal, with a median interferon score across the group of 16.99 (interquartile range [IQR]: 10.64–25.71) compared with controls (median: 0.93, IQR: 0.57–1.30) (Fig. 4). Positive scores were observed up to 25 years after disease onset. We also tested 20 interferon scores from 16 parental carriers of a recessive mutation in *ADAR1*. Two samples from seven parents heterozygous for the recurrent p.Pro193Ala mutation demonstrated a positive interferon score, versus six samples from nine parents carrying a different mutation (Supplementary Fig. S1, online-only).

Discussion

In 2012, *ADAR1* mutations were described in the context of the early-onset encephalopathy AGS, associated with the presence of intracranial calcification, white matter disease, and severe developmental delay.[5] Subsequently, in 2014, mutations in *ADAR1* were also shown to underlie cases of apparently nonsyndromic BSN, and of isolated spastic para-paresis with

normal neuroimaging.[6,7] Here, we confirm these associations, thus emphasizing the need to consider ADAR1-related disease in several distinct clinical scenarios triggering different investigative algorithms. Furthermore, we now describe a patient with a dominant-negative mutation in *ADAR1* demonstrating an adult-onset phenotype evocative of "idiopathic" basal ganglia calcification characterized by intracranial calcification and subtle psychological disturbance. Our clinical and radiological findings highlight the propensity of ADAR1-related disease to incur basal ganglia dysfunction, and the value of basal ganglia calcification, frequently only appreciated on computed tomography, as a diagnostic indicator. In general, mutations in *ADAR1* should be considered in the context of a motor disorder characterized by spasticity and dystonia. The onset of disease can occur after a period of normal development, sometimes associated with a rapid loss of skills, or a much slower progression over many years. Assessments using the GMFCS, MACS, and CFCS rating scales indicate that disease outcome in the cases that we have ascertained is frequently severe. It is of note that we observed cases with completely preserved intellect +/− normal neuroimaging in the face of significant motor disability.

Our own research focus is biased toward the ascertainment of pediatric disease. However, Tojo et al described a female patient with the dominant-negative p.Gly1007Arg mutation, presenting at the age of 17 years with gait disturbance and dystonic posturing of the legs, who experienced intellectual deterioration from 21 years of age, and became wheelchair bound a year later.[13] Together with our observation of an adult female whose clinical phenotype only became evident at the age of 30 years, it is clear that later onset disease can occur due to ADAR1 deficiency. This latter case also illustrates the significant intrafamilial variability which can be seen in association with ADAR1 dysfunction, the mother presenting in adulthood with subtle psychological disturbance, while her son experienced a devastating early-onset encephalopathy.

ADAR1-related neurological disease can be inherited as either an autosomal recessive or autosomal dominant trait. We observed homozygosity for a missense mutation in five of 28 families segregating recessive disease. As previously suggested, the absence of patients with homozygous null mutations indicates that, as for the *ADAR1* null mouse, complete loss of ADAR1 protein activity is likely embryonic lethal.[5] Our molecular data reveal a remarkably high frequency of the p.Pro193Ala substitution, seen in 22 of 23 families with compound heterozygous molecular lesions in *ADAR1*. This mutation, which is recorded on 602 of 282,636 alleles in the gnomAD database, was not observed in the homozygous state in our cohort. That this variant was seen in combination with a null mutation in 13 families suggests that homozygosity for the p.Pro193Ala allele leads to a milder, later onset, or distinct phenotype not ascertained here, or may not be associated with disease. Perhaps of note, the gnomAD database includes one individual homozygous for this mutation. Finally, our molecular data highlight the dominant-negative p.Gly1007Arg mutation, which can occur de novo, or be inherited with variable expression and/or nonpenetrance at least into mid-adult life. The proximity of Gly1007 to the backbone of its RNA ligand, and the possibility for an arginine residue to make polyvalent interactions there suggests a mechanism whereby Arg1007 might bind more tightly to RNA and thus act as a competitive inhibitor of wild-type protein, while being itself catalytically inactive.[14] In keeping with this model, we previously demonstrated that a plasmid expressing Gly1007Arg showed stronger

inhibition of wild-type ADAR1 than equivalent amounts of a plasmid expressing catalytic inactive ADAR1.[5]

More than 130 different *ADAR1* mutations have been documented in patients with DSH, an autosomal-dominant disorder characterized by the childhood onset of hypopigmented and hyperpigmented macules on the face and dorsal aspects of the extremities.[15] DSH has only very rarely been reported outside of Japan and China, and even within identified families, a marked variability in expression is well recognized. In our series, five patients were noted to demonstrate pigmentary lesions consistent with DSH. The frequent observation of stop and frameshift variants in DSH indicates haploinsufficiency as the likely molecular pathology, consistent with the recent confirmation of our previous suggestion that two individuals with DSH would be at one in four risk of a pregnancy with ADAR1-related neurological disease.[16]

Loss-of-function mutations in *ADAR1* have been classified within the so-called type I interferonopathy grouping, a novel set of inborn errors of immunity where it is proposed that an upregulation of type I interferon signaling is central to disease pathogenesis.[17,18] The AGS phenotype can arise due to mutations in any one of seven genotypes within this grouping (*AGS1–7: TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1*, and *IFIH1*), and apparently isolated spastic paraparesis has been reported in patients mutated in three of these genes (*RNASEH2B, ADAR1*, and *IFIH1*). In contrast, in an overview of 374 patients from 299 families with mutations in *AGS1–7*, BSN, the most frequently ascertained phenotype in the current series, was only recorded in the context of ADAR1-related disease, suggesting discrete factors relevant to gene/protein expression and disease mechanism consequent upon ADAR1 dysfunction.[19] Also possibly reflective of this apparent specificity, in comparison to other genotypes, is the frequency of clinical progression, and the low risk of developing glaucoma and chilblain-like lesions (since we recorded no examples of the former and only two cases of the latter in our cohort).

The consistent finding of a positive interferon signature in peripheral blood in the series of patients reported here indicates the potential utility of this biomarker as a screening test for ADAR1-related disease, for the interpretation of *ADAR1* genetic sequence variants of uncertain significance, and in the possible monitoring of treatment efficacy as anti-interferon therapies is developed.[20,21] We emphasize that the interferon signature remains elevated many years after disease onset, providing evidence of ongoing pathology. ADAR1 is expressed throughout the brain including the basal ganglia (http://www.brain-map.org), and it has been shown that a loss of ADAR1 renders cells more susceptible to apoptosis following stress, including infection.[22] We cannot rule out the possibility that the occurrence of fevers prior to frank neurological regression represents a prodrome in some cases. However, a history of vaccination or an apparently discrete infectious episode in several patients considered to be completely developmentally normal prior to disease onset, of whom 12 demonstrated BSN on neuroimaging, raises the possibility that the acute degeneration of striatal tissue seen in many patients with *ADAR1* mutations might relate to a rapid induction of apoptosis triggered by viral infection/metabolic stress. Beyond this possibility, there is strong evidence that interferon is a neurotoxin,[23–27] and we consider it likely that inappropriate and chronic exposure to type I interferons may be directly relevant

to the ADAR1-related neurological phenotypes described here, perhaps induced by dsRNA species which are normally edited by ADAR1, thereby rendering them as immunology inert/marking them as self.[1,3,4,28] These observations highlight the potential utility of treatments for ADAR1-related disease, which recent data suggest might be usefully targeted at antagonism of type I interferon signaling.[29]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Liddicoat BJ, Chalk AM, Walkley CR. ADAR1, inosine and the immune sensing system: distinguishing self from non-self. Wiley Interdiscip Rev RNA. 2016; 7(2):157–172. [PubMed: 26692549]
2. Hartner JC, Walkley CR, Lu J, Orkin SH. ADAR1 is essential for the maintenance of hematopoiesis and suppression of interferon signaling. Nat Immunol. 2009; 10(1):109–115. [PubMed: 19060901]
3. Liddicoat BJ, Piskol R, Chalk AM, et al. RNA editing by ADAR1 prevents MDA5 sensing of endogenous dsRNA as nonself. Science. 2015; 349(6252):1115–1120. [PubMed: 26275108]
4. Pestal K, Funk CC, Snyder JM, Price ND, Treuting PM, Stetson DB. Isoforms of RNA-editing enzyme ADAR1 independently control nucleic acid sensor MDA5-driven autoimmunity and multi-organ development. Immunity. 2015; 43(5):933–944. [PubMed: 26588779]
5. Rice GI, Kasher PR, Forte GM, et al. Mutations in ADAR1 cause Aicardi-Goutières syndrome associated with a type I interferon signature. Nat Genet. 2012; 44(11):1243–1248. [PubMed: 23001123]
6. Livingston JH, Lin JP, Dale RC, et al. A type I interferon signature identifies bilateral striatal necrosis due to mutations in ADAR1. J Med Genet. 2014; 51(2):76–82. [PubMed: 24262145]
7. Crow YJ, Zaki MS, Abdel-Hamid MS, et al. Mutations in ADAR1, IFIH1, and RNASEH2B presenting as spastic paraplegia. Neuropediatrics. 2014; 45(6):386–393. [PubMed: 25243380]
8. La Piana R, Uggetti C, Olivieri I, et al. Bilateral striatal necrosis in two subjects with Aicardi-Goutières syndrome due to mutations in ADAR1 (AGS6). Am J Med Genet A. 2014; 164A(3):815–819. [PubMed: 24376015]
9. La Piana R, Uggetti C, Roncarolo F, et al. Neuroradiologic patterns and novel imaging findings in Aicardi-Goutières syndrome. Neurology. 2016; 86(1):28–35. [PubMed: 26581299]
10. Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. Dev Med Child Neurol. 1997; 39(4):214–223. [PubMed: 9183258]
11. Eliasson AC, Krumlinde-Sundholm L, Rösblad B, et al. The Manual Ability Classification System (MACS) for children with cerebral palsy: scale development and evidence of validity and reliability. Dev Med Child Neurol. 2006; 48(7):549–554. [PubMed: 16780622]
12. Hidecker MJ, Paneth N, Rosenbaum PL, et al. Developing and validating the Communication Function Classification System for individuals with cerebral palsy. Dev Med Child Neurol. 2011; 53(8):704–710. [PubMed: 21707596]
13. Tojo K, Sekijima Y, Suzuki T, et al. Dystonia, mental deterioration, and dyschromatosis symmetrica hereditaria in a family with ADAR1 mutation. Mov Disord. 2006; 21(9):1510–1513. [PubMed: 16817193]
14. Heale BS, Keegan LP, McGurk L, et al. Editing independent effects of ADARs on the miRNA/siRNA pathways. EMBO J. 2009; 28(20):3145–3156. [PubMed: 19713932]
15. Hayashi M, Suzuki T. Dyschromatosis symmetrica hereditaria. J Dermatol. 2013; 40(5):336–343. [PubMed: 22974014]
16. Kono M, Matsumoto F, Suzuki Y, et al. Dyschromatosis symmetrica hereditaria and Aicardi-Goutières syndrome 6 are phenotypic variants caused by ADAR1 mutations. J Invest Dermatol. 2016; 136(4):875–878. [PubMed: 26802932]
17. Crow YJ. Type I interferonopathies: a novel set of inborn errors of immunity. Ann N Y Acad Sci. 2011; 1238:91–98. [PubMed: 22129056]

18. Crow YJ, Manel N. Aicardi-Goutières syndrome and the type I interferonopathies. Nat Rev Immunol. 2015; 15(7):429–440. [PubMed: 26052098]
19. Crow YJ, Chase DS, Lowenstein Schmidt J, et al. Characterization of human disease phenotypes associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR, and IFIH1. Am J Med Genet A. 2015; 167A(2):296–312. [PubMed: 25604658]
20. Rice GI, Forte GM, Szynkiewicz M, et al. Assessment of interferon-related biomarkers in Aicardi-Goutières syndrome associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, and ADAR: a case-control study. Lancet Neurol. 2013; 12(12):1159–1169. [PubMed: 24183309]
21. Rice GI, Melki I, Frémond ML, et al. Assessment of type I interferon signaling in pediatric inflammatory disease. J Clin Immunol. 2017; 37(2):123–132. [PubMed: 27943079]
22. Toth AM, Li Z, Cattaneo R, Samuel CE. RNA-specific adenosine deaminase ADAR1 suppresses measles virus-induced apoptosis and activation of protein kinase PKR. J Biol Chem. 2009; 284(43):29350–29356. [PubMed: 19710021]
23. Kavanagh D, McGlasson S, Jury A, et al. Type I interferon causes thrombotic microangiopathy by a dose-dependent toxic effect on the microvasculature. Blood. 2016; 128(24):2824–2833. [PubMed: 27663672]
24. Akwa Y, Hassett DE, Eloranta ML, et al. Transgenic expression of IFN-alpha in the central nervous system of mice protects against lethal neurotropic viral infection but induces inflammation and neurodegeneration. J Immunol. 1998; 161(9):5016–5026. [PubMed: 9794439]
25. Campbell IL, Krucker T, Steffensen S, et al. Structural and functional neuropathology in transgenic mice with CNS expression of IFN-alpha. Brain Res. 1999; 835(1):46–61. [PubMed: 10448195]
26. Barlow CF, Priebe CJ, Mulliken JB, et al. Spastic diplegia as a complication of interferon Alfa-2a treatment of hemangiomas of infancy. J Pediatr. 1998; 132(3 Pt 1):527–530. [PubMed: 9544915]
27. Michaud AP, Bauman NM, Burke DK, Manaligod JM, Smith RJ. Spastic diplegia and other motor disturbances in infants receiving interferon-alpha. Laryngoscope. 2004; 114(7):1231–1236. [PubMed: 15235352]
28. Vitali P, Scadden AD. Double-stranded RNAs containing multiple IU pairs are sufficient to suppress interferon induction and apoptosis. Nat Struct Mol Biol. 2010; 17(9):1043–1050. [PubMed: 20694008]
29. Frémond ML, Rodero MP, Jeremiah N, et al. Efficacy of the Janus kinase 1/2 inhibitor ruxolitinib in the treatment of vasculopathy associated with TMEM173-activating mutations in 3 children. J Allergy Clin Immunol. 2016; 138(6):1752–1755. [PubMed: 27554814]

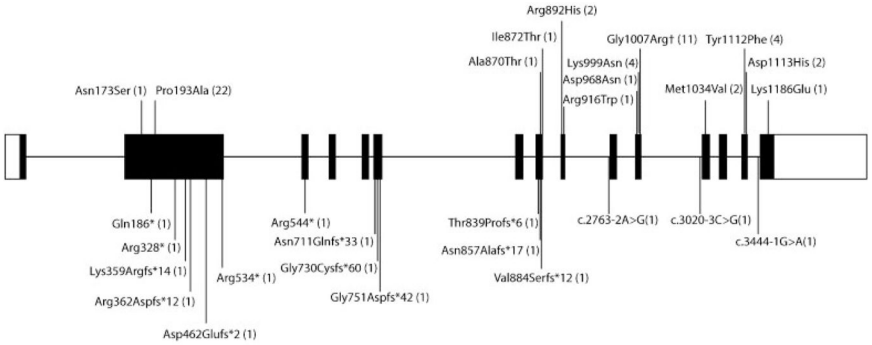

Fig. 1.

Schematic of *ADAR1* gene showing mutations (according to protein nomenclature) ascertained in the present study. Missense and nonsense mutations are annotated above and below, respectively. Numbers in brackets indicate the number of families in which each mutation was observed. †Indicates mutation acting as a dominant negative.

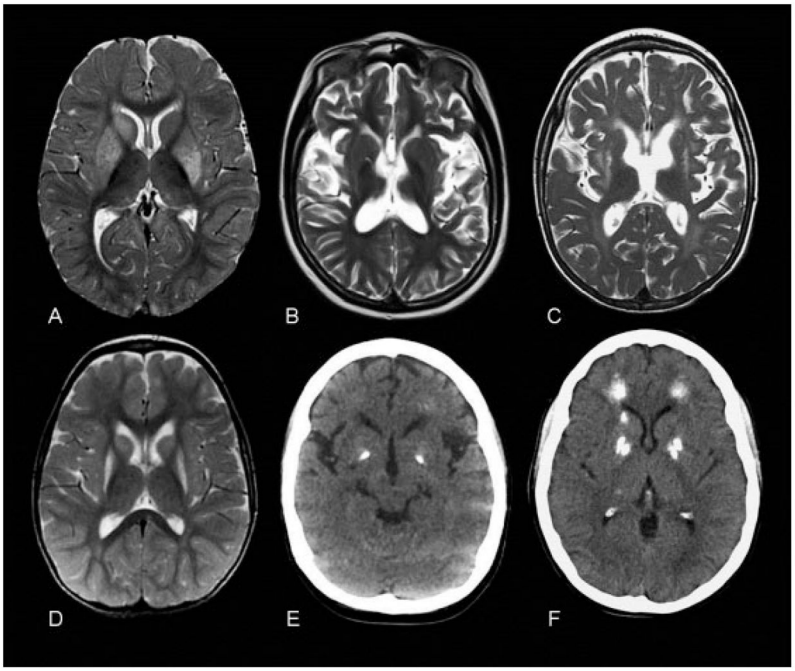

Fig. 2.

Characteristic neuroradiological features of ADAR1-related disease. Images (**A**) and (**D**) are axial T2 images of AGS251, presenting at 9 months of age with bilateral striatal necrosis following varicella zoster infection, showing characteristic high signal and swelling of head of caudate and putamen (**A**). (**D**) Follow-up at 35 months shows persisting signal change and shrinkage of caudate and putamen. Images (**B**) and (**E**) are from AGS150, a 10-year-old child presenting with an Aicardi–Goutières syndrome phenotype. (**B**) T2 axial MR shows cerebral atrophy with mildly increased signal in white matter. (**E**) CT shows dense bilateral globus pallidus calcification. Image (**C**) is of a patient presenting with an Aicardi–Goutières syndrome phenotype (AGS810_P1). (**C**) T2 axial MR at 5 years shows marked cerebral atrophy, white matter high signal, and signal change and shrinkage of the putamen. (**F**) CT scan of his mother (AGS810_P2) aged 34 years shows dense calcification of globus pallidus, head of caudate, and deep frontal white matter. Her MR (not shown) was normal. CT, computed tomography; MR, magnetic resonance.

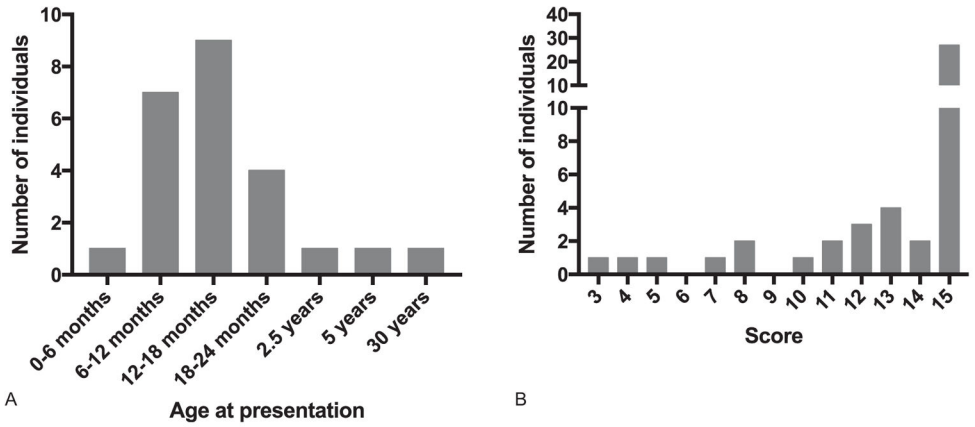

Fig. 3.

Age at presentation and associated disability. (**A**) Age at presentation in patients developing disease after a period of clearly normal development. (**B**) Assessment of gross motor function, manual ability, and communication status in living patients with mutations in *ADAR1* over 1 year of age.

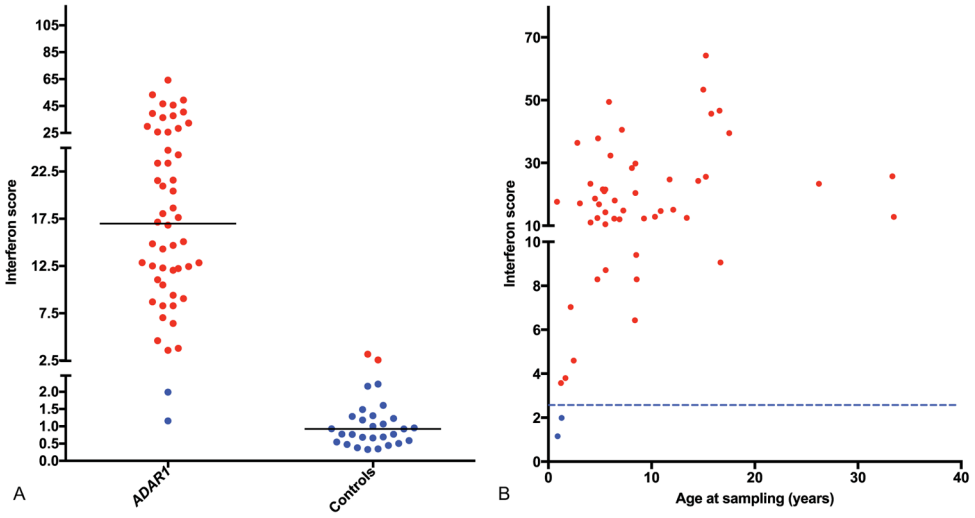

Fig. 4.

Interferon score data in *ADAR1*-mutated patients and controls. Summary of interferon score data (**A**) in *ADAR1*-mutated patients and controls and (**B**) in *ADAR1*-mutated patients by age. Circles indicate results above +2 SD of the mean of 29 controls (= 2.466, considered "positive"). Solid horizontal lines indicate median value of *ADAR1*-mutated and control groups. Dotted line indicates positive/negative boundary (2.466) of interferon score.

Table 1

Family structure, ethnicity, and molecular data of ascertained *ADAR1* mutation-positive cases

AGS number	Individuals tested	Consanguinity	Ethnicity	cDNA	Protein	Allelic status	Inheritance	SIFT	Polyphen2	CADD Phred	ExAc frequency	gnomAD frequency
AGS081	3A, M, F	No	White European	c.577C>G	p.Pro193Ala	Het	Maternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.2675G>A	p.Arg892His	Het	Paternally inherited	Deleterious 0.01	Probably damaging 1.000	35	Novel	1/252010
AGS093	1A, M, F	No	Italian	c.577C>G	p.Pro193Ala	Het	Paternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.2608G>A	p.Ala870Thr	Het	Maternally inherited	Deleterious 0	Probably damaging 1.000	34	Novel	Novel
AGS107	2A, M, F	Yes	Pakistani	c.3337G>C	p.Asp1113His	Hom	Both parents het	Deleterious 0.02	Probably damaging 1.000	33	Novel	Novel
AGS150	1A, M, F	No	Brazilian	c.3019G>A	p.Gly1007Arg	Het	De novo (paternity confirmed)	Deleterious 0	Probably damaging 1.000	34	Novel	Novel
AGS219	1A	Yes	Pakistani	c.3335A>T	p.Tyr1112Phe	Hom	Not tested	Tolerated 0.17	Probably damaging 1.000	33	Novel	Novel
AGS228	1A, M, F	No	Indian	c.2997G>T	p.Lys999Asn	Hom	Both parents het	Deleterious 0.03	Probably damaging 1.000	34	Novel	Novel
AGS251	1A, M, F	No	White European	c.577C>G	p.Pro193Ala	Het	Maternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.2615T>C	p.Ile872Thr	Het	Paternally inherited	Deleterious 0.01	Probably damaging 1.000	26.9	1/121342	1/252270
AGS327	1A, M, F	No	Italian/ Hispanic	c.577C>G	p.Pro193Ala	Het	Maternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.1076_1080del	p.Lys359Argfs*14	Het	Paternally inherited	Frameshift	Frameshift	Frameshift	Novel	Novel
AGS430	2A, M, F	No	Spanish	c.577C>G	p.Pro193Ala	Het	Maternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.2675G>A	p.Arg892His	Het	Paternally inherited	Deleterious 0.01	Probably damaging 1.000	35	Novel	1/252010
AGS474	1A, M, F	No	White European	c.3019G>A	p.Gly1007Arg	Het	De novo (paternity confirmed)	Deleterious 0	Probably damaging 1.000	34	Novel	Novel
AGS530	2A, M	No	White European	c.3019G>A	p.Gly1007Arg	Het	Presumed inherited from asymptomatic Father	Deleterious 0	Probably damaging 1.000	34	Novel	Novel
AGS550	1A, M, F	No	White European	c.577C>G	p.Pro193Ala	Het	Paternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.2565_2568del	p.Asn857Alafs*17	Het	Maternally inherited	Frameshift	Frameshift	Frameshift	Novel	1/30224
AGS567	1A, M, F	No	Greek/ Lebanese	c.518A>G	p.Asn173Ser	Het	Paternally inherited	N/A	Probably damaging 0.999	24.3	34/121366	144/282658 1 hom
				c.2515del	p.Thr839Profs*6	Het	Maternally inherited	Frameshift	Frameshift	Frameshift	Novel	Novel
AGS582	1A	No	White European	c.577C>G	p.Pro193Ala	Het	Not known	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.2647_2648dup	p.Val884Serfs*12	Het	Not known	Frameshift	Frameshift	Frameshift	Novel	Novel
AGS663	2A, M, F	No	White European	c.577C>G	p.Pro193Ala	Het	Paternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.1630C>T	p.Arg544*	Het	Maternally inherited	Stop	Stop	Stop	Novel	2/252366
AGS679	1A	No	White European	c.577C>G	p.Pro193Ala	Het	Not known	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.3556A>G	p.Lys1186Glu	Het	Not known	Tolerated 0.11	Probably damaging 0.999	31	Novel	Novel

AGS number	Individuals tested	Consanguinity	Ethnicity	cDNA	Protein	Allelic status	Inheritance	SIFT	Polyphen2	CADD Phred	ExAc frequency	gnomAD frequency
AGS699	1A, M, F	No	White European	c.3019G>A	p.Gly1007Arg	Het	De novo (genotyping not undertaken)	Deleterious 0	Probably damaging 1.000	34	Novel	Novel
AGS703	1A	No	Asian/ White European	c.577C>G	p.Pro193Ala	Het	Not known	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.3100A>G	p.Met1034Val	Het	Not known	Deleterious 0.03	Possibly damaging 0.760	25.8	Novel	Novel
AGS720	1A, M, F	No	White European	c.577C>G	p.Pro193Ala	Het	Maternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.2250del	p.Gly751Aspfs*42	Het	De novo (genotyping not undertake n)	Frameshift	Frameshift	Frameshift	Novel	Novel
AGS759	1A, M, F	No	White European	c.577C>G	p.Pro193Ala	Het	Paternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.2902G>A	p.Asp968Asn	Het	Maternally inherited	Tolerated 0.06	Probably damaging 1.000	34	Novel	Novel
AGS765	1A	No	White European	c.577C>G	p.Pro193Ala	Het	Not known	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.556C>T	p.Gln186*	Het	Not known	Stop	Stop	Stop	Novel	Novel
AGS788	1A, M, F	No	White European	c.577C>G	p.Pro193Ala	Het	Maternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.1386_1390del	p.Asp462Glufs*2	Het	De novo (paternity confirmed)	Frameshift	Frameshift	Frameshift	Novel	Novel
AGS810	1A, MA, F	No	White European	c.3019G>A	p.Gly1007Arg	Het	Inherited from symptomatic mother	Deleterious 0	Probably damaging 1.000	34	Novel	Novel
AGS943	1A, M, F	No	North African	c.3019G>A	p.Gly1007Arg	Het	De novo (genotyping not undertaken)	Deleterious 0	Probably damaging 1.000	34	Novel	Novel
AGS1115	1A, M, F	Yes	Persian	c.2997G>T	p.Lys999Asn	Hom	Both parents het	Deleterious 0.03	Probably damaging 1.000	34	Novel	Novel
AGS1170	1A	No	Asian	c.577C>G	p.Pro193Ala	Het	Not known	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.3100A>G	p.Met1034Val	Het	Not known	Deleterious 0.03	Possibly damaging 0.760	25.8	Novel	Novel
AGS1315	2A, M, F (mosaic)	No	White European	c.3019G>A	p.Gly1007Arg	Het	Father mosaic	Deleterious 0	Probably damaging 1.000	34	Novel	Novel
AGS1456	1A	No	White European	c.577C>G	p.Pro193Ala	Het	Not known	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.3020–3C>G	Splicing	Het	Not known	Splicing	Splicing	Splicing	Novel	Novel
AGS1507	1A, M, F	No	Asian/ White European	c.577C>G	p.Pro193Ala	Het	Maternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.2763–2A>G	Splicing	Het	Paternally inherited	Splicing	Splicing	Splicing	Novel	Novel
AGS1537	1A	No	White European	c.3019G>A	p.Gly1007Arg	Het	Not known	Deleterious 0	Probably damaging 1.000	34	Novel	Novel
AGS1542	2A, M, F	Yes	Asian	c.3335A>T	p.Tyr1112Phe	Hom	Both parents het	Tolerated 0.17	Probably damaging 1.000	33	Novel	Novel
AGS1824	1A	No	White European	c.577C>G	p.Pro193Ala	Het	Paternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.1084_1085del	p.Arg362Aspfs*12	Het	Maternally inherited	Frameshift	Frameshift	Frameshift	Novel	Novel
AGS1980	1A	No	White European	c.577C>G	p.Pro193Ala	Het	Not known	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom

AGS number	Individuals tested	Consanguinity	Ethnicity	cDNA	Protein	Allelic status	Inheritance	SIFT	Polyphen2	CADD Phred	ExAc frequency	gnomAD frequency
				c.2130dupC	p.Asn711Glnfs*33	Het	Not known	Frameshift	Frameshift	Frameshift	Novel	Novel
AGS1989	1A, M, F	No	South American	c.577C>G	p.Pro193Ala	Het	Paternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.2187_2198delinsGT	p.Gly730Cysfs*60	Het	Maternally inherited	Frameshift	Frameshift	Frameshift	Novel	Novel
AGS2007	1A	No	White European	c.577C>G	p.Pro193Ala	Het	Not known	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.982C>T	p.Arg328*	Het	Not known	Stop	Stop	Stop	Novel	1/252210
AGS2009	1A, M, F	No	White European	c.577C>G	p.Pro193Ala	Het	Paternally inherited	Deleterious 0	Probably damaging 1.000	23.9	260/ 121402	602/282636 1 hom
				c.2746C>T	p.Arg916Trp	Het	Maternally inherited	Deleterious 0	Probably damaging 1.000	35	Novel	Novel
AGS2010	1A, M	No	Hispanic	c.3019G>A	p.Gly1007Arg	Het	M WT, F not tested	Deleterious 0	Probably damaging 1.000	34	Novel	Novel

Abbreviations: A, affected; F, father; Het, heterozygous; Hom, homozygous; M, mother; MA, mother affected; WT, wild type.

Note: Nucleotide numbering based on transcript *ADAR1* NM_001111.4. ExAc browser Beta version accessed on October 28, 2016 (http://exac.broadinstitute.org), gnomAD browser β version accessed on October 28, 2016 (http://gnomad.broadinstitute.org).

Table 2

Clinical and radiological data relating to ascertained *ADAR1* mutation-positive cases

AGS number	Individual	Sex	Developmental status prior to onset	Possible trigger	Age at initial ascertainment	Features at presentation	Current age/ age at death (cause were known)	Progressive course	Status at last contact	Neuroimaging	Interferon scores (age, decimalized years)	GMFCS	MACS	CFCS	Summary
AGS081	P1	F	Delayed	No	5 mo	DD, dystonia, irritability	Died aged 17 y	Yes	SDT with severe ID	Characteristic of AGS	24.267 (14.53); 53.356 (15.01); 45.676 (15.78)	V	V	V	AGS
	P2	F	Delayed	No	5 mo	DD, dystonia, irritability, microcephaly	Died aged 23 mo	Yes	SDT with severe ID	Characteristic of AGS	NT	V	V	V	AGS
	P3	M	Diagnosed at birth	No	Neonatal	Raised CSF IFN at birth with transient thrombocytopenia and petechiae	9 y	Not obvious	SDT with severe ID	Characteristic of AGS	37.822 (4.82); 21.590 (5.28)	V	V	V	AGS
AGS093	P1	M	Delayed	No	1 mo	DD, irritability, sleep and feeding disturbance	20 y	Not obvious	SDT with severe ID	Characteristic of AGS and BSN	25.608 (15.26); 46.665 (16.59)	V	V	V	AGS/BSN
AGS107	P1	F	Delayed	No	< 7 mo	DD, dystonia, irritability, microcephaly	Died aged 19 y	Not obvious	SDT with severe ID; AIHA	Characteristic of AGS	64.22 (15.26)	V	V	V	AGS
	P2	F	Delayed	No	Neonatal	DD, dystonia, irritability, microcephaly	14 y	Not obvious	SDT with severe ID; AIHA	Characteristic of AGS	NT	V	V	V	AGS
AGS150	P1	F	Mild delay	No	18 mo	Loss of head control, sitting and speech	15 y	No	SDT with some ID	Some white matter disease and calcification of GP	14.69 (10.88)	V	V	V	AGS
AGS219	P1	M	Delayed	No	< 6 mo	DD, poor head control	Died aged 6 y	Not obvious	SDT with severe ID; AIHA	Characteristic of AGS	NT	V	V	V	AGS
AGS228	P1	F	Delayed	No	Prenatal	IUGR, thrombocytopenia, HSM	Died aged 10 mo	Not obvious	SDT with severe ID	Characteristic of AGS	NT	< 1 year	< 1 year	< 1 year	AGS
AGS251	P1	F	Normal	Varicella infection	9 mo	Loss of skills over a few weeks	12 y	Not obvious	SDT with some ID; CB	BSN	28.367 (8.1); 12.301 (9.27)	V	V	IV	BSN
AGS327	P1	M	Delayed	Possible viral infection (otitis media)	8 mo	DD, encephalopathy, irritability	8 y	Not obvious	SDT with severe ID; DSH	AGS with features of BSN	23.382 (4.07); 9.402 (8.52)	V	V	V	AGS/BSN
AGS430	P1	F	Delayed	No	< 2 mo	DD, dystonia, irritability, microcephaly	Died aged 6 y	Uncertain	SDT with severe ID	Characteristic of AGS	8.296 (4.75); 21.538 (5.53)	V	V	V	AGS
	P2	F	Delayed	No	< 2 mo	DD, dystonia, irritability, microcephaly	9 y	Not obvious	SDT with severe ID	Characteristic of AGS	12.444 (4.75); 14.306 (5.53)	V	V	V	AGS
AGS474	P1	M	Normal	Vaccination	4 mo	Nystagmus, gross and fine motor delay	8 y	Yes, with worsening respiratory function and overall neurological deterioration	SDT with severe ID	Characteristic of AGS	20.961 (5.42); 32.319 (5.88); 49.463 (6.02)	V	V	V	AGS
AGS530	P1	F	Normal	No	5 y	Subacute loss of skills becoming rigid over a few months	17 y	Yes	SDT with some understanding	BSN	12.502 (13.41)	V	V	V	BSN
	P2	F	Normal	No	1 y	Subacute loss of skills becoming rigid over a few months	29 y	Yes	SDT with some understanding	BSN	23.385 (26.21)	V	IV	IV	BSN

AGS number	Individual	Sex	Developmental status prior to onset	Possible trigger	Age at initial ascertainment	Features at presentation	Current age/ age at death (cause were known)	Progressive course	Status at last contact	Neuroimaging	Interferon scores (age, decimalized years)	GMFCS	MACS	CFCS	Summary
AGS550	P1	M	Normal	D & V	16 mo	Sudden-onset motor regression	Died aged 9 y (pneumonia)	Yes	SDT with some understanding	BSN	6.429 (8.39)	V	V	V	BSN
AGS567	P1	M	Mild delay	Bronchiolitis	9 mo	Sudden onset motor regression	6 y	Yes	SDT with moderate ID; DSH	BSN	36.387 (2.81)	V	V	V	BSN
AGS582	P1	M	Normal	No	14 mo	Loss of skills	Died aged 10 y	Yes	SDT with moderate ID	BSN	NT	V	V	V	BSN
AGS663	P1	M	Normal	URTI	11 mo	Sudden onset motor regression	12 y	No	SDT moderate ID	BSN	NT	IV	III	III	BSN
	P2	M	Normal	URTI	11 months	Sudden onset motor regression	Died age 18 years	Not obvious	SDT	BSN	38.13 (17.53)	V	V	IV	BSN
AGS679	P1	F	Normal	Unspecified viral infection	18 mo	Sudden onset motor regression	4 y	Yes, then some recovery	Dystonic gait and clumsy hand finger movements; intellectually normal	BSN	3.802 (1.66)	II	III	III	BSN
AGS699	P1	M	Normal	No	2 y	Falling	8 y	Yes	Major LL spasticity; intellectually normal	Normal	16.833 (4.91)	II	I	I	SP
AGS703	P1	M	Mild delay	No	2 y	Loss of skills over a few weeks	11 y	Yes	SDT with severe ID	Initially structurally normal MRI; BG calcification noted 2 years later	20.427 (8.44); 29.817 (8.44)	V	IV	III	SDT
AGS720	P1	F	Normal	Unspecified viral infection	18 mo	Rapid loss of skills	9 y	No	SDT; intellectually normal; DSH	BSN	12.057 (6.90)	V	V	III	BSN
AGS759	P1	F	Normal	URTI	14 mo	Motor regression and speech arrest	6 y	No	SDT; intellectually normal	Calcification of caudate and putamen	11.048 (4.09); 18.633 (4.53)	III	II	III	SDT
AGS765	P1	F	Normal	URTI	11 mo	Rapid loss of skills	7 y	No	SDT with some ID	BG signal changes and atrophy with subcortical hypomyelination	NT	IV	V	IV	SDT
AGS788	P1	F	Normal	URTI/meningitis C vaccination	15 mo	Acute regression, dystonia, extrapyramidal movements, orofacial dyskinesia	3 y	Not obvious	SDT with severe ID	BSN	1.99 (1.29); 4.59 (2.46)	V	V	V	BSN
AGS810	P1 (son to P2)	M	Mild delay	URTI	12 mo	Rapid psychomotor regression, axial hypotonia, spastic dystonic tetraparesis	9 y	Not obvious	SDT with severe ID	Characteristic of AGS	40.571 (7.13); 14.851 (7.27)	V	V	V	AGS/BSN
	P2 (mother to P1)	F	Normal	No	30 y	Pain, fatigue, anxiety, sleep problems	35 y	Possibly	Normal clinical examination; subtle psychological difficulties	Normal except for BG, WM, and Cb calcification	25.743 (33.34); 12.836 (33.48)	I	I	I	ICC with psychiatric features
AGS943	P1	M	Normal	Vaccination	22 mo	SP	13 y	Yes, developing asymmetric dystonia of upper limbs 7 y after initial presentation	SDT; intellectually normal	Some cortical atrophy with BG and WM calcification	24.753 (11.75); 15.074 (12.11)	I	III	I	SP becoming SDT with preserved intellect
AGS1115	P1	M	Unknown	No	4 mo	Hypotonia and dystonia	2 y	Not obvious	SDT with severe ID	Characteristic of AGS	NT	V	V	V	AGS
AGS1170	P1	F	Normal	URTI	9 mo	Acute regression with dystonia necessitating ICU admission	2 y	Not obvious	SDT with severe ID	Initial bilateral high signal and swelling of BG progressing to extensive WM and	17.627 (0.84); 1.158 (0.90); 3.578 (1.23)	V	V	V	AGS/BSN

AGS number	Individual	Sex	Developmental status prior to onset	Possible trigger	Age at initial ascertainment	Features at presentation	Current age/ age at death (cause were known)	Progressive course	Status at last contact	Neuroimaging	Interferon scores (age, decimalized years)	GMFCS	MACS	CFCS	Summary
										cortical atrophy and severely atrophied putamina (no CT)					
AGS1315	P1 (brother to P2, son of P3)	M	Normal	No	2.5 y	ST with normal intellect	6 y	Fluctuations	ST; intellectually normal;	Normal MRI (at 2 years) and CT (at 4 years)	10.506 (5.53)	IV	II	I	ST
	P2 (brother to P1, son of P3)	M	Delayed	No	DD obvious by 1 y	ST and speech delay	4 y	Yes, age 2.5 y episode of definite regression	ST with severe ID	MRI normal, BG and PV calcification on CT	17.147 (3.06)	IV	IV	IV	ST
	P3 (Father to P1 and P2; mosaic)	M	Always normal	NR	Always normal	Always normal	31 y	No	Normal	No imaging	2.692 (30.18)	I	I	I	Normal
AGS1456	P1	M	Normal	Otitis media	15 mo	Lethargy, dystonia, global regression	17 y	Yes, with intermittent flares of encephalopathy and slowly progressive dystonia	SDT with severe ID; DSH	Mild hyper- intensity of the BG (no CT)	9.063 (16.68)	V	V	V	SDT
AGS1507	P1	M	Moderate delay	No	13 mo	Developmental arrest with onset of generalized dystonia	9 y	Yes, episode of definite regression at age 4 years	SDT with some ID; DSH	BSN	8.293 (8.56)	V	V	V	BSN
AGS1537	P1	F	Delayed	No	15 mo	Motor delay with spastic tetraparesis	11 y	No	SDT with some ID; AIHA	BG calcification (CT); normal MRI at age 10 years	12.865 (10.33)	V	IV	III	SDT
AGS1542	P1	M	Normal	No	21 mo	Rapidly progressive SP	7 y	Yes, with progressive involvement of UL and spastic dystonia	SDT with some ID	Normal (no CT)	12.24 (6.38); 18.051 (6.43)	IV	III	IV	SP becoming SDT
	P2	M	Likely delayed	No	14 mo	Onset of dystonia and loss of skills	19 mo	No	SDT with severe ID	No imaging	7.031 (2.17)	V	V	V	Clinically AGS-like (but no imaging)
AGS1824	P1	M	Normal	Unspecified viral infection	11 mo	Acute regression with dystonia necessitating ICU admission	5 y	No	SDT with severe ID; CB	BSN with BG calcification	8.713 (5.55)	V	V	V	BSN
AGS1980	P1	M	Normal	Febrile illness	14 mo	Left hemiparesis with loss of ambulation	2 y	Yes, from uni- to bi- lateral; however, some skills (e.g., crawling, pulling to stand) subsequently reacquired	SDT with some ID	BSN (no CT)	NT	IV	IV	III	BSN
AGS1989	P1	M	Normal	Otitis media	12 mo	Tremor and rapid loss of skills	4 y	No	SDT with severe ID	BSN with BG calcification	NT	V	V	V	BSN
AGS2007	P1	M	Possible mild delay	Febrile respiratory illness	15 mo	Developmental regression with loss of crawling and other skills	3 y	No	SDT with severe ID	Characteristic of AGS	NT	V	V	V	AGS
AGS2009	P1	F	Normal	MMR and varicella vaccination	13 mo	Developmental regression with loss of skills	6 y	No	SDT with severe ID	BSN with BG calcification	NT	IV	IV	IV	BSN

AGS number	Individual	Sex	Developmental status prior to onset	Possible trigger	Age at initial ascertainment	Features at presentation	Current age/ age at death (cause were known)	Progressive course	Status at last contact	Neuroimaging	Interferon scores (age, decimalized years)	GMFCS	MACS	CFCS	Summary
AGS2010	P1	F	Normal	Vaccination	6 mo	Lost all acquired skills over 6 mo period	9 y	No	SDT with severe ID	Some white matter disease and calcification of GP	NT	V	V	V	AGS

Abbreviations: AGS, Aicardi–Goutières syndrome; AIHA, autoimmune hemolytic anemia; BG, basal ganglia; BSN, bilateral striatal necrosis; CB, chilblains; CFCS, Communication Function Classification System; CSF, cerebrospinal fluid; CT, computed tomography; DD, developmental delay; DSH, dyschromatosis symmetrica hereditaria; D & V, diarrhea and vomiting; GMFCS, Gross Motor Function Classification System; GP, globus pallidus; HSM, hepatosplenomegaly; ICC, intracranial calcification; ICU, intensive care unit; ID, intellectual disability; IFN, interferon; IUGR, intrauterine growth retardation; LL, lower limb; MACS, Manual Ability Classification System; MRI, magnetic resonance imaging; NR, not relevant; NT, not tested; PV, periventricular; SD, spastic dystonia; SDT, spastic dystonic tetraparesis; SP, spastic paraparesis; ST, spastic tetraparesis; UL, upper limb; URTI, upper respiratory tract infection; WM, white matter.

Note: AGS1315_P3 (different shading) is not included in the patient data analysis because of mosaic status; disability scales were not calculated for AGS228 because of age < 1 year at last contact.