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

DOI:
10.1055/s-0037-1601449

Document Version
Accepted author manuscript

Published in:
Neuropediatrics

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Journal:	*Neuropediatrics*
Manuscript ID	neuped-01-2017-1625-OA.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
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Keyword:	Aicardi-Goutières syndrome, Bilateral striatal necrosis, Spastic paraparesis, Dystonia, Idiopathic basal ganglia calcification
Please select the subject area of your manuscript::	Genetics, Inflammatory
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Genetic, phenotypic and interferon biomarker status in ADAR1-related neurological disease

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Word count: 2,506

Key words:

Aicardi-Goutières syndrome

Bilateral striatal necrosis

Spastic paraparesis

Dystonia

Idiopathic basal ganglia calcification

Short title:

Neurological disease due to ADAR1 dysfunction

Abstract

We investigated the genetic, phenotypic and interferon status of 46 patients from 37 families with neurological disease due to mutations in *ADAR1*. The clinico-radiological phenotype encompassed a spectrum of Aicardi-Goutières syndrome (AGS), isolated bilateral striatal necrosis (BSN), 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 to 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.

Introduction

Adenosine deaminases acting on RNA (ADARs) catalyse 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-

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copy gene that 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, and demonstrated that, similar to the *Adar1*-null mouse, the mutant genotype was associated with an upregulation of type I interferon signalling[5]. Further to this, in 2014, we described both bilateral striatal necrosis, sometimes occurring after a trivial childhood infection, and otherwise non-syndromic, slowly progressive spastic paraparesis associated with normal intellect to occur due 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 Aicardi-Goutières syndrome was suggested by characteristic clinical and neuroimaging features including cerebral atrophy, white matter disease and intracranial calcification[9]. Bilateral striatal necrosis was diagnosed in the context of an acute or subacute onset of a dystonic / rigid motor disorder associated with magnetic resonance imaging (MRI) 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 non-specific 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 1). Purified 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* programmes 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 polymerase chain reaction (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 6 probes, individual data were expressed relative to a single calibrator. RQ (relative quantification) is equal to $2^{-\Delta\Delta Ct}$ i.e. the normalized fold change relative to the control data. The median fold change of the 6 genes compared to 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 i.e. 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; Figure 1). In four families the p.Gly1007A mutation was considered to have occurred *de novo*, whilst in three families inheritance was confirmed or inferred (2 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 summarised in Table 2, and characteristic radiological appearances summarised in Figure 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 Aicardi-Goutières syndrome (15 patients), bilateral striatal necrosis (16 patients), apparently isolated spastic paraparesis (one patient) / tetraparesis (two patients) and a progressive spastic dystonic motor disorder (seven patients). In two of these latter cases the initial presentation was of isolated lower limb spasticity, with a

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dystonic component and involvement of the upper limbs only becoming evident several years later. Four patients demonstrated radiological features of both Aicardi-Goutières syndrome and bilateral striatal necrosis. The mother of a child with an Aicardi-Goutières syndrome 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.

Twenty five patients were considered to have demonstrated normal development prior to disease onset, in 18 of whom there was a history of either vaccination (four patients) or a notable infectious episode (14 patients) in the period shortly preceding the development of clinical signs (Figure 3A). A number of 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 Aicardi-Goutières syndrome.

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)(Figure 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

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considered to have normal intellectual function.

Five patients were reported to demonstrate hypo / hyperpigmentation consistent with dyschromatosis symmetrica hereditaria 1 (DSH), and two patients were described with chilblain-like vasculitic lesions. Four patients were documented with autoimmune haemolytic anaemia. 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 to controls (median: 0.93, IQR: 0.57 – 1.30)(Figure 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 Figure 1).

Discussion

In 2012, *ADAR1* mutations were described in the context of the early-onset encephalopathy Aicardi-Goutières syndrome, 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 non-syndromic bilateral striatal necrosis, and of isolated spastic paraparesis with normal neuroimaging[6,7]. Here we confirm these associations, thus emphasising the need to consider ADAR1-related disease in a number of distinct clinical scenarios

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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 characterised 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. More generally, mutations in *ADAR1* should be thought of in the context of a motor disorder characterised 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 towards the ascertainment of paediatric disease. However, Tojo *et al.* described a female patient with the dominant-negative p.Gly1007Arg mutation, presenting at age 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 age 30 years, it is clear that later onset disease can occur due to ADAR1 deficiency. This latter case also illustrates the significant intra-familial variability which can be seen in association with ADAR1 dysfunction, the mother presenting in adulthood with subtle psychological disturbance, whilst her son experienced a devastating early-onset encephalopathy.

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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 non-penetrance 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, whilst 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,

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an autosomal-dominant disorder characterised 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 recognised. 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 signalling is central to disease pathogenesis[17,18]. The Aicardi-Goutières syndrome 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*, bilateral striatal necrosis, 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]. Possibly also 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).

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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 are developed[20,21]. We emphasise 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 bilateral striatal necrosis 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 signalling[29].

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Acknowledgements

We are grateful to the affected families for their involvement in our research, and to all clinicians who contributed samples and clinical data not included here. We thank Marie-Louise Frémond for critical reading of the manuscript. We would like to thank the Exome Aggregation Consortium (ExAC), the Genome Aggregation Database (gnomAD) and the groups that provided exome variant data for comparison.

Author 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 manuscript and agreed to its publication.

Financial Disclosure/Conflict of Interest

None of the authors have any financial disclosures to report.

Funding

YJC acknowledges funding from the European Research Council (GA 309449: Fellowship to Y.J.C), ERA-NET Neuron (MR/M501803/1) and a state subsidy managed by the National Research Agency (France) under the "Investments for the

Future" (ANR-10-IAHU-01). TAB acknowledges funding from the NIHR. V.N. and K.M.R. acknowledge the clinical support of the C4RCD Research Group.

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Figure legends

Figure 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.

Figure 2. Characteristic neuroradiological features of ADAR1-related disease. 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). Follow up (D) 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. T2 axial MR (B) shows cerebral atrophy with mildly increased signal in white matter. CT (E) shows dense bilateral globus pallidus calcification. Image C is of a patient presenting with an Aicardi-Goutières syndrome phenotype (AGS810_P1). T2 axial MR at 5 years (C) shows marked cerebral atrophy, white matter high signal and signal change and shrinkage of the putamen. CT scan of his mother (F)(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.

Figure 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.

Figure 4. Interferon score data in *ADAR1*-mutated patients and controls. Summary of interferon score data in *ADAR1*-mutated patients and controls (A), and in *ADAR1*-mutated patients by age (B). Red 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.

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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
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 undertaken)	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	c.577C>G	p.Pro193Ala	Het	Maternally inherited	Deleterious 0	Probably damaging	23.9	260/121402	602/282636 1 hom

			European	c.2763-2A>G	Splicing	Het	Paternally inherited	Splicing	1.000 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
				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

A affected; F Father; Het heterozygous; Hom Homozygous; WT wild-type; M Mother; MA Mother affected. Nucleotide numbering based on transcript *ADAR1* NM_001111.4. ExAc browser Beta version accessed on 28/10/2016 (http://exac.broadinstitute.org), gnomAD browser beta version accessed on 28/10/2016 (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	Possible trigger	Age at initial ascertainment	Features at presentation	Current age / age at	Progressive course	Status at last contact	Neuroimaging	Interferon scores (age,	GMFCS	MACS	CFCS	Summary

			onset				death (cause where known)				decimalized years)				
AGS081	P1	F	Delayed	No	5 months	DD, dystonia, irritability	Died age 17 years	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 months	DD, dystonia, irritability, microcephaly	Died aged 23 months	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 years	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 month	DD, irritability, sleep and feeding disturbance	20 years	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 months	DD, dystonia, irritability, microcephaly	Died aged 19 years	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 years	Not obvious	SDT with severe ID; AIHA	Characteristic of AGS	NT	V	V	V	AGS
AGS150	P1	F	Mild delay	No	18 months	Loss of head control, sitting and speech	15 years	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 months	DD, poor head control	Died aged 6 years	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 months	Not obvious	SDT with severe ID	Characteristic of AGS	NT	< 1 year	< 1 year	< 1 year	AGS
AGS251	P1	F	Normal	Varicella infection	9 months	Loss of skills over a few weeks	12 years	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 months	DD, encephalopathy, irritability	8 years	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 months	DD, dystonia, irritability, microcephaly	Died aged 6 years	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 months	DD, dystonia, irritability, microcephaly	9 years	Not obvious	SDT with severe ID	Characteristic of AGS	12.444 (4.75); 14.306	V	V	V	AGS

											(5.53)				
AGS474	P1	M	Normal	Vaccination	4 months	Nystagmus, gross and fine motor delay	8 years	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 years	Subacute loss of skills becoming rigid over a few months	17 years	Yes	SDT with some understanding	BSN	12.502 (13.41)	V	V	V	BSN
	P2	F	Normal	No	1 year	Subacute loss of skills becoming rigid over a few months	29 years	Yes	SDT with some understanding	BSN	23.385 (26.21)	V	IV	IV	BSN
AGS550	P1	M	Normal	D & V	16 months	Sudden onset motor regression	Died age 9 years (pneumonia)	Yes	SDT with some understanding	BSN	6.429 (8.39)	V	V	V	BSN
AGS567	P1	M	Mild delay	Bronchiolitis	9 months	Sudden onset motor regression	6 years	Yes	SDT with moderate ID; DSH	BSN	36.387 (2.81)	V	V	V	BSN
AGS582	P1	M	Normal	No	14 months	Loss of skills	Died age 10 years	Yes	SDT with moderate ID	BSN	NT	V	V	V	BSN
AGS663	P1	M	Normal	URTI	11 months	Sudden onset motor regression	12 years	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 months	Sudden onset motor regression	4 years	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 years	Falling	8 years	Yes	Major LL spasticity; intellectually normal	Normal	16.833 (4.91)	II	I	I	SP
AGS703	P1	M	Mild delay	No	2 years	Loss of skills over a few weeks	11 years	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 months	Rapid loss of skills	9 years	No	SDT; intellectually normal; DSH	BSN	12.057 (6.90)	V	V	III	BSN
AGS759	P1	F	Normal	URTI	14 months	Motor regression and speech arrest	6 years	No	SDT; intellectually normal	Calcification of caudate and putamine	11.048 (4.09); 18.633 (4.53)	III	II	III	SDT

AGS765	P1	F	Normal	URTI	11 months	Rapid loss of skills	7 years	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 months	Acute regression, dystonia, extra-pyramidal movements, orofacial dyskinesia	3 years	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 months	Rapid psychomotor regression, axial hypotonia, spastic dystonic tetraparesis	9 years	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 years	Pain, fatigue, anxiety, sleep problems	35 years	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 months	SP	13 years	Yes, developing asymmetric dystonia of upper limbs 7 years 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 months	Hypotonia and dystonia	2 years	Not obvious	SDT with severe ID	Characteristic of AGS	NT	V	V	V	AGS
AGS1170	P1	F	Normal	URTI	9 months	Acute regression with dystonia necessitating ICU admission	2 years	Not obvious	SDT with severe ID	Initial bilateral high signal and swelling of BG progressing to extensive WM and cortical atrophy and severely atrophied putamina (no CT)	17.627 (0.84); 1.158 (0.90); 3.578 (1.23)	V	V	V	AGS/BSN
AGS1315	P1 (brother to P2, son of P3)	M	Normal	No	2.5 years	ST with normal intellect	6 years	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 year	ST and speech delay	4 years	Yes, age 2.5 years 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	M	Always normal	NR	Always normal	Always normal	31 years	No	Normal	No imaging	2.692	I	I	I	Normal

	to P1 and P2; mosaic)										(30.18)				
AGS1456	P1	M	Normal	Otitis media	15 months	Lethargy, dystonia, global regression	17 years	Yes, with intermittent flares of encephalopathy and slowly progressive dystonia	SDT with severe ID; DSH	Mild hyperintensity of the BG (no CT)	9.063 (16.68)	V	V	V	SDT
AGS1507	P1	M	Moderate delay	No	13 months	Developmental arrest with onset of generalized dystonia	9 years	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 months	Motor delay with spastic tetraparesis	11 years	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 months	Rapidly progressive SP	7 years	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 months	Onset of dystonia and loss of skills	19 months	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 months	Acute regression with dystonia necessitating ICU admission	5 years	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 months	Left hemiparesis with loss of ambulation	2 years	Yes, from uni- to bilateral; 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 months	Tremor and rapid loss of skills	4 years	No	SDT with severe ID	BSN with BG calcification	NT	V	V	V	BSN
AGS2007	P1	M	Possible mild delay	Febrile respiratory illness	15 months	Developmental regression with loss of crawling and other skills	3 years	No	SDT with severe ID	Characteristic of AGS	NT	V	V	V	AGS
AGS2009	P1	F	Normal	MMR and varicella vaccination	13 months	Developmental regression with loss of skills	6 years	No	SDT with severe ID	BSN with BG calcification	NT	IV	IV	IV	BSN
AGS2010	P1	F	Normal	Vaccination	6 months	Lost all acquired skills over 6 month period	9 years	No	SDT with severe ID	Some white matter disease and calcification of GP	NT	V	V	V	AGS

AGS Aicardi-Goutières syndrome; AIHA Autoimmune haemolytic anaemia; 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 Diarrhoea 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.

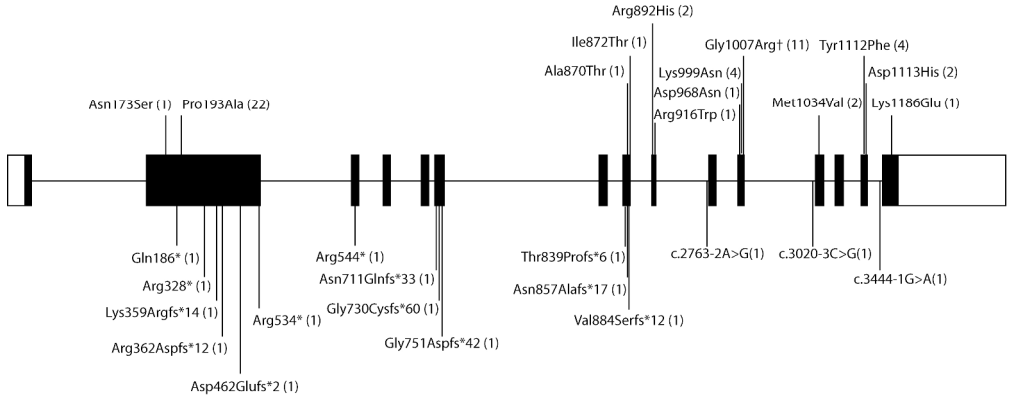

Figure 1. Schematic of ADAR1 gene showing mutations (according to protein nomenclature) ascertained in the present study.

282x110mm (300 x 300 DPI)

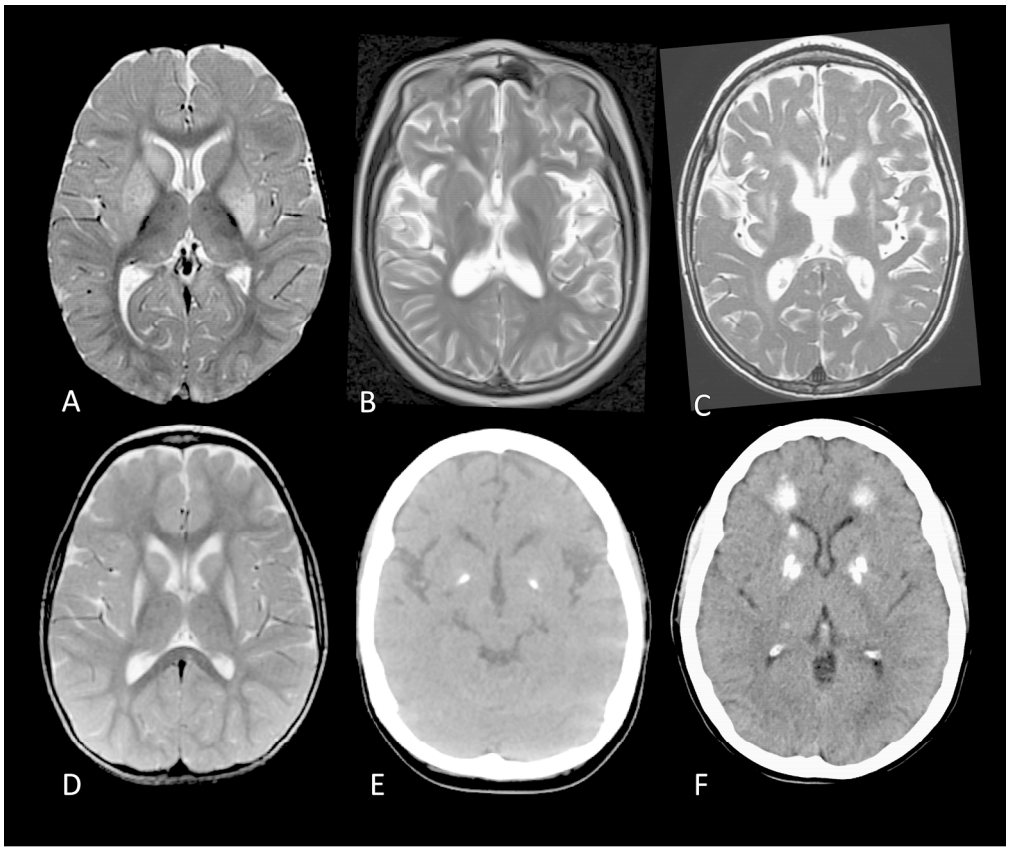

Figure 2. Characteristic neuroradiological features of ADAR1-related disease.

199x168mm (300 x 300 DPI)

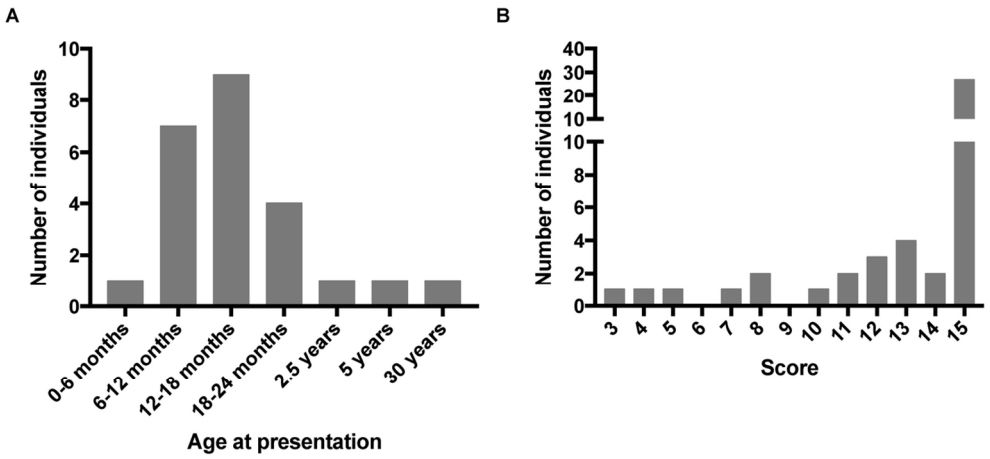

Figure 3. Age at presentation and associated disability.

122x58mm (300 x 300 DPI)

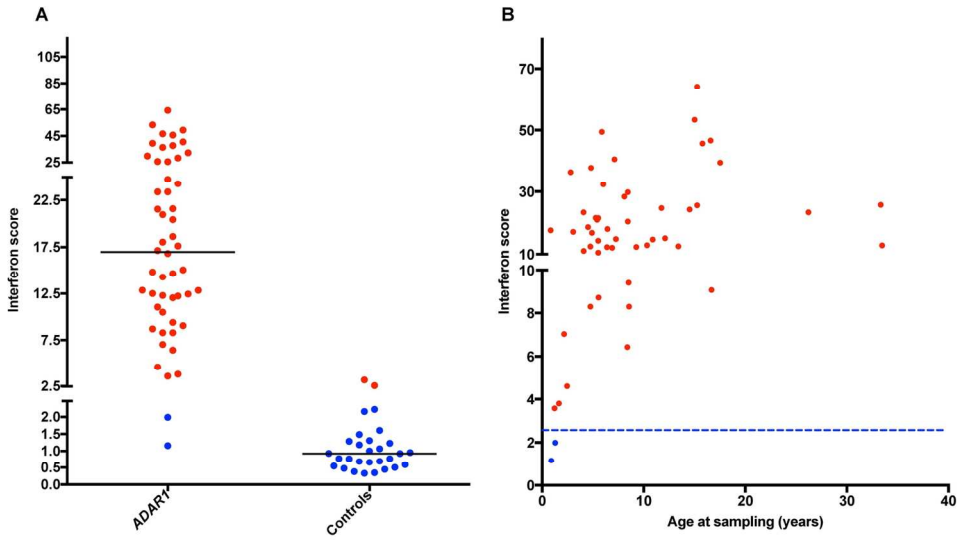

Figure 4. Interferon score data in ADAR1-mutated patients and controls.

149x83mm (300 x 300 DPI)

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Supplementary Table 1. *ADAR1* primer sequences.

Exon	Primer name	Sequence
Exon 1 001 (p150)	adar001E1F	AGCGGAGGGGTTCGACTT
	adar001E1R	ACACTCACAAGACGCACACG
Exon 1 201 (p110)	adar201E1F	ACAGCGGCATTAACACAACG
	adar201E1R	CAACGCCAGAGACTGGAGAG
Exon 2	adar1E2aF	TCACCCTAGCCAAGTCATAAGC
	adar1E2aR	GGACACCCCTGATGTCCACT
	adar1E2bF	CACCTTCCCTCCCAGGACT
	adar1E2bR	GTCTTCCGGTTCCAAACTCG
	adar1E2cF	CGCGGTCTCCACTCAGGCTTGG
	adar1E2cR	CCCTGCCTTTCCATGTCAAT
	adar1E2dF	CTCCTCTGCCCTGAATTTGG
	adar1E2dR	GAAGGAGGGCATCTCCATGA
	adar1E2eF	GCCACAGATGACATCCCAGA
	adar1E2eR	AATCAGCCAAGACTGCGTCA
Exon 3	adar1E3F	AAAGCCCCTTAGCGTAGCAG
	adar1E3R	TTGCCTCAAGGGAGTCAGTT
Exon 4	adar1E4F	CCTTGACAGGTGGTGGGAAT
	adar1E4R	CCTGAGGAGGCAAGGAAGAA
Exon 5	adar1E5F	TGACTGCACAGAGGTGATGTG
	adar1E5R	TGAGGGAGTCACTGGCAATC
Exon 6	adar1E6F	TGAGTGGTTTAAGATTGCCAGTG
	adar1E6R	ACAGACCATCCCCAACTGGT
Exon 7	adar1E7F	ACTGGCCACATCTTCAGCAA
	adar1E7R	ACTCGCATGACAGCAAGAGC
Exon 8	adar1E8F	GATGACTCACACGACAAGTAGAGC
	adar1E8R	GACTCCAGGGGAGGATGAGA
Exon 9	adar1E9F	TGAGGCTGTTTCTGCCTTGA
	adar1E9R	GATTCAGAGGCCGTGTCAGA
Exon 10	adar1E10F	TGTGAAACGTCTGCAACATTGA
	adar1E10R	ACAGGCCAGGAGAGCATTTG
Exon 11	adar1E11F	GCTGTCCACCTCCAGTCTCC
	adar1E11R	TCTGTGCCCAGTGACTAATGG
Exon 12	adar1E12F	GGCCCAAGCTTAAGGAGGAT
	adar1E12R	CCTGGCAATAAAGCATGCAG
Exon 13	adar1E13F	CTCCAAAATCCCCACATGCT
	adar1E13R	GCTACCACTGTGGGCAATGT
Exon 14	adar1E14F	TCATGACCCCACACTTCCTC
	adar1E14R	GCCCTGAGACTGCAGAGGTA
Exon 15	adar1E15aF	GACTTGCAAGGGTGCATCA
	adar1E15aR	ATCCCCTGACCATGTGATGA

Supplementary Figure 1. Summary of interferon score data in parents heterozygous for a recessive *ADAR1*-mutation, either p.Pro193Ala or non-p.Pro193Ala, and in controls.