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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 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-

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

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

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

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.

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,

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).

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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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.

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None of the authors have any financial disclosures to report.

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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.