Genetic and phenotypic spectrum associated with IFIH1 gain-of-function

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Genetic and phenotypic spectrum associated with IFIH1 gain-of-function

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1 | INTRODUCTION

In 2014, heterozygous gain-of-function mutations in IFIH1 were reported to cause a spectrum of neuroimmune phenotypes including classical Aicardi–Goutières syndrome (AGS; Oda et al., 2014; Rice et al., 2014). IFIH1 encodes interferon-induced helicase C domain-containing protein 1 (IFIH1; also known as melanoma differentiation associated gene 5 protein: MDA5) which senses viral double-stranded (ds) RNA in the cytosol, leading to the induction of a type I interferon-mediated antiviral response. Consequent to Mendelian determined gain-of-function, it is suggested that IFIH1 inappropriately senses self-derived nucleic acid as viral, leading to an autoinflammatory state classified as a type I interferonopathy (Ahmad et al., 2018; Crow & Manel, 2015). In 2015, a p.Arg822Gln substitution in IFIH1 was shown to cause Singleton Merten syndrome (SMS), an autosomal dominant trait variably characterized by a deforming arthropathy, abnormal tooth development and cardiac valve calcification, again in association with enhanced type I interferon signaling (Rutsch et al., 2015). Although it was initially considered that SMS was a distinct, mutation-specific disorder, subsequent reports indicate that SMS and the neuroinflammatory phenotypes seen in the context of IFIH1 gain-of-function constitute part of the same disease spectrum (Buers, Rice, Crow, & Rutsch, 2017; Burszttein et al., 2015).

Type I interferonopathy associated IFIH1 mutations are either absent from control databases, or only present at very low frequency. However, we have noted previously that in silico algorithms are not always reliable in differentiating IFIH1 disease-causing variants from benign polymorphisms (Ruaud et al., 2018). Such difficulty in assigning molecular pathogenicity is compounded by marked variability in disease expression, sometimes even within the same family, and the observation of complete non-penetrance in certain pedigrees (Rice et al., 2014). Given this background, we considered it important to provide an update of our experience of sequencing individuals for pathogenic IFIH1 mutations associated with a type I interferonopathy state. In total, we describe molecular and clinical data relating to 74 individuals from 51 families, identifying 27 likely pathogenic mutations that cluster close to the ATP binding region of the protein. Our data confirm variable expression and non-penetrance as important characteristics of these mutant genotypes, and the consistent association with enhanced type I interferon signaling as assessed by interferon-stimulated gene (ISG) expression, referred to as the interferon score.

2 | MATERIALS AND METHODS

2.1 | Subjects

Patients were ascertained through direct contact and/or collaborating physicians across clinical research laboratories in the UK and France (Crow), the USA (Vanderver), and Italy (Orcesi). The study was approved by the Leeds (East) Research Ethics Committee (10/H1307/132), the Comité de Protection des Personnes (ID-RCB/EUDRACT: 2014-A01017-40), IRB study protocol (Myelin Disorders Bioregistry Project: IRB# 14-011236) and the local ethics committee of the IRCCS Mondino Foundation, Pavia, Italy (3549/2009 of 30/9/2009 and 11/12/2009;
Amino acid substitutions were considered as pathogenic mutations when they were seen in the context of a neuroimmune/autoinflammatory state (including AGS, a spastic-dystonic syndrome, nonsyndromic spastic paraparesis or SMS), and when two or more of the following applied: observation of the same variant in an unrelated family; de novo occurrence; documented increase in ISG expression; in vitro data consistent with IFIH1 gain-of-function.

2.2 Mutational analysis

Mutations were identified on a variety of next-generation sequencing platforms. Where Sanger sequencing was undertaken, primers were designed to amplify the coding exons of IFIH1, with mutation annotation based on the reference cDNA sequence NM_002168.2. Variants were assessed using the in silico programs SIFT (http://sift.jcvi.org), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/), and CADD (https://cadd.gs.washington.edu), summarized in VarCards (http://varcards.biols.ac.cn/). Population allele frequencies were obtained from the gnomAD database (http://gnomad.broadinstitute.org).

2.3 Protein modeling

Molecular graphics figures were generated with PyMOL (Schrödinger) using the PDB coordinates (4GL2).

2.4 Interferon score

Interferon scores were calculated on the basis of the expression of ISGs according to previously published protocols. In brief, this involved either a quantitative reverse transcription-polymerase chain reaction (qPCR) analysis using TaqMan probes (Crow laboratory: Rice et al., 2013), or testing on a Nanostring platform (Vandervar laboratory: Adang et al., 2018+). In the former, the relative abundance of IFI27 (Hs01086370_m1), IFI44L (Hs00199115_m1), IFIT1 (Hs00356631_g1), ISG15 (Hs00192713_m1), RSAD2 (Hs01057264_m1), and SIGLEC1 (Hs00988063_m1) transcripts was normalized to the expression levels of Hprt1 (Hs03929096_g1) and 18s (Hs99999901_s1). The median fold change of the six genes, compared to the median of 29 previously collected healthy controls, was then 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. Alternatively, the copy number of mRNA transcripts of the six ISGs listed above, and four housekeeping genes (ALAS1, HPRT1, TBP, and TUBB), was quantified using a Nanostring nCounter™ Digital Analyzer. The raw copy number of mRNA transcripts of each ISG was standardized using the geometric mean of the four housekeeping genes for each individual, and the six-gene interferon signature for each individual calculated using the median of the Z scores, with the result considered positive if ≥1.96 (>98th centile; one tail analysis).

2.5 Interferon reporter assay

The pFLAG-CMV4 plasmid encoding IFIH1 has been described elsewhere (Rice et al., 2014). Indicated mutations were introduced using Phusion HiFi DNA polymerase. HEK 293T cells (ATCC) were maintained in 48-well plates in DMEM (Cellgro) supplemented with 10% fetal bovine serum and 1% L-glutamine. At 80% confluence, cells were cotransfected with pFLAGCMV4 plasmids encoding wild-type or mutant IFIH1 (5 ng, unless indicated otherwise), interferon β (IFNβ) promoter-driven firefly luciferase reporter plasmid (100 ng), and a constitutively expressed Renilla luciferase reporter plasmid (pRL-TK, 10 ng), by using Lipofectamine 2000 (Life Technologies) according to the manufacturer’s protocol. The medium was changed 6 hr after transfection, and cells were subsequently incubated for 18 hr with or without stimulation with poly(I–C) (500 ng; InvivoGen) using Lipofectamine 2000. Cells were lysed with Passive Lysis Buffer (Promega), and IFNβ promoter activity was measured using a Dual-Luciferase Reporter Assay (Promega) and a Synergy 2 plate reader (BioTek). Firefly luciferase activity was normalized to Renilla luciferase activity. Each experiment was performed in triplicate and data are presented as mean ± standard mean of error. Statistical significance was determined by two-tailed, unpaired Student’s t-test with * , **, and *** indicating p values <.05, <.01, and <.001, respectively. Expression levels of individual constructs were tested by western blot analysis.

3 RESULTS

3.1 Molecular data

We collected data on 74 individuals from 51 families, identifying 27 distinct mutations in total (Figure 1; Table 1). Fourteen mutations were recorded in a single proband, seven in more than one individual belonging to a single family, and six in more than one family. Of these six recurrent mutations, the p.Arg720Gln, p.Arg779Cys, and p.Arg779His substitutions were observed most frequently (6, 8, and 10 times, respectively). Twenty-two mutations were recorded to have occurred de novo in at least one individual, whilst four mutations were only ascertained in familial cases demonstrating autosomal dominant transmission (two mutations, p.Ala489Thr and p.Gly495Arg, were transmitted from a father in whom the mutation arose de novo). Three mutations, p Thr331Arg, p.Arg779Cys, and p.Arg779His substitutions, were documented to have occurred both de novo, in association with severe, AGS-like, neurological disease, and in families with transmission across two or more generations.

For six putative mutations (p.Gly389Arg; p.Asn449Lys; p.Ile583Val; p.Ile803Phe; p.Asp848GlU; p.Ile956Val), in silico predictions using both SIFT and Polyphen2 suggested that the substitutions were benign, with relatively poor evolutionary conservation (Figure S1). However, all of these variants were novel (i.e., not recorded in gnomAD), and assays of interferon signaling (ISG expression and in vitro testing) indicate that they represent pathogenic mutations conferring gain-of-function (Table S1; Figure S2). Of note, four of these variants were seen in the context of a spastic paraparesis phenotype with no or minimal cognitive impairment.
Clinical nonpenetrance was observed in three of these families (the other three variants arising in the proband de novo).

### 3.2 Clinical phenotype

Consistent with previous data, we observed a spectrum of phenotypes in our cohort, encompassing classical AGS, less easily defined rapid neuroregression, a spastic-dystonic syndrome, spastic paraparesis, SMS, and clinical nonpenetration (Figure 2; Table 2; Table S2). A single individual, AGS2222, experienced neonatal hepatitis and then developed chronic fibrotic liver disease in the absence of any other clinical features (note that this same variant was seen in another proband, AGS735, presenting with neuroregression at age 1 year). Unequivocal episodes of rapid neuroregression were noted in at least 20 patients, in seven of whom an acute loss of skills occurred after the age of 1 year on a background of completely normal development. Recognition/onset of symptoms was frequently later in patients with a spastic paraparesis phenotype, with one patient experiencing the development of lower limb spasticity beginning at 13 years of age (AGS531_P4). Ten individuals were reported as asymptomatic mutation carriers, across five mutations (p.Gly389Arg, p.Arg779Cys, p.Arg779His, p.Asp848Glu, and p.Ile956Val), with seven aged over 50 years.

### 3.3 Interferon status

Where tested, all mutations (i.e., 26 of 27) were associated with increased expression of ISGs in peripheral blood (Table 1). Samples were unavailable for the single patient carrying the p.Glu773Gln substitution. This variant is not recorded in gnomAD, occurring de novo in the context of a phenotype compatible with IFIH1 upregulation, and conferring a gain-of-function in our in vitro assay (Figure S2). Considering all (51) mutation-positive individuals tested for ISG expression in the Crow laboratory (given that a direct comparison of results across laboratories is not possible), 109 of 117 values were positive (Table S3; Figure S3). Only one clinically symptomatic patient (AGS2154_1) demonstrated a negative interferon signature (on two of three occasions tested). The phenotype, in this case, was unusual; a child with white matter disease confined to the right cerebral hemisphere on MRI and no abnormal neurological signs on examination, having presented at age 8 years with headaches. We leave open the possibility that these two normal results, and three normal results from his mother, might be due to technical artifact, given that the samples had been stored for many months before testing. Sixteen samples from seven clinically nonpenetrant subjects exhibited an upregulation of interferon signaling, with two asymptomatic mutation carriers demonstrating normal interferon signatures (each tested on three occasions).

### 3.4 Modeling of IFIH1 gain-of-function mutations

Modeling of the 27 mutations described here showed that most residues cluster near the ATP binding site within the helicase domain.
| cDNA change | Protein change | Families (de novo inheritance; or, number of symptomatic and non-penetrant individuals where familial) | Associated phenotypes (’/’ within family); (’/’ between families) | Upregulation of interferon signalling | Assessment by interferon reporter assay | gnomAD | SIFT | Polyphen2 | CADD score | Var-cards |
|------------|----------------|-----------------------------------------------|-------------------------------------------------|----------------------------------|---------------------------------|--------|------|--------|----------|---------|
| c.992C>G   | p.Thr331Arg    | AGS674 (de novo); AGS1972 (2;0)               | AGS-SMS; SMS                                    | Yes                              | Yes (de Carvalho et al., 2017) | Novel  | Deleterious 0 | Probably damaging 1000 | 29.7 | 22:23    |
| c.992C>T   | p.Thr331Ile    | AGS1938 (3;0)                                 | SMS                                             | Yes                              | Yes (de Carvalho et al., 2017) | Novel  | Deleterious 0 | Probably damaging 1000 | 31   | 22:23    |
| c.1009A>G  | p.Arg337Gly    | AGS237 (de novo)                              | NR                                              | Yes                              | Yes (Rice et al., 2014)         | Novel  | Tolerated 012 | Probably damaging 1000 | 26.8 | 17:23    |
| c.1165G>A  | p.Gly389Arg    | AGS848 (2;1)                                  | AGS/SP/CNP                                      | Yes                              | Yes (this paper)                | Novel  | Tolerated 088 | Benign 0.108 | 5.325 | 01:23    |
| c.1178A>T  | p.Asp393Val    | AGS626 (de novo)                              | NR                                              | Yes                              | Yes (Rice et al., 2014)         | Novel  | Deleterious 001 | Probably damaging 0.998 | 28.6 | 16:23    |
| c.1178A>C  | p.Asp393Ala    | AGS2586 (de novo)                             | AGS                                             | Yes                              | No                              | Novel  | Deleterious 003 | Possibly damaging 0.913 | 24.8 | 12:23    |
| c.1331A>G  | p.Glu444Gly    | AGS2669 (de novo)                             | AGS                                             | Yes                              | Yes (this paper)                | Novel  | Deleterious 0   | Probably damaging 1     | 31   | 23:23    |
| c.1347C>G  | p.Asn449Lys    | AGS1001 (de novo)                             | SP                                              | Yes                              | Yes (this paper)                | Novel  | Tolerated 064 | Benign 0.163 | 13.91 | 03:23    |
| c.1465G>A  | p.Ala489Thr    | AGS755 (3;0)                                  | CLL/AGS-SMS/SM5                                 | Yes                              | Yes (Bursztejn et al., 2015)    | Novel  | Deleterious 0   | Probably damaging 1000 | 32   | 21:23    |
| c.1483G>A  | p.Gly495Arg    | AGS524 (2;0)                                  | SP-LLD/SP                                      | Yes                              | Yes (Rice et al., 2014)         | Novel  | Deleterious 001 | Probably damaging 0.982 | 23.3 | 14:23    |
| c.1747A>G  | p.Ile583Val    | AGS2369 (de novo)                             | AGS                                             | Yes                              | Yes (this paper)                | Novel  | Tolerated 048 | Benign 0.000 | 0.573 | 5:23     |
| c.2156C>T  | p.Ala719Val    | Hm_1 (de novo)                                | AGS                                             | Yes                              | No                              | Novel  | Tolerated 007 | Possibly damaging 0.949 | 27.1 | 09:23    |
| c.2159G>A  | p.Arg720Gln    | AGS102 (de novo); AGS647 (de novo); AGS1504 (de novo); AGS2422 (NPDT); AGS2548 (de novo); LD_0982.0 (de novo) | AGS; SP                                          | Yes                              | Yes (Rice et al., 2014)         | Novel  | Deleterious 0   | Probably damaging 0.992 | 34   | 17:23    |

(Continues)
| cDNA change | Protein change | Families (de novo inheritance; or, number of symptomatic and non-penetrant individuals where familial) | Associated phenotypes (‘/’ within family); (‘;’ between families) | Upregulation of interferon signalling | Assessment by interferon reporter assay | gnomAD | SIFT | Polyphen2 | CADD score | Var-cards |
|-------------|----------------|-------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|----------------------------------------|----------------------------------------|--------|------|---------|-----------|----------|
| c.2317G>C  | p.Glu773Gln     | AGS2399 (de novo)                                                                                  | NR                                                              | NA                                     | Yes (this paper)                       | Novel  | Tolerated 0.27 | Possibly damaging 0.743 | 24.8     | 13:23     |
| c.2335C>T  | p.Arg779Cys     | AGS376 (NPDT); AGS723 (NPDT); AGS1004 (de novo); AGS1156 (de novo); AGS2154 (1:1); AGS2180 (de novo); AGS2507 (de novo); LD_1030.0 (de novo) | AGS; LLD; SP; ICC; NR; unilateral white matter disease/CNP; AGS | Yes                                    | Yes (Rice et al., 2014)                | Novel  | Deleterious 0.01 | Probably damaging 1.000 | 34       | 21:23     |
| c.2336G>A  | p.Arg779His     | AGS163 (de novo); AGS259 (3:2); AGS1351 (de novo); AGS1509 (de novo); AGS2177 (1:2); Berg_1 (de novo); Orc_0098 (de novo); LD_1199.0 (de novo); LD_1381 (3:1); LD_1585.0 (de novo) | AGS; CNP; NR; SP | Yes                                    | Yes (Rice et al., 2014)                | 1/244230 | Tolerated 0.05 | Probably damaging 0.994 | 28.9     | 19:23     |
| c.2336G>T  | p.Arg779Leu     | LD_1067.0 (de novo)                                                                                | AGS                                                              | Yes                                    | No                                     | Novel  | Tolerated 0.06 | Probably damaging 1.000 | 35       | 21:23     |
| c.2342G>A  | p.Gly781Glu     | LD_0940.0 (de novo); LD_0943.0 (de novo)                                                        | NR; SP                                                           | Yes                                    | No                                     | Novel  | Deleterious 0     | Probably damaging 1.000 | 32       | 19:23     |
| c.2404A>G  | p.Asn802Asp     | AGS2662 (de novo)                                                                                  | NR                                                               | Yes                                    | No                                     | Novel  | Tolerated 0.22 | Probably damaging 1.000 | 28.1     | 18:23     |
| c.2407A>T  | p.Ile803Phe     | LD_1488.0 (de novo)                                                                                | AGS                                                              | Yes                                    | Yes (this paper)                       | Novel  | Tolerated 0.24 | Benign 0.043           | 11.8     | 04:23     |
| c.2465G>A  | p.Arg822Gln     | AGS1514 (de novo)                                                                                  | SD-ICC                                                           | Yes                                    | Yes (Rutsch et al., 2015)              | 6/244096 | Deleterious 0     | Probably damaging 1.000 | 35       | 23:23     |
| c.2471G>A  | p.Arg824Lys     | AGS735 (de novo); AGS2222 (de novo)                                                              | NR; Isolated liver disease                                      | Yes                                    | No                                     | Novel  | Deleterious 0     | Probably damaging 1.000 | 34       | 22:23     |
| c.2486C>G  | p.Thr829Ser     | AGS1290 (2 siblings and NPDT)                                                                       | AGS                                                              | Yes                                    | No                                     | Novel  | Tolerated 0.73 | Possibly damaging 0.512 | 16.61    | 12:23     |
| c.2544T>G  | p.Asp848Glu     | AGS531 (3:2)                                                                                       | SP; ICC/CNP                                                       | Yes                                    | Yes (Ruaud et al., 2018)               | Novel  | Tolerated 0.4    | Benign 0.004           | 10.08    | 02:23     |
Three mutations, p.Ileu583Val, p.Ileu956Val, and p.Leu979Trp, were the only residues not situated in the cluster (colored cyan; only p.Ileu583Val and p.Leu979Val are shown since residue p.Ileu956 is disordered in the crystal structure). Within this main cluster, residues can be further categorized into three groups: those at the ATP binding pocket (magenta spheres), those in the double stranded RNA (dsRNA) binding surface (colored blue) and those not directly involved in either ATP or RNA binding (colored green). Three published mutations (p.Leu372phe; p.Ala452Thr; p.Glu813Asp; Table S4) not ascertained in our cohort are also located within the main cluster (colored orange), further supporting the importance of this region in the regulation of IFIH1 signaling activity.

### DISCUSSION

Here we present data on 74 individuals, 41 previously unreported, from 51 families, with a putative gain-of-function mutation in IFIH1. Consistent with previous descriptions, we observed a spectrum of phenotypes, encompassing AGS, spastic-dystonia, spastic paraparesis, SMS and clinical nonpenetration. Phenotypic variability was common, both in the context of familial inheritance and mutations seen recurrently across families so that no obvious genotype-phenotype correlations could be ascertained.

Acute regression was noted in almost one-third of symptomatic mutation carriers, occurring after the age of 1 year in seven patients demonstrating completely normal development to that time. Beyond acute regression, a slower onset of disease, and subsequent progression, was seen in patients demonstrating a spastic paraparesis phenotype. Together with the observation of clinical nonpenetration (10: 13.5% of 74 mutation-positive individuals in our series), with seven individuals identified to be apparently disease-free beyond the age of 50 years, these data suggest the importance of additive genetic factors and/or environmental triggers in determining phenotypic status. Although we did not formally record neuroimaging features in our cohort, white matter disease and intracranial calcification were observed frequently. Such imaging characteristics can be seen in the absence of overt neurological signs (see Bursztejn et al., 2015 and de Carvalho et al., 2017). Conversely, significant neurological disease, most typically spastic paraparesis, can occur in the context of normal brain and spinal imaging (e.g., the father in family AGSS24).

| cDNA change | Protein change | Families (de novo inheritance; or, number of individuals where familial) | Associated phenotypes and classification ("/") within family ("/") between families) | Upregulation of interferon signaling reporter assay | gnomAD | SIFT | Polyphen2 | CADD score | Varcards | 44.12% | 14.71% | 20.59% | 10.29% | 10.29% |
|-------------|----------------|------------------------------------------------------------------------------|---------------------------------------------------------------------------------|-----------------------------------------------|-----|------|---------|----------|---------|---------|--------|--------|--------|--------|--------|
| c.2561T>A   | p.Met854Lys     | AGS2081 (de novo)                                                            | AGS2; AGS/SMS                                                                   | Yes                                           | No  | Novel| Tolerated| 0.77     | 26.6    | 0.01    | 0.77   | 0.01   | 0.77   | 0.01   |
| c.2866A>G   | p.Ile956Val     | AGS1430 (2:1)                                                                | AGS2; ICC/CNP                                                                   | Yes                                           | Yes | Yes (this paper) | Yes (this paper) | 0.004 | 3.576  | 26.6   | 0.004  | 3.576  | 26.6   | 0.004  |
| c.2936T>G   | p.Leu979Trp     | LD_1346.0 (de novo)                                                          | AGS2; AGS                                                                     | Yes                                           | Yes | Yes (this paper) | Yes (this paper) | 0.01  | 0.01   | 0.01   | 0.01   | 0.01   | 0.01   | 0.01   |

Note: IFIH1 mutation annotation based on the reference complementary DNA sequence NM_022168.2. Upregulation of interferon signaling reporter assay. gnomAD, Polyphen2, and CADD score for deleterious predictions. Varcards, Polyphen2, and CADD score for deleterious predictions.

**FIGURE 2** Overview of phenotypes observed in the IFIH1-mutation-positive cohort. Classification of 68 of 74 individuals according to phenotype. For clarity, six individuals displaying characteristics difficult to classify were omitted from this analysis (Figure 3). Three mutations, p.Ileu583Val, p.Ileu956Val, and p.Leu979Trp were the only residues not situated in the cluster (colored cyan; only p.Ileu583Val and p.Leu979Trp are shown since residue p.Ileu956 is disordered in the crystal structure). Within this main cluster, residues can be further categorized into three groups: those at the ATP binding pocket (magenta spheres), those in the double stranded RNA (dsRNA) binding surface (colored blue) and those not directly involved in either ATP or RNA binding (colored green). Three published mutations (p.Leu372phe; p.Ala452Thr; p.Glu813Asp; Table S4) not ascertained in our cohort are also located within the main cluster (colored orange), further supporting the importance of this region in the regulation of IFIH1 signaling activity.
| Family   | Individual | Sex | cDNA       | Protein          | Inheritance (number of mutation-positive individuals) | Previously reported (reference) | Clinical phenotype                                                                 | Status at last contact (age in years) |
|----------|------------|-----|------------|------------------|-----------------------------------------------------|-------------------------------|-----------------------------------------------------------------------------------|---------------------------------------|
| AGS102   | P1         | M   | c.2159G>A  | p.Arg720Gln      | De novo                                             | Rice et al. (2014)             | AGS                                                                              | Deceased (2)                          |
| AGS163   | P1         | M   | c.2336G>A  | p.Arg779His      | De novo                                             | Rice et al. (2014)             | AGS                                                                              | Alive (13)                            |
| AGS237   | P1         | M   | c.1009A>G  | p.Arg337Gly      | De novo                                             | Rice et al. 2014; Adang et al. 2018 | Neuroregression and SD starting at age 15 months                                | Deceased (16)                         |
| AGS259   | P1         | M   | c.2336G>A  | p.Arg779His      | Familial (3)                                        | Rice et al. (2014)             | AGS                                                                              | Alive (13)                            |
| P2 (father of P1) | M   |     |            |                  |                                                     |                               | Clinical nonpenetrant                                                            | Alive (54)                            |
| P3 (mother of P2) | F   |     |            |                  |                                                     |                               | Clinical nonpenetrant                                                            | Deceased (84)                         |
| AGS376   | P1         | M   | c.2335C>T  | p.Arg779Cys      | No parental testing                                 | Rice et al. (2014)             | AGS with LLĐ                                                                     | Deceased (3)                          |
| AGS524   | P1         | F   | c.1483G>A  | p.Gly495Arg      | Familial (2)(shown to have occurred de novo in P2) | Rice et al. (2014); Hacohen et al. 2015; Crow et al. 2015; McLellan et al. 2018 | SP with LLĐ and AQP4 + TM                                                          | Alive (10)                            |
| P2 (father of P1) | M   |     |            |                  |                                                     |                               | Pure SP                                                                          | Alive (39)                            |
| AGS531   | P1         | F   | c.2544T>G  | p.Asp848Glu      | Familial (5)                                        | Ruaud et al. (2018)            | SP with ICC                                                                      | Alive (13)                            |
| P2 (brother of P1) | M   |     |            |                  |                                                     |                               | Clinical nonpenetrant                                                            | Alive (13)                            |
| P3 (father of P1 and P2) | M   |     |            |                  |                                                     |                               | SP with ICC                                                                      | Alive (40)                            |
| P4 (brother of P3) | M   |     |            |                  |                                                     |                               | SP with ICC                                                                      | Alive (38)                            |
| P5 (father of P3 and P4) | M   |     |            |                  |                                                     |                               | Clinically non-penetrant                                                        | Alive (66)                            |
| AGS626   | P1         | M   | c.1178A>T  | p.Asp393Val      | De novo                                             | Rice et al. (2014)             | Neuroregression and SD starting at 13 months                                      | Alive (13)                            |
| AGS647   | P1         | M   | c.2159G>A  | p.Arg720Gln      | De novo                                             | Rice et al. (2014)             | AGS                                                                              | Alive (2)                             |
| AGS674   | P1         | M   | c.992C>G   | p.Thr331Arg      | De novo                                             | Unreported                     | SP-SMS overlap                                                                   | Alive (14)                            |
| AGS723   | P1         | F   | c.2335C>T  | p.Arg779Cys      | Mother negative; no paternal DNA                    | Unreported                     | SP with ICC                                                                      | Alive (19)                            |
| AGS735   | P1         | M   | c.2471G>A  | p.Arg824Lys      | De novo                                             | Galli et al. 2018              | Neuroregression and SD starting at 12 months                                      | Alive (19)                            |
| AGS755   | P1         | M   | c.1465G>A  | p.Ala489Thr      | Familial (3)                                        | Bursztejn et al. (2015)        | CLL                                                                              | Alive (4)                             |
| P2 (brother of P1) | M   |     |            |                  |                                                     |                               | AGS-SMS overlap                                                                  | Alive (3)                             |
| P3 (father of P1 and P2) | M   |     |            |                  |                                                     |                               | SMS-like                                                                        | Alive (41)                            |
| AGS848   | P1         | M   | c.1165G>A  | p.Gly389Arg      | Familial (3)                                        | Unreported                     | AGS                                                                              | Alive (8)                             |
| P2 (father of P1) | M   |     |            |                  |                                                     |                               | SP                                                                               | Alive (42)                            |
| P3 (maternal grandmother of P2) | F   |     |            |                  |                                                     |                               | Clinically nonpenetrant                                                          | Alive (84)                            |
| AGS1001  | P1         | M   | c.1347C>G  | p.Asn449Lys      | De novo                                             | Unreported                     | SP                                                                               | Alive (19)                            |

(Continues)
| Family    | Individual | Sex | cDNA | Protein | Inheritance (number of mutation-positive individuals) | Previously reported (reference) | Clinical phenotype                                           | Status at last contact (age in years) |
|-----------|------------|-----|------|---------|-----------------------------------------------------|----------------------------------|-------------------------------------------------------------|--------------------------------------|
| AGS1004   | P1         | F   | c.2335C>T | p.Arg779Cys | De novo                                           | Unreported                      | AGS (neuroregression with onset at age 8 months)             | Alive (8)                           |
| AGS1156   | P1         | M   | c.2335C>T | p.Arg779Cys | De novo                                           | Kothur et al. 2018               | AGS (neuroregression with onset at age 8 months)             | Alive (5)                           |
| AGS1290   | P1         | M   | c.2486C>G | p.Thr829Ser | 2 affected (no parental DNA)                      | Unreported                      | AGS                                                          | Alive (6)                           |
| AGS1351   | P1         | M   | c.2336G>A | p.Arg779His | De novo                                           | Unreported                      | AGS                                                          | Alive (4)                           |
| AGS1430   | P1         | F   | c.2866A>G | p.Le956Val  | Familial (3)                                      | Unreported                      | SP with ICC with onset at age 6 years                        | Alive (14)                          |
| AGS1504   | P1         | F   | c.2159G>A | p.Arg720Gln | De novo                                           | Unreported                      | AGS                                                          | Alive (10)                          |
| AGS1509   | P1         | M   | c.2336G>A | p.Arg779His | De novo                                           | Unreported                      | AGS                                                          | Alive (8)                           |
| AGS1514   | P1         | M   | c.2465G>A | p.Arg822Gln | De novo                                           | Buers et al. (2017)             | SD with ICC                                                  | Alive (6)                           |
| AGS1938   | P1         | F   | c.992C>T  | p.Thr331Ile | Familial (3)                                      | de Carvalho et al. (2017)       | SMS                                                          | Alive (18)                          |
| AGS1972   | P1         | F   | c.992C>G  | p.Thr331Arg | Familial (2)                                      | de Carvalho et al. (2017)       | SMS                                                          | Alive (9)                           |
| AGS2081   | P1         | M   | c.2561T>A | p.Met854LyS | De novo                                           | Unreported                      | SP-SMS overlap                                               | Alive (12)                          |
| AGS2154   | P1         | M   | c.2335C>T | p.Arg779Cys | Familial (2)                                      | Unreported                      | Unilateral white matter disease with normal development      | Alive (13)                          |
| AGS2177   | P1         | M   | c.2336G>A | p.Arg779His | Familial (3)                                      | Neuroregression and SD starting at age 12 months | Alive (29)                                                   |                                      |
| AGS2180   | P1         | F   | c.2335C>T | p.Arg779Cys | De novo                                           | Unreported                      | AGS                                                          | Alive (4)                           |
| AGS2222   | P1         | M   | c.2471G>A | p.Arg824LyS | De novo                                           | Unreported                      | Isolated liver disease                                        | Alive (9)                           |
| AGS2369   | P1         | M   | c.1747A>G | p.Ile583Val | De novo                                           | Unreported                      | AGS                                                          | Alive (10)                          |
| Family     | Individual | Sex | cDNA | Protein                      | Inheritance (number of mutation-positive individuals) | Previously reported (reference) | Clinical phenotype                              | Status at last contact (age in years) |
|------------|------------|-----|------|------------------------------|--------------------------------------------------------|---------------------------------|-----------------------------------------------|--------------------------------------|
| AGS2399    | P1         | M   | c.2317G>C | p.Glu773Gln                  | De novo                                                | Unreported                        | Neuroregression and SD starting at age 16 months | Alive (8)                           |
| AGS2422    | P1         | F   | c.2159G>A | p.Arg720Gln                  | No parental testing                                    | Unreported                        | SP                                           | Alive (38)                          |
| AGS2507    | P1         | F   | c.2335C>T | p.Arg779Cys                   | De novo                                                | Unreported                        | AGS                                           | Alive (1)                           |
| AGS2548    | P1         | M   | c.2159G>A | p.Arg720Gln                  | De novo                                                | Unreported                        | AGS                                           | Alive (3)                           |
| AGS2586    | P1         | M   | c.1178A>C | p.Asp393Ala                   | De novo                                                | Unreported                        | AGS-like with frank regression at age 21 months | Alive (3)                           |
| AGS2662 (LD_1640) | P1 | F   | c.2404A>G | p.Asn802Asp                   | De novo                                                | Unreported                        | Neuroregression and SD starting at age 11 months | Alive (1)                           |
| AGS2669    | P1         | M   | c.1331A>G | p.Glu444Gly                   | De novo                                                | Unreported                        | AGS                                           | Deceased (0.5)                      |
| Hm_1       | P1         | F   | c.2156C>T | p.Ala719Val                  | De novo                                                | Unreported                        | AGS                                           | Alive (2)                           |
| Berg_1     | P1         | F   | c.2336G>A | p.Arg779His                   | De novo                                                | Unreported                        | Neuroregression and SD starting at age 9 months | Alive (7)                           |
| Orc_0098   | P1         | M   | c.2336G>A | p.Arg779His                   | De novo                                                | Unreported                        | AGS                                           | Alive (4)                           |
| LD_09400   | P1         | M   | c.2342G>A | p.Gly781Glu                   | De novo                                                | Unreported                        | Neuroregression and SD starting at age 15 months | Alive (5)                           |
| LD_09430   | P1         | F   | c.2342G>A | p.Gly781Glu                   | De novo                                                | Unreported                        | SP                                           | Alive (14)                          |
| LD_09820   | P1         | M   | c.2159G>A | p.Arg720Gln                  | De novo                                                | Adang et al. (2018); Case 2       | AGS                                           | Alive (9)                           |
| LD_10300   | P1         | F   | c.2335C>T | p.Arg779Cys                   | De novo                                                | Unreported                        | AGS                                           | Alive (5)                           |
| LD_10670   | P1         | M   | c.2336G>T | p.Arg779Leu                   | De novo                                                | Unreported                        | AGS                                           | Alive (8)                           |
| LD_11990   | P1         | F   | c.2336G>A | p.Arg779His                   | De novo                                                | Unreported                        | AGS                                           | Alive (4)                           |
| LD_13460   | P1         | M   | c.2936T>G | p.Leu979Trp                   | De novo                                                | Adang et al. (2018); Case 3       | AGS                                           | Deceased (0.4)                      |
| LD_1381 (Hart) | P1 | F   | c.2336G>A | p.Arg779His                   | Familial (4)                                             | Unreported                        | SP                                           | Alive (4)                           |
| P2 (brother of P1) | M |       |       |                              |                                                        |                                 | SP                                           | Alive (3)                           |
| P3 (father of P1 and P2) | M |       |       |                              |                                                        |                                 | SP                                           | Alive (32)                          |
| P4 (father of P3) | M |       |       |                              |                                                        |                                 | Clinically nonpenetrant                     | Alive (68)                          |
| LD_14880   | P1         | F   | c.2407A>T | p.Ile803Phe                   | De novo                                                | Unreported                        | AGS                                           | Alive (2)                           |
| LD_15850   | P1         | F   | c.2336G>A | p.Arg779His                   | De novo                                                | Unreported                        | AGS                                           | Alive (5)                           |

Note: IFIH1 mutation annotation based on the reference complementary DNA sequence NM_022168.2.
Abbreviations: AGS, Aicardi–Goutières syndrome; CLL, Chilblain-like lesions; F, Female; ICC, intracranial calcification; LLD, Lupus-like disease; M, Male; SD, spastic dystonia; SP, spastic paraparesis; SMS: Singleton Merten syndrome; TM, transverse myelitis.
Despite documented clinical nonpenetrance in some cases, all putative IFIH1 gain-of-function substitutions are rare, with only two of the 30 discrete mutations described here and in previous reports recorded in gnomAD. Furthermore, all ascertained type I interferonopathy associated mutations are missense variants, likely conferring increased sensitivity to a self-derived nucleic acid. Although premature termination mutations in the helicase domain are seen in control populations as common polymorphisms, none has been associated with a type I interferonopathy phenotype, further supporting the role of nucleic acid binding by the helicase domain in disease pathogenesis. Substitutions of the arginine residues at positions 720 and 779 were seen in six and 19 probands, respectively, in our series. Given the focus of our laboratories on pediatric neurological disease, our data are likely to subject to ascertainment bias. Indeed, although only observed once by us, the p.Arg822Gln mutation has been reported in an additional five pedigrees demonstrating a classical SMS phenotype (Pettersson et al., 2017; Rutsch et al., 2015).

IFIH1 is a member of the retinoic acid-inducible gene I (RIG-I) receptor family (del Toro Duany, Wu, & Hur, 2015). Recognition of cytoplasmic viral dsRNA by IFIH1 induces filament assembly along the dsRNA axis, with the helicase domains and C terminal domain responsible for RNA recognition. Filament formation then induces oligomerization of the tandem CARD domains (2CARD) of IFIH1, leading to the interaction with mitochondrial MAVS and subsequent induction of interferon and other proinflammatory cytokines. IFIH1 filament stability is intrinsically regulated by ATP hydrolysis, which is stimulated upon dsRNA binding. Mutations that impair ATP hydrolysis generally increase filament stability and, often, but not always, confer gain-of-function signaling activity. The clustering of mutations that we ascertained, and of a further three unique published mutations, near the ATP binding region likely highlights common mechanisms, perhaps increasing RNA binding affinity or decreasing the efficiency of ATP hydrolysis and the rate of filament disassembly.

Summarizing, IFIH1 gain-of-function is associated with a spectrum of phenotypes, occurring due to de novo mutations or transmitted as an autosomal dominant trait. Testing for an interferon signature in blood represents a useful biomarker in this context, which can aid in the interpretation of identified sequence variants.

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CONFLICT OF INTERESTS

Y. J. Crow has undertaken consultancy work with Biogen on behalf of the University of Edinburgh.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions. Identified variants submitted to ClinVar (Submission ID: SUB6667166; Organization ID: 507341).

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SUPPORTING INFORMATION

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