Christiane Zweier ORCID iD: 0000-0001-8002-2020

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

Gillian I Rice,[1*] Sehoon Park,[2,3*] Francesco Gavazzi,[4] Laura A Adang,[4] Loveline A Ayuk,[5] Lien Van Eyck,[6] Luis Seabra,[6] Christophe Barrea,[7] Roberta Battini,[8,9] Alexandre Belot,[10,11] Stefan Berg,[12] Thierry Billette de Villemeur,[13] Annette E Bley,[14] Lubov Blumkin,[15,16] Odile Boespflug-Tanguy,[17,18] Tracy A Briggs,[1,19] Elise Brimble,[20] Russell C Dale,[21] Niklas Darin,[22,23] François-Guillaume Debray,[24] Valentina De Giorgis,[25] Jonas Denecke,[14] Diane Doummar,[26] Gunilla Drake af Hagelsrum,[27] Despina Eleftheriou,[28] Margherita Estienne,[29] Elisa Fazzi,[30,31] François Feillet,[32] Jessica Galli,[30,31] Nicholas Hartog,[33] Julie Harvengt,[34] Bénédicte Heron,[35] Delphine Heron,[36] Diedre A Kelly,[37] Dorit Lev,[16,38] Virginie Levrat,[39] John H Livingston,[40] Itxaso Marti,[41] Cyril Mignot,[42] Fanny Mochel,[43] Marie-Christine Nougues,[44] Ilena Oppermann,[14] Belén Pérez-Dueñas,[45] Bernt Popp,[46] Mathieu P Rodero,[6] Diana Rodriguez,[47,48] Veronica Saletti,[49] Cia Sharpe,[50] Davide Tonduti,[51] Gayatri Vadlamani,[40] Keith Van Haren,[20] Miguel Tomas Vila,[52] Julie Vogt,[53] Evangeline Wassmer,[54] Arnaud Wiedemann,[32] Callum J Wilson,[55] Ayelet Zerem,[15,16] Christiane Zweier,[46] Sameer M Zuberi,[56,57] Simona Orcesi,[25,58] Adeline L Vanderver,[4] Sun Hur,[2,3] Yanick J Crow,[6,59,60]

[1]Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, M13 9PT, United Kingdom

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/humu.23975.

[2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA

[3]Program in Cellular and Molecular Medicine, Boston Children's Hospital, MA 02115, USA

[4]Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA, USA

[5]Paediatric Department, Dumfries and Galloway Royal Infirmary, Cargenbridge, United Kingdom

[6]Laboratory of Neurogenetics and Neuroinflammation, Institut Imagine, Paris, France

[7]Department of Neuropaediatrics, CHU & University of Liège, Belgium

[8]Dept. Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

[9]IRCCS Fondazione Stella Maris, Pisa, Italy

[10]Université de Lyon, INSERM U1111, CIRI, Lyon, France

[11]Centre International de Recherche en Infectiologie, CIRI, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, École Normale Supérieure de Lyon, Université de Lyon, Lyon, France

[12]Pediatric Immunology and Rheumatology, The Queen Silvia Children's Hospital, Goteborg, Sweden

[13]Sorbonne Université, Neuropédiatrie, AP-HP, Hôpital Trousseau, Centre de référence Neurogénétique, Inserm U 1141, Paris, France

[14]University Children's Hospital, University Medical Center Hamburg Eppendorf, Hamburg, Germany

[15]Pediatric Neurology Unit, Metabolic Neurogenetic Service, Wolfson Medical Center, Holon, Israel

[16]Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

[17]Génétique Médicale, Université Paris Diderot, Paris, France

[18]Service de Neuropédiatrie et des Maladies Métaboliques, Centre de Référence Maladies Rares "Leucodystrophies", Hopital Robert Debré, Paris, France

[19]Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester University NHS Foundation Trust, Manchester, United Kingdom

[20]Department of Neurology, Stanford University School of Medicine, Stanford, California, USA

[21]Kids Neuroscience Centre, Brain and Mind Centre, Children's Hospital at Westmead, Faculty of Medicine and Health, University of Sydney, Australia

[22]Department of Pediatrics, Institute of Clinical Sciences, Sahlgrenska University Hospital, University of Gothenburg, Sweden

[23]The Queen Silvia Children's Hospital, Sahlgrenska University Hospital, Gothenburg, Sweden

[24]Metabolic Unit, Department of Medical Genetics, CHU & University of Liège, Belgium

[25]Child Neurology and Psychiatry Unit, IRCCS Mondino Foundation, Pavia, Italy

[26]GHUEP, département de neuropédiatrie, Centre de référence neurogénétique mouvement anormaux de l'enfant, Hôpital Armand Trousseau, Paris, France

[27]Pediatric Neurology, The Queen Silvia Children's Hospital, Goteborg, Sweden

[28]Paediatric rheumatology, University College London (UCL) Great Ormond Street Hospital, Institute of Child Health, ARUK Centre for adolescent rheumatology, London, United Kingdom

[29]U.O. Neuropsichiatria infantile, Fondazione IRCCS, Istituto Neurologico Carlo Besta, Milan, Italy

[30]Unit of Child Neurology and Psichiatry, ASST Spedali Civili of Brescia, Italy

[31]Department of Experimental and Clinical Sciences, University of Brescia, Italy

[32]Service de Médecine Infantile, Centre de Référence des maladies métaboliques de Nancy, CHU Brabois Enfants, Unité INSERM NGERE U1256, Nancy, France

[33]Department of Allergy/Immunology, Spectrum Health Helen Devos Children's Hospital, Michigan State University College of Human Medicine, Michigan, USA

[34]Department of Medical Genetics, CHU & University of Liège, Belgium

[35]Service de Neuropédiatrie, Centre Référence des Maladies Lysosomales, Hôpital Trousseau, Paris, France

[36]UF Génétique Médicale et Centre de Référence "Déficiences Intellectuelles", UMR-S975, Groupe Hospitalier Pitié-Salpêtrière, Paris, France

[37]The Liver Unit, Birmingham Women's and Children's Hospital NHS Foundation Trust, Birmingham, United Kingdom

[38]The Rina Mor Institute of Medical Genetics, Metabolic Neurogenetic Service, Wolfson Medical Center, Holon, Israel

[39]Service de pédiatrie, Centre Hospitalier Annecy Genevois, Pringy, France

[40]Department of Paediatric Neurology, Leeds General Infirmary, Leeds, United Kingdom

[41]Pediatric Neurology, Hospital Universitario Donostia, Universidad del País Vasco UPV-EHU, San Sebastian, Spain

[42]Departement de Génétique & Centre de Référence Déficience Intellectuelle de cause rare, GH Pitié-Sapêtrière, Paris, France

[43]INSERM U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Institut du Cerveau et de la Moelle épinière, ICM, F-75013, Paris, France

[44]Service de Neuropédiatrie, GHUEP, Hôpital Armand Trousseau, APHP, Paris, France

[45]Pediatric Neurology Research Group, Hospital Vall d'Hebron – Research Institute (VHIR), Universitat Autonoma de Barcelona, Barcelona, Spain

[46]Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany

[47]Sorbonne Université, GRC n°19, pathologies Congénitales du Cervelet-LeucoDystrophies, CRMR maladies neurogénétiques, Paris, France

[48]Inserm U1141, Groupe Hospitalier HUEP, Service de Neuropédiatrie, Hôpital Trousseau, Paris, France

[49]Developmental Neurology Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

[50]Paediatric Neurology, Starship Children's Hospital, Auckland, New Zealand

[51]Pediatric Neurology Unit, V. Buzzi Children's Hospital, Milan, Italy

[52]Neuropediatría, Hospital Universitari i Pôlitecnic La Fe, Valencia, Spain

[53]West Midlands Regional Clinical Genetics Service and Birmingham Health Partners, Birmingham Women's and Children's Hospitals NHS Foundation Trust, Birmingham, United Kingdom

[54]Department of Paediatric Neurology, Birmingham Women's and Children's Hospitals NHS Foundation Trust, Birmingham, United Kingdom

[55]National Metabolic Service, Starship Children's Hospital, Auckland, New Zealand

[56]Paediatric Neurosciences Research Group, Royal Hospital for Children, Glasgow, United Kingdom

[57]School of Medicine, University of Glasgow, Glasgow, United Kingdom

[58]Department of Brain and Behavioural Sciences, University of Pavia, Pavia, Italy

[59]Paris Descartes University, Sorbonne-Paris-Cité, Institut Imagine, Paris, France

[60]Centre for Genomic and Experimental Medicine, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom

*Denotes equal contributions

Corresponding authors

Yanick J. Crow (yanickcrow@mac.com)

Centre for Genomic and Experimental Medicine, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom

Sun Hur (Sun.Hur@childrens.harvard.edu)

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA; Program in Cellular and Molecular Medicine, Boston Children's Hospital, MA 02115, USA

Funding sources

YJC acknowledges the European Research Council (786142 - E-T1IFNs), a state subsidy managed by the National Research Agency (France) under the 'Investments for the Future' program bearing the reference ANR-10-IAHU-01 and the MSDAvenir fund (DEVO-DECODE Project). TAB acknowledges the National Institute for Health Research (NIHR) (NIHR Transitional Research Fellowship, TRF-2016-09-002; with the views expressed being those of the author and not necessarily those of the NHS, the NIHR or the Department of Health). ALV is supported by the Kamens endowed chair for Translational Neurotherapeutics, and the Myelin Disorders Bioregistry Project. ALV and LA acknowledge the CURE Pennsylvania Frontiers in Leukodystrophy grant and U01HD082806. LA also acknowledges the National Center for Advancing Translational Sciences of the National Institutes of Health under award number KL2TR001879. LVE received funding from Research Foundation Flanders (FWO).

Declarations

YJC has undertaken consultancy work with Biogen on behalf of the University of Edinburgh.

Total word count

2670

Abstract

IFIH1 gain-of-function has been reported as a cause of a type I interferonopathy encompassing a spectrum of autoinflammatory phenotypes including Aicardi-Goutières syndrome and Singleton Merten syndrome. Ascertaining patients through a European and North American collaboration, we set out to describe the molecular, clinical and interferon status of a cohort of individuals with pathogenic heterozygous mutations in *IFIH1*. We identified 74 individuals from 51 families segregating a total of 27 likely pathogenic mutations in *IFIH1*. Ten adult individuals, 13.5% of all mutation carriers, were clinically asymptomatic (with seven of these aged over 50 years). All mutations were associated with enhanced type I interferon signaling, including six variants (22%) which were predicted as benign according to multiple *in silico* pathogenicity programs. The identified mutations cluster close to the ATP binding region of the protein. These data confirm variable expression and non-penetrance as important characteristics of the *IFIH1* genotype, a consistent association with enhanced type I interferon signaling, and a common mutational mechanism involving increased RNA binding affinity or decreased efficiency of ATP hydrolysis and filament disassembly rate.

Key words:

IFIH1

MDA5

Type I interferonopathy

Aicardi-Goutières syndrome

Singleton Merten syndrome

Short title:

IFIH1 gain-of-function: genetic and phenotypic spectrum

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 (IFHI1)(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 anti-viral response. Consequent upon 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; Bursztejn 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 which 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.

Materials and methods

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; n.20170035275 of 23/10/2017). 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, non-syndromic 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.

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 *IFH1*, with mutation annotation based on the reference cDNA sequence NM_022168.2. 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).

Protein modelling

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

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 (Vanderver 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* (Hs999999001_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 i.e.

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).

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 co-transfected with pFLAGCMV4 plasmids encoding wild-type or mutant *IFIH1* (5 ng, unless indicated otherwise), interferon beta (*IFNb*) 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 hours after transfection, and cells were subsequently incubated for 18 hours with or without stimulation with poly(I-C) (500 ng; InvivoGen) using Lipofectamine 2000. Cells were lysed with Passive Lysis Buffer (Promega), and *IFNb* 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 ± SEM. Statistical significance was determined by two-tailed, unpaired Student's t-test with *, ** and *** indicating P values <0.05, <0.01 and <0.001 respectively. Expression levels of individual constructs were tested by western blotting.

Results

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, 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 Poyphen2 suggested that the substitutions were benign, with relatively poor evolutionary conservation (Supp. 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 (Supp. Table S1; Supp. 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 non-penetrance was observed in three of these families (the other three variants arising in the proband *de novo*).

Clinical phenotype

Consistent with previous data, we observed a spectrum of phenotypes in our cohort, encompassing classical AGS, less easily defined rapid neuro-regression, a spastic-dystonic syndrome, spastic paraparesis, SMS and clinical non-penetrance (Figure 2; Table 2; Supp. 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 neuro-regression at age 1 year). Unequivocal episodes of rapid neuro-regression were noted in at least 20 patients, in seven of whom an acute loss of skills occurred after the age of one 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). Six symptomatic patients were recorded to have died. Five of these individuals demonstrated a severe AGS phenotype with features obvious at, or soon after, birth i.e. indicating prenatal onset. One further deceased patient presented with neuro-regression at age 15 months, and died suddenly of a cardio-respiratory arrest at 16 years of age, with pulmonary hypertension documented on post-mortem examination. 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.

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 (Supp. 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 (Supp. Table S3; Supp. 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 non-penetrant subjects exhibited an upregulation of interferon signaling, with two asymptomatic mutation carriers demonstrating normal interferon signatures (each tested on three occasions).

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 (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.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 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; Supp. 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 non-penetrance. 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 one 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 non-penetrance (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) for examples). 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 AGS524).

Clinically manifest extra-neurological illness was uncommon in our series, but there appears to be a real association between IFIH1 gain-of-function and a lupus-like illness, auto-immune hepatitis and hypothyroidism. Furthermore, a psoriatic-like skin

disease is a well-recognized feature of the SMS phenotype. As recently described (Adang et al., 2018), two patients included here were diagnosed with pulmonary hypertension, a feature which was not searched for in most patients and may be under-recognized.

We observed a strong association of mutation status with an enhanced expression of ISGs, with 109 of 117 samples from 51 patients being positive in the experience of one laboratory. A similar conclusion can be drawn from *in vitro* testing. As such, upregulated interferon signaling represents a reliable biomarker of IFIH1 gain-of-function, and can serve as an indicator of variant pathogenicity where doubt exists as to the significance of a molecular lesion. This is important given that we show here that *in silico* algorithms do not always accurately predict pathogenicity (involving 22% of the mutations that we recorded). Where tested, clinical non-penetrance was also associated with a persistent upregulation of interferon signaling, with only two of nine such individuals non-penetrant on ISG testing in blood. Whether these individuals demonstrate fluctuations in ISG expression is not known at this time.

Despite documented clinical non-penetrance 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 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 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 (CTD) 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 pro-inflammatory 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.

Data Availability Statement

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

Acknowledgements

YJC acknowledges The University of Maryland Brain and Tissue Bank of the NIH NeuroBioBank.

References

Adang, L. A., Frank, D. B., Gilani, A., Takanohashi, A., Ulrick, N., Collins, A.,... Vanderver, A. L. (2018). Aicardi goutieres syndrome is associated with pulmonary hypertension. *Mol Genet Metab, 125*(4), 351-358. doi:10.1016/j.ymgme.2018.09.004

Ahmad, S., Mu, X., Yang, F., Greenwald, E., Park, J. W., Jacob, E.,... Hur, S. (2018). Breaching Self-Tolerance to Alu Duplex RNA Underlies MDA5-Mediated Inflammation. *Cell, 172*(4), 797-810 e713. doi:10.1016/j.cell.2017.12.016

Buers, I., Rice, G. I., Crow, Y. J., & Rutsch, F. (2017). MDA5-Associated Neuroinflammation and the Singleton-Merten Syndrome: Two Faces of the Same Type I Interferonopathy Spectrum. *J Interferon Cytokine Res, 37*(5), 214-219. doi:10.1089/jir.2017.0004

Bursztejn, A. C., Briggs, T. A., del Toro Duany, Y., Anderson, B. H., O'Sullivan, J., Williams, S. G.,... Crow, Y. J. (2015). Unusual cutaneous features associated with a heterozygous gain-of-function mutation in IFIH1: overlap between Aicardi-Goutieres and Singleton-Merten syndromes. *Br J Dermatol, 173*(6), 1505-1513. doi:10.1111/bjd.14073

Crow, Y. J., & Manel, N. (2015). Aicardi-Goutieres syndrome and the type I interferonopathies. *Nat Rev Immunol, 15*(7), 429-440. doi:10.1038/nri3850

de Carvalho, L. M., Ngoumou, G., Park, J. W., Ehmke, N., Deigendesch, N., Kitabayashi, N.,... Crow, Y. J. (2017). Musculoskeletal Disease in MDA5-Related Type I Interferonopathy: A Mendelian Mimic of Jaccoud's Arthropathy. *Arthritis Rheumatol, doi: 10.1002/art.40179.* doi:10.1002/art.40179

del Toro Duany, Y., Wu, B., & Hur, S. (2015). MDA5-filament, dynamics and disease. *Curr Opin Virol, 12*, 20-25. doi:10.1016/j.coviro.2015.01.011

Oda, H., Nakagawa, K., Abe, J., Awaya, T., Funabiki, M., Hijikata, A.,... Heike, T. (2014). Aicardi-Goutieres syndrome is caused by IFIH1 mutations. *Am J Hum Genet, 95*(1), 121-125. doi:S0002-9297(14)00269-9 [pii]

10.1016/j.ajhg.2014.06.007

Pettersson, M., Bergendal, B., Norderyd, J., Nilsson, D., Anderlid, B. M., Nordgren, A., & Lindstrand, A. (2017). Further evidence for specific IFIH1 mutation as a cause of Singleton-Merten syndrome with phenotypic heterogeneity. *Am J Med Genet A, 173*(5), 1396-1399. doi:10.1002/ajmg.a.38214

Rice, G. I., Del Toro Duany, Y., Jenkinson, E. M., Forte, G. M., Anderson, B. H., Ariaudo, G.,... Crow, Y. J. (2014). Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling. *Nat Genet, 46*(5), 503-509. doi:ng.2933 [pii]

10.1038/ng.2933

Rice, G. I., Forte, G. M., Szynkiewicz, M., Chase, D. S., Aeby, A., Abdel-Hamid, M. S.,... Crow, Y. J. (2013). Assessment of interferon-related biomarkers in

Aicardi-Goutieres syndrome associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, and ADAR: a case-control study. *Lancet Neurol, 12*(12), 1159-1169. doi:S1474-4422(13)70258-8 [pii]

10.1016/S1474-4422(13)70258-8

Ruaud, L., Rice, G. I., Cabrol, C., Piard, J., Rodero, M., van Eyk, L.,... Van Maldergem, L. (2018). Autosomal-dominant early-onset spastic paraparesis with brain calcification due to IFIH1 gain-of-function. *Hum Mutat, 39*(8), 1076-1080. doi:10.1002/humu.23554

Rutsch, F., MacDougall, M., Lu, C., Buers, I., Mamaeva, O., Nitschke, Y.,... Hennekam, R. C. (2015). A Specific IFIH1 Gain-of-Function Mutation Causes Singleton-Merten Syndrome. *Am J Hum Genet, 96*(2), 275-282. doi:10.1016/j.ajhg.2014.12.014

Figure legends

Figure 1. Schematic showing the positions of protein domains and their amino acid boundaries within the 1,025-residue IFIH1 protein. The 27 mutations ascertained in the present study are annotated, with the numbers in brackets indicating the number of families in which each mutation was observed. Three previously published mutations (p.Leu372Phe; p.Ala452Thr; p.Glu813Asp), not ascertained in our series, are also denoted (below the cartoon).

CARD, caspase activation recruitment domain; Hel, helicase domain, where Hel1 and Hel2 are the two conserved core helicase domains and Hel2i is an insertion domain that is conserved in the RIG-I–like helicase family; P, pincer or bridge region connecting Hel2 to the C-terminal domain (CTD) involved in binding dsRNA.

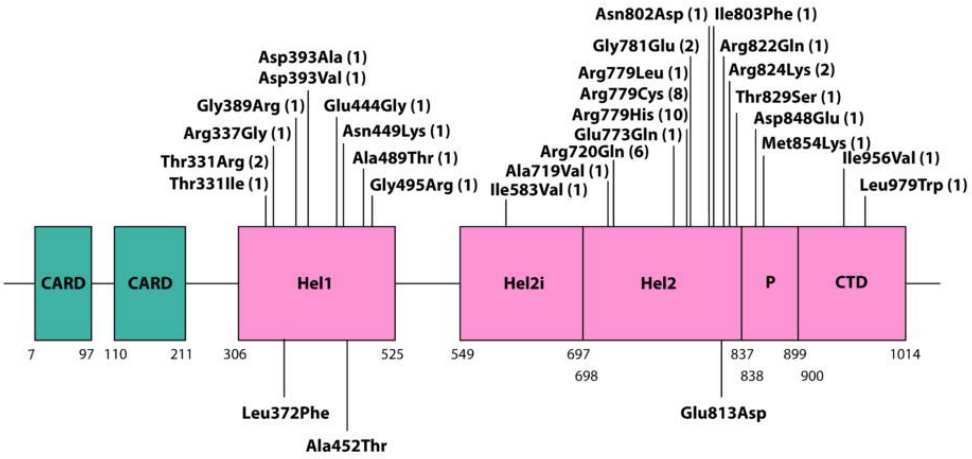

Figure 2. Overview of phenotypes observed in the *IFIH1*-mutation positive cohort. Classification of 68 of 74 individuals according to phenotype. For clarity, six individuals displaying characteristics difficult to classify were omitted from this analysis.

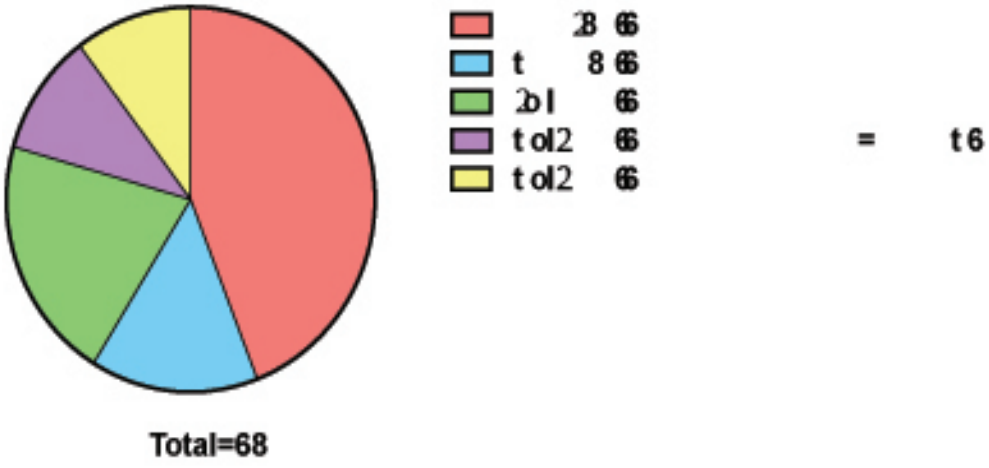

Figure 3. Mutation mapping. Structure of human IFIH1 (4GL2) in complex with dsRNA (blue stick model in the center). Only the RNA binding domain (helicase domain and C-terminal domain (CTD)) are included in the crystal structure. Note that the helicase domain consists of Hel1, Hel2i and Hel2. Mutations are indicated by spheres using the following color code: residues in the ATP binding pocket (magenta), residues in the dsRNA binding surface (blue), residues within the main cluster but not directly involved in RNA binding or ATP binding (green), residues outside the main cluster (cyan), and residues previously reported by others but not in our cohort (orange). We considered all 27 mutations reported here plus three previously published mutations (p.Leu372Phe; p.Ala452Thr; p.Glu813Asp) not ascertained in our series. Residues p.Arg822, p.Arg824 and p.Ile956 are not shown because they are disordered in the crystal structure, but are expected to be located in the ATP binding (p.Arg822 and p.Arg824) and RNA binding (p.Ile956) pockets.

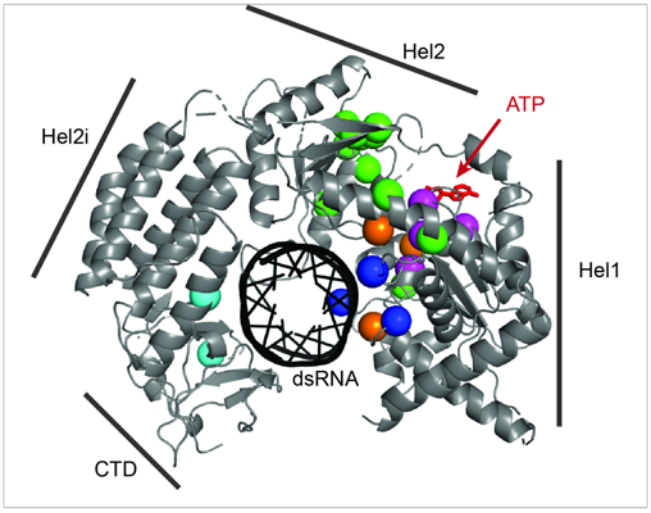

Table 1. Details of individual *IFIH1* mutations identified in the families included in the present data set

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	Polyphen 2	CADD score	Varcards
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 1.000	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 1.000	31	22:23
c.1009A>G	p.Arg337Gly	AGS237 (de novo)	NR	Yes	Yes (Rice et al. 2014)	Novel	Tolerated 0.12	Probably damaging 1.000	26.8	17:23
c.1165G>A	p.Gly389Arg	AGS848 (2;1)	AGS/SP/CNP	Yes	Yes (this paper)	Novel	Tolerated 0.88	Benign 0.108	5.325	01:23
c.1178A>T	p.Asp393Va	AGS626 (de	NR	Yes	Yes (Rice et al.	Novel	Deleterious	Probably dama	28.6	16:23

	l	novo)			2014)		0.01	ging 0.998		
c.1178A>C	p.Asp393Ala	AGS2586 (de novo)	AGS	Yes	No	Novel	Deleterious 0.03	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 0.64	Benign 0.163	13.91	03:23
c.1465G>A	p.Ala489Thr	AGS755 (3;0)*	CLL/AGS-SMS/SMS	Yes	Yes (Burszt ejn et al. 2015)	Novel	Deleterious 0	Probably damaging 1.000	32	21:23
c.1483G>A	p.Gly495Arg	AGS524 (2;0)*	SP-LLD/SP	Yes	Yes (Rice et al. 2014)	Novel	Deleterious 0.01	Probably damaging 0.982	23.3	14:23
c.1747A>G	p.Ile583Val	AGS2369 (de novo)	AGS	Yes	Yes (this paper)	Novel	Tolerated 0.48	Benign 0.00	0.573	5.23
c.2156C>T	p.Ala719Val	Hm_1 (de novo)	AGS	Yes	No	Novel	Tolerated 0.07	Possibly damaging 0.949	27.1	09:23
c.2159G>A	p.Arg720Gln	AGS102 (de novo); AGS647 (de novo); AGS1504	AGS; SP	Yes	Yes (Rice et al. 2014)	Novel	Deleterious 0	Probably damaging 0.992	34	17:23

		(de novo); AGS2422 (NPDT); AGS2548 (de novo); LD_0982.0 (de novo)								
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	NR	Yes	No	Novel	Tolerated 0.22	Probably damaging	28.1	18:23

		novo)						1.000		
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
c.2561T>A	p.Met854Lys	AGS2081 (de novo)	AGS/SMS	Yes	No	Novel	Deleterious 0	Probably damaging 1.000	31	18:23
c.2866A>G	p.Ile956Val	AGS1430 (2;1)	SP-ICC/CNP	Yes	Yes (this paper)	Novel	Tolerated 0.77	Benign 0.004	3.576	06:23
c.2936T>G	p.Leu979Trp	LD_1346.0 (de novo)	AGS	Yes	Yes (this paper)	Novel	Deleterious 0.01	Probably damaging 1.000	26.6	16:23

IFIH1 mutation annotation based on the reference cDNA sequence NM_022168.2

*This mutation was shown to have been paternally inherited by the proband, and to have occurred *de novo* in the proband's father.

NPDT: No parental DNA testing.

AGS: Aicardi-Goutières syndrome; CLL: Chilblain-like lesions; CNP: Clinical non-penetrance; ICC: Intracranial calcification; LLD: Lupus-like disease; NR: Neuro-regression; SD: Spastic dystonia; SP: Spastic paraparesis; SMS: Singleton Merten syndrome

Table 2. Molecular and clinical data by family

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.	AGS	Deceased (2)
AGS163	P1	M	c.2336G>A	p.Arg779His	De novo	Rice et al.	AGS	Alive (13)
AGS237 (LD_0762)	P1	M	c.1009A>G	p.Arg337Gly	De novo	Rice et al.; Adang et al.	Neuroregression and SD starting at age 15 months	Deceased (16)
AGS259	P1	M	c.2336G>A	p.Arg779His	Familial (3)	Rice et al.	AGS	Alive (13)
	P2 (father of P1)	M					Clinically non-penetrant	Alive (54)

	P3 (mother of P2)	F					Clinically non-penetrant	Deceased (84)
AGS376	P1	M	c.2335C>T	p.Arg779Cys	No parental testing	Rice et al.	AGS with LLD	Deceased (3)
AGS524	P1	F	c.1483G>A	p.Gly495Arg	Familial (2)(shown to have occurred de novo in P2)	Rice et al.; Hacohen et al.; Crow et al.; McLellan et al.	SP with LLD 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.	SP with ICC	Alive (13)
	P2 (brother of P1)	M					Clinically non-penetrant	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	M					Clinically non-penetrant	Alive (66)

	P4)							
AGS626	P1	M	c.1178A>T	p.Asp393Val	De novo	Rice et al.	Neuroregression and SD starting at 13 months	Alive (13)
AGS647	P1	M	c.2159G>A	p.Arg720Gln	De novo	Rice et al.	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.	Neuroregression and SD starting at 12 months	Alive (19)
AGS755	P1	M	c.1465G>A	p.Ala489Thr	Familial (3)	Burszt ejn et al.	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 non-penetrant	Alive (84)
AGS1001	P1	M	c.1347C>G	p.Asn449Lys	De novo	Unreported	SP	Alive (19)
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.	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)
	P2 (brother of P1)	M					AGS	Alive (4)
AGS1351	P1	F	c.2336G>A	p.Arg779His	De novo	Unreported	AGS	Deceased (2)
AGS1430	P1	M	c.2866A>G	p.Ile956Val	Familial (3)	Unreported	SP with ICC with onset at age 6	Alive (14)

							years	
	P2 (father of P1)	M					SP with onset at age 2 years	Alive (50)
	P3 (father of P2)	M					Clinically non-penetrant	Alive (72)
AGS1504 (LD_1175)	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.	SD with ICC	Alive (6)
AGS1938	P1	F	c.992C>T	p.Thr331Ile	Familial (3)	de Carvalho et al.	SMS	Alive (18)
	P2 (mother of P1)	F					SMS	Alive (45)
	P3 (sister of P2)	F					SMS	Alive (27)
AGS1972	P1	F	c.992C>G	p.Thr331Arg	Familial (2)	de Carvalho et al.	SMS	Alive (9)
	P2 (father of P1)	M					SMS	Alive (47)

AGS2081	P1	M	c.2561T>A	p.Met854Lys	De novo	Unreported	SP-SMS overlap	Alive (12)
AGS2154 (LD_1240)	P1	M	c.2335C>T	p.Arg779Cys	Familial (2)	Unreported	Unilateral white matter disease with normal development	Alive (13)
	P2 (mother of P1)	F					Clinically non-penetrant	Alive (40)
AGS2177	P1	M	c.2336G>A	p.Arg779His	Familial (3)	Popp et al. 2017	Neuroregression and SD starting at age 12 months	Alive (29)
	P2 (mother of P1)	F					Clinically non-penetrant	Alive (62)
	P3 (sister of P1)	F					Clinically non-penetrant	Alive (33)
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)
AGS2399	P1	M	c.2317G>C	p.Glu773Gln	De novo	Unreported	Neuroregression and SD starting at	Alive (8)

							age 16 months	
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_0940.0	P1	M	c.2342G>A	p.Gly781Glu	De novo	Unreported	Neuroregression and SD starting at age 15 months	Alive (5)
LD_0943.0	P1	F	c.2342G>A	p.Gly781Glu	De novo	Unreported	SP	Alive (14)
LD_0982.0	P1	M	c.2159G>A	p.Arg720Gln	De novo	Adang et al. (Case 2)	AGS	Alive (9)
LD_1030.0	P1	F	c.2335C>T	p.Arg779Cys	De novo	Unreported	AGS	Alive (5)
LD_1067.0	P1	M	c.2336G>T	p.Arg779Leu	De novo	Unreported	AGS	Alive (8)
LD_1199.0	P1	F	c.2336G>A	p.Arg779His	De novo	Unreported	AGS	Alive (4)
LD_1346.0	P1	M	c.2936T>G	p.Leu979Trp	De novo	Adang et al. (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 non-penetrant	Alive (68)
LD_1488.0	P1	F	c.2407A>T	p.Ile803Phe	De novo	Unreported	AGS	Alive (2)
LD_1585.0	P1	F	c.2336G>A	p.Arg779His	De novo	Unreported	AGS	Alive (5)

IFIH1 mutation annotation based on the reference cDNA sequence NM_022168.2

AGS: Aicardi-Goutières syndrome; CLL: Chilblain-like lesions; F: Female; LLD: Lupus-like disease; M: Male; ICC: Intracranial calcification; SD: Spastic dystonia; SP: Spastic paraparesis; SMS: Singleton Merten syndrome; TM: Transverse myelitis