Gain-of-function mutations in *IFIH1* cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling

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Supplementary Materials

Supplementary Note

F102. This male child was born to non-consanguineous European Italian parents with no family history of note. He was delivered at 34 weeks gestation by caesarean section indicated because of intrauterine growth retardation (birth weight 1.5 kg; head circumference 29 cm; length 29 cm). In the neonatal period he demonstrated congenital microcephaly, hepatosplenomegaly and thrombocytopenia. He was irritable and fed poorly in the first months of life, and was recognized to have profound developmental delay with spastic-dystonic tetraparesis. Cranial imaging at age 8 months revealed cerebral atrophy with basal ganglia calcifications and white matter disease. At the age of 12 months, interferon activity was recorded as 150 IU/ml and 25 IU/ml in the CSF and serum respectively. At 13 months of age he presented with seizures which were difficult to control. He was also recorded to have hypertension of unknown cause. Autoimmune screening (including ANA, ANCA and ENA antibody testing) at 22 months of age was negative. He died of pneumonia at the age of 2 years.

His phenotype is characteristic of Aicardi-Goutières syndrome (AGS).

F163. This male child was born to non-consanguineous European French parents with no family history of note. After an unremarkable pregnancy and delivery at 39 weeks gestation (birth weight 2.9 kg; head circumference 34 cm), he presented before the age of 1 month with irritability, feeding difficulties, axial hypotonia and abnormal limb posturing. Cranial CT and MRI scanning around the age of 2 years demonstrated cerebral and cerebellar atrophy with periventricular calcification and abnormal high T2 signal of the deep white matter. At age 52 months his CSF interferon activity was 37 IU/ml. Two months later the level was 25 IU/ml, with serum interferon activity recorded as 50 IU/ml. Further serum samples at the ages of 58 months and 8 years 6 months were 25 and 37 IU/ml respectively. At age 12 years he presented with generalized urticaria and associated oedema which was treated with steroids and antihistamines. Autoimmune investigations revealed only a mildly positive antinuclear factor. Now, at the age of 13 years, he demonstrates a severe quadriplegia, with no head control, no speech, abnormal ocular movements and microcephaly. He is fed by gastrostomy.

His phenotype is characteristic of AGS.

F237. This male child was born to non-consanguineous white North American parents with no family history of note. His development was considered normal until the age of 15 months, at which time he was riding a push car and had 6 - 10 words. After this point he developed intermittent posturing and rigidity of his legs, and then of the upper extremities. He also developed exaggerated startles which have persisted. He subsequently experienced a relentless loss of motor and intellectual skills, so that by age 24 months he was unable to sit unsupported and had lost the ability to swallow. Between 15 months and 4 years of age he demonstrated a fluctuating pattern of poor sleep, with persistent whining and crying. CSF neopterin was 4 x and 3 x the upper limit of normal at 2 years and 7 years of age respectively. Calcification of the basal ganglia and white matter were observed on cranial CT imaging at age 2 years, with abnormal high signal of the deep white matter seen on T2 weighted MRI. Extensive immunological testing at age 3 years was normal. Now, at the age of 12 years, he has no useful hand function, cannot sit independently and has limited words, although his understanding is relatively preserved.

His phenotype conforms to one of rapid onset of neuro-regression with spasticity and dystonia beginning at age 15 months.

F259_1. This male child was born to non-consanguineous European Italian parents with no family history of note. After an unremarkable pregnancy and delivery at 40 weeks gestation (birth weight 3.1 kg; head circumference 34 cm), and an apparently normal early neonatal history, at age 6 months he was noted to demonstrate axial hypotonia and lower limb spasticity. Cranial CT scan at 2 years 6 months of age demonstrated cerebral and cerebellar atrophy with periventricular calcification. MRI at the same age revealed abnormal high T2 signal of the deep white matter. At age 31 months his CSF showed a normal white cell count, but a raised level of interferon activity (50 IU/ ml: serum level of <2IU/ml). He developed chilblains aged 6 years. Now, at the age of 8 years, he is severely delayed with a head circumference of 48 cm. He has never acquired the ability to sit, has minimal hand function (he will reach out for some objects), feeds via gastrostomy, and cannot communicate – although he can smile and cry when apparently content / upset. He has abnormal eye movements with nystagmus, but seems to have some useful vision. He demonstrates marked dystonic posturing of the limbs with axial hypotonia.

His phenotype is characteristic of AGS.

F259_2. At the age of 48 years, the father of AGS259_1 is completely asymptomatic with no recognized clinical signs. He has not undergone any neuro-imaging.

F259_3. Up to the age of 79 years, the paternal grandmother of AGS259_1 has remained healthy throughout her life apart from a squint recognized in infancy, and a left hip replacement for osteoarthritis at age 78 years. She has not undergone any neuro-imaging.

F376. This male child was born to non-consanguineous white British parents with no family history of note. He was delivered at 35 weeks gestation indicated because of significant oligohydramnios and intra-uterine growth retardation (birth weight 1.9 kg). The child was irritable, fed poorly, and experienced recurrent chest infections. Developmental delay was obvious by age 4 months, with central hypotonia, peripheral hypertonia and poor head growth. He presented at age 29 months with a 4 week history of recurring fevers, irritability, extensor spasms, developmental regression, weight loss and diarrhea. On examination, in addition to the preexisting neurological signs, he exhibited a florid ulcerative photosensitive vasculitic rash over the face and trunk, hepatosplenomegaly, generalized lymphadenopathy, and arthritis of the knees and ankles. He had serositis with pericardial effusion, a pronounced inflammatory response with raised ESR and CRP, haemolytic anaemia, and markedly abnormal autoantibody profile (ANA >1:640, dsDNA 1200 IU/ml, raised anti-cardiolipin antibodies, low complement C30.53, C4 0.11 g/l, and strongly positive pANCA). An infection and malignancy screen, including for HIV and hepatitis, was negative. At age 30 months his CSF interferon activity was 6 IU/L, with a serum level of 9 IU/L (normal <2). Cranial CT at the same age demonstrated gross cerebral atrophy with basal ganglia and white matter calcification, together with abnormal high signal in the periventricular and deep white matter on T2 weighted imaging. Treatment with a variety of immunosuppressive drugs (steroids, Cyclophosphamide, Infliximab, Rituximab, immunoglobulins and Azathioprine) was associated with an improvement in the quality of his social interactions, skin rash, lymphadenopathy, and blood indices. However, therapeutic weaning was impossible due recurrent flares, particularly of gastrointestinal vasculitis with rectal bleeds. He subsequently died at age 3 years 6 months after possible hemophagocytic lymphohistiocytosis with severe liver derangement (pancytopenia, ferritin of 3700, low fibrinogen).

His phenotype is characteristic of AGS associated with a severe lupus-like disease.

F524_1. This female child was born to non-consanguineous white British parents. There was no other family history of note beyond the problems affecting her father (see below). She was delivered at term (birth weight 3 kg). Her motor development was delayed, so that at age 21 months she was only just able to maintain a stable sitting position, and she

demonstrated markedly increased tone in her legs. Intellectually she was considered to be normal. In light of her father's condition a working diagnosis of hereditary spastic paraparesis was suggested. At this point her MRI brain scan revealed non-specific high T2 signal in a periventricular distribution, with no atrophy. Spinal cord MRI was normal. After this time she became non-specifically unwell, with bouts of fever, irritability, vomiting, general lethargy and a suggestion of motor regression. A repeat MRI brain revealed obvious cerebral atrophy and accentuation of white matter changes. At age 3 years and 7 months, she suffered an episode of urinary retention and loss of upper limb function. She was treated empirically with steroids for a diagnosis of transverse myelitis confirmed on MRI which demonstrated the new appearance of increased T2 signal in her cervical and thoracic cord, leading to a diagnosis of transverse myelitis, and obvious cerebral atrophy. She was positive for Aquaporin 4, antinuclear (ANA) and anti dsDNA antibodies. Her CSF neopterin was 15 x the upper limit of normal with no increase in white cells. Further assessment identified evidence of a multisystem inflammatory process with a history of hair loss, marker bilateral palmar erythema, a livedo rash, raised ESR with normal CRP, mildly elevated transaminases, positive lupus anticoagulant and a raised urinary protein/creatinine ratio. She was diagnosed as having a lupus-like illness. She was treated with high dose immunosuppression (pulsed steroids followed by Rituximab) to which she had a marked positive response, and was subsequently maintained on oral prednisolone and mycophenolate mofetil. A repeat MRI brain showed some resolution of the atrophic changes associated with her deterioration. Now, at the age of 5 years, she can commando crawl across the floor but has not acquired the ability to walk independently. She demonstrates increased tone with spasticity in all 4 limbs and clawing of the hands. She has good understanding, but her speech remains dysarthric, and she is considered to have a degree of intellectual delay.

Her phenotype conforms to one of congenital lower-limb spasticity, with acute neurological deterioration beginning at age 3 years, and an associated lupus-like disease.

F524_2. This male, the father of F524_1, was born to non-consanguineous white British parents. The birth and perinatal period were uneventful, and he acquired all of his early milestones appropriately – sitting at age 6 months and walking independently at just under 1 year old. At age 2 years it was noted that he was toe-walking, and he started to fall more than previously. By the age of 3 years he had been given a diagnosis of cerebral palsy, and between the ages of 4 and 15 years he underwent multiple tendon lengthening operations. His disorder has been very slowly progressive, so that in his teens he was able to play as a goal-keeper for a local football team, whilst he can now only walk with the aid of sticks. At the age of 33 years he demonstrates significant lower limb spasticity, with no involvement of the upper limbs. He is cognitively fully intact, and has experienced no other problems with his health. At this time, he had a borderline positive ANA result (titre of 1 in 160), with normal anti-DNA, anti-Ro, La, Sm, RNP, Scl-70, Jo-1, centromere and ANCA antibody titres. Cardiolipin Ab (IgG) and (IgM), Rheumatoid Factor, ESR and CRP were also normal. He had a normal cranial and spinal MRI at age 29 years.

His phenotype conforms to one of childhood-onset lower-limb spasticity which has been very slowly progressive.

F626. This male child was born to non-consanguineous European Italian parents with no family history of note. He was delivered at term by elective caesarean section (birth weight 3.3 kg; head circumference 34 cm; length 52 cm). Initial concerns were raised at age 3 months because of poor feeding, for which he was admitted to hospital and noted to have a mild transaminitis. Microvesicular steatosis was seen on a liver biopsy taken at 1 year of age. Serological and infection-related investigations were consistently negative. Transaminase values gradually normalized by the age of 4 years. He sat at age 7 months, started to babble at age 8 - 9 months, and could walk with support by 1 year of age. At age 13 months he experienced a rapid loss of motor and intellectual skills over a period of 1

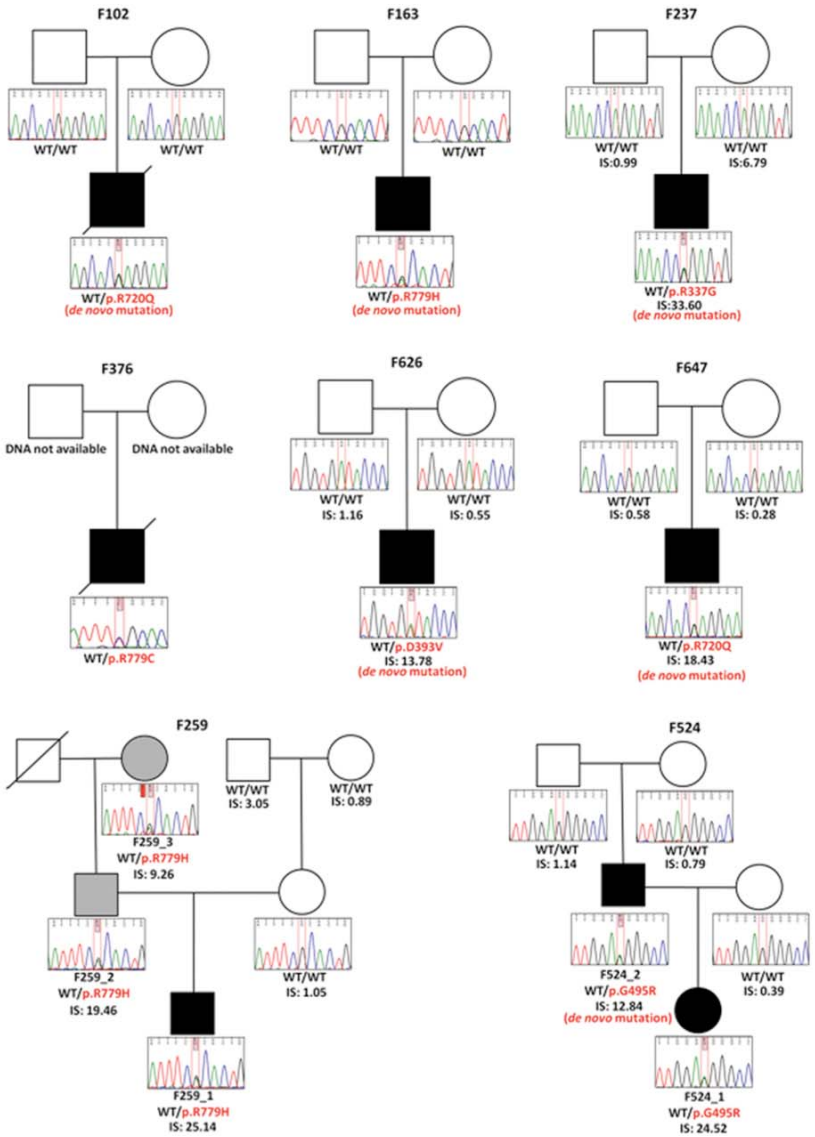
month resulting in severe spastic tetraparesis, axial hypotonia and a loss of speech. Cranial imaging undertaken at this time revealed non-specific subcortical areas of T2 high signal. These changes were still present on MRI at age 15 years, at which time low signal in the basal ganglia, subsequently confirmed as calcification on CT, was also seen. Of note, there was no brain atrophy. CSF neopterin has been consistently raised (5 – 10 x the upper limit of normal when measured on 8 occasions between the ages of 5 and 16 years). At 16 years of age he was admitted to hospital with a persistent fever, rash, and seizures. He was subsequently diagnosed with an inflammatory alveolitis in the absence of an obvious infectious agent, which responded to steroids. Extensive autoimmune testing was essentially normal apart from a single positive ANA antibody titre (1:80). At the age of 17 years his head circumference is between the 3rd - 10th centiles, with weight and height < 3rd centile. He demonstrates a spastic tetraplegia with mild dystonic movements in all 4 limbs. He feeds orally. Communication is limited to eye movements and he has no useful hand function.

His phenotype conforms to one of rapid onset of neuro-regression at age 13 months.

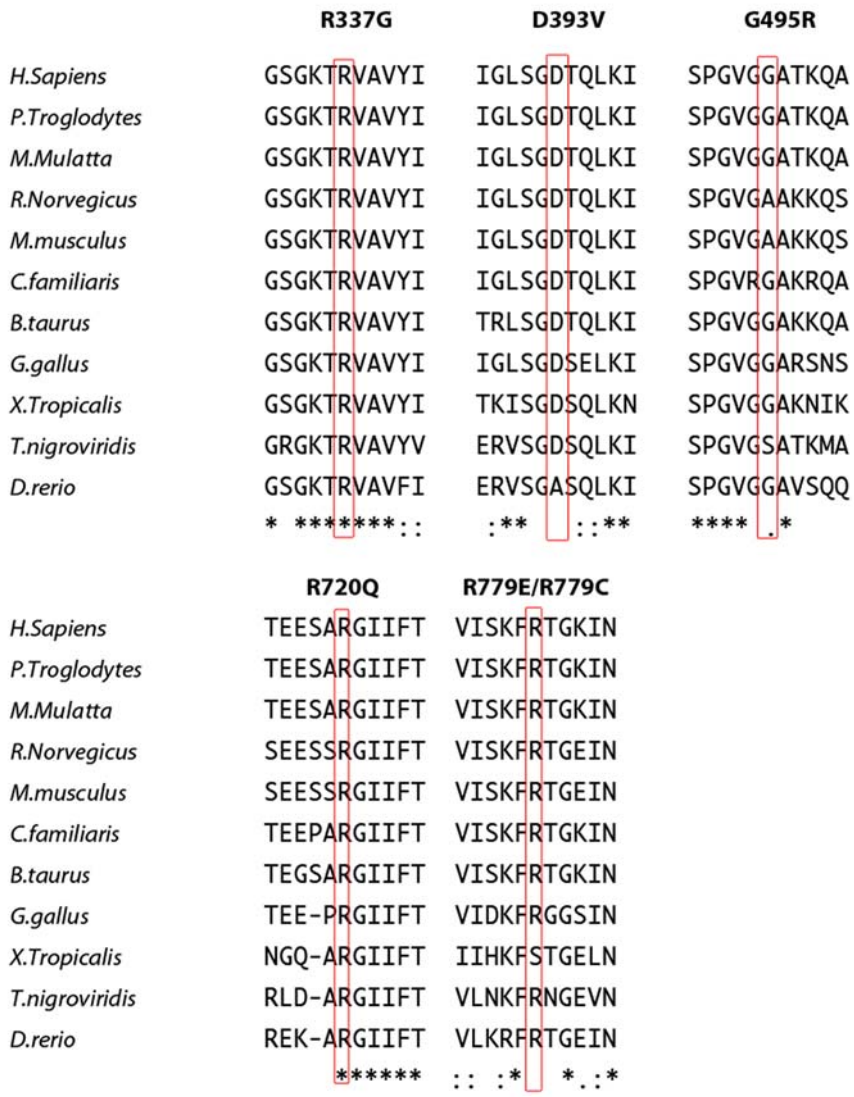
F647. This male child was born to non-consanguineous white Irish and Ukranian parents with no family history of note. He was delivered at 34 weeks gestation by caesarean section indicated because of oligohydramnios. He was small for dates (birth weight 1.73 kg; head circumference 30 cm). He was noted to be anaemic at birth and demonstrated a severe thrombocytopenia. He subsequently received 19 platelet transfusions and 4 red cell transfusions over the first 6 weeks of life. Extensive investigations, including bone marrow biopsy, were non-contributory. Developmental delay was obvious by the age of 3 months, at which time he was exhibiting dystonic movements, limb hypertonia with axial hypotonia, and microcephaly. Following an episode of bloody diarrhoea at age 9 months, he was identified to have concentric hypertrophy of the left cardiac ventricle. Further investigations at this time for underlying mitochondrial disease, including liver and muscle biopsy, were normal (except for features of steatosis in the liver). At 10 months of age scrotal oedema was noted. Renal biopsy was consistent with focal glomerular sclerosis, with the presence of tubuloreticular inclusions also noted. He was diagnosed with acute nephrotic syndrome, and responded to a 3 month course of steroids. He was found to be possibly hypothyroid at 18 months of age and is now on replacement thyroxine therapy. At age 2 years, following a prodrome of vomiting with blood, he underwent endoscopy. Biopsy of the stomach demonstrated features consistent with atrophic gastritis. Cranial ultrasound at age 1 week revealed several small foci of hyperintensity indicative of calcification. Cranial MRI at this time was normal. However, repeat MRI at age 1 year demonstrated marked cerebral atrophy, delayed myelination, and calcification in the basal ganglia, white matter and cortex. Now, at the age of 2 years, he is severely developmentally delayed, with poor head control, no useful hand function and limited social interaction.

His phenotype is characteristic of AGS with marked bone marrow suppression in the neonatal period, hypertrophic cardiomyopathy, hypothyroidism and nephrotic syndrome.

Supplementary Figures

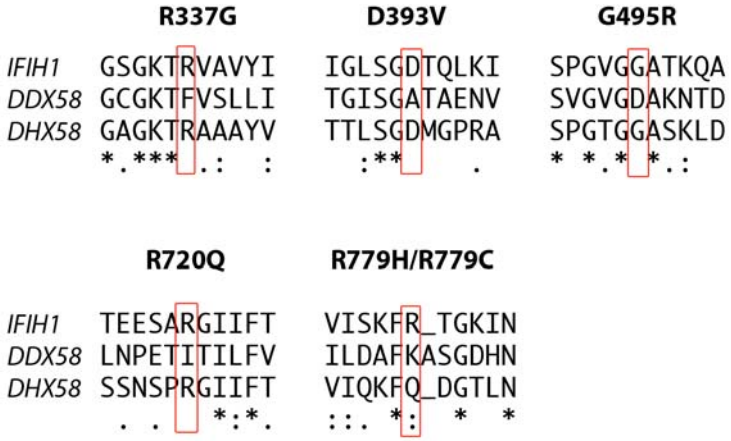


Supplementary Figure 1. Families with mutations in *IFIH1*. Family number is shown above each pedigree. Sequence chromatograms are given where available. IFIH1 protein status is stated if known, with WT denoting wild-type. *De novo* mutations are annotated in brackets. Uncolored shapes represent unaffected individuals. Black filled shapes represent clinically affected individuals. Grey filled shapes represent clinically asymptomatic mutation-positive individuals. Diagonal line indicates deceased status. IS denotes interferon score (for participants with repeat samples, the mean combined measurement is given).

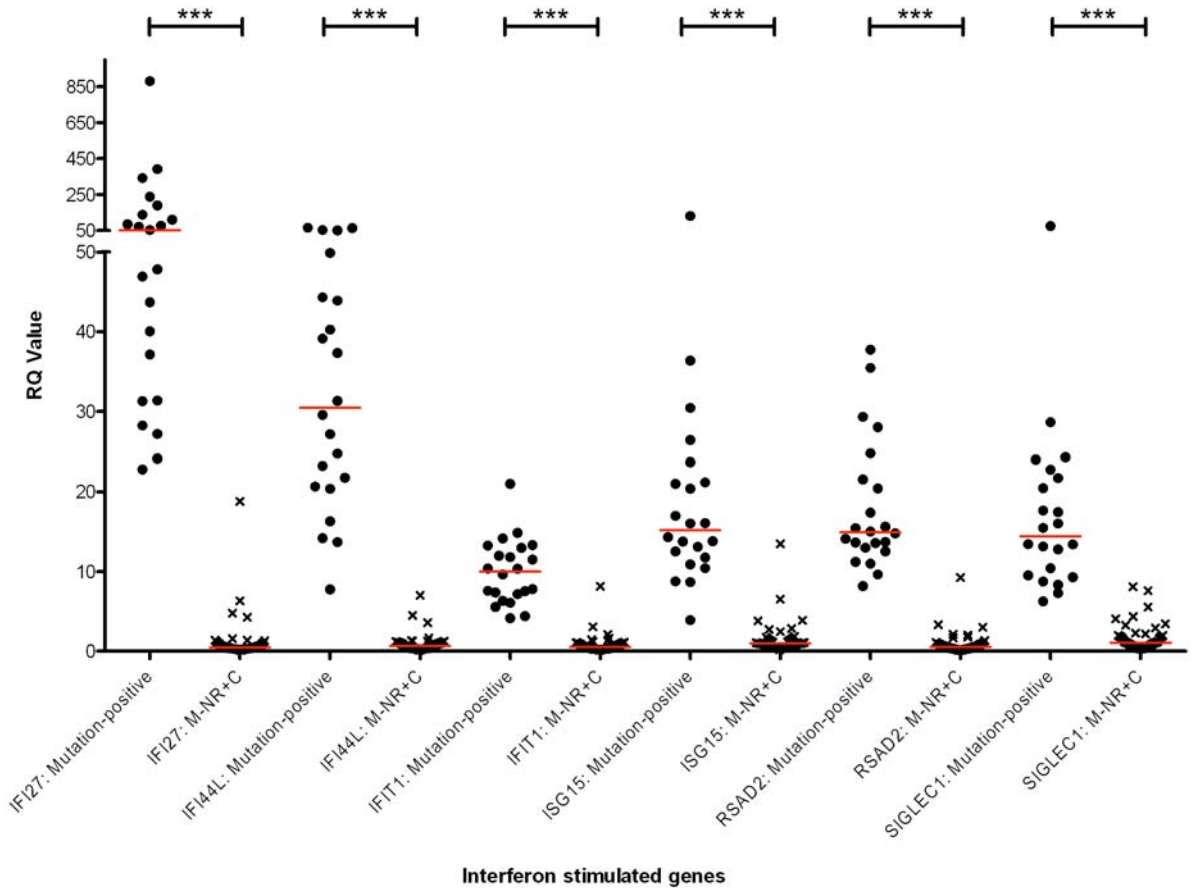


Supplementary Figure 2. **CLUSTAL Omega alignment of IFIH1 homologues.** IFIH1 homologues were identified on Ensembl and aligned using CLUSTAL Omega. Amino acids altered by *IFIH1* mutations are highlighted by a red box. H.Sapiens: *Homo sapiens* (ENSP00000263642); P.troglodytes: *Pan troglodytes* (ENSPTRG00000012582); M.mulatta: *Macaca mulatta* (ENSMMUG00000003202); R.Norvegicus: *Rattus norvegicus* (ENSRNOG00000006227); M.Musculus: *Mus musculus* (ENSMUSG00000026896); C.Familiaris: *Canis familiaris* (ENSCAFG00000010438); B.Taurus: *Bos Taurus* (ENSBTAG00000008142); G.gallus: *Gallus gallus* (ENSGALG00000011089); X.Tropicalis: *Xenopus tropicalis* (ENSXETG00000013176); T.nigroviridis: *Tetraodon nigroviridis* (ENSTNIG00000016500); D. Rerio: *Danio rerio* (ENSDARG00000018553).

Homology to the human IFIH1 reference sequence (ENSP00000263642): Human: *P.troglodytes* 100%; Human: *M.mulatta* 98%; Human: *B.torus* 84%; Human: *C.familiaris* 83%; Human: *M.musculus* 80%; Human: *R.Norvegicus* 80%; Human: *G.Gallus* 56%; Human: *X.tropicalis* 55%; Human: *D.rerio* 49%; Human: *T.nigroviridis* 44%.



Supplementary Figure 3. CLUSTAL Omega alignment of RNA helicases. RNA helicases were identified on Ensembl and aligned using CLUSTAL Omega. Amino acids altered by *IFIH1* mutations are highlighted by a red box. IFIH1 (ENSP00000263642); DDX58 (RIG-I) (ENSP00000369213); DHX58 (LGP2) (ENSP00000251642). Homology of human IFIH1 (reference sequence ENSP00000263642) to DDX58 (RIG-I) and DHX58 (LGP2) is 31% and 41% respectively.



Supplementary Figure 4. **Individual interferon stimulated gene (ISG) transcript levels in *IFIH1* mutation-positive individuals, their *IFIH1* mutation-negative relatives, and a cohort of 29 controls.** Quantitative reverse transcription PCR of a panel of six ISGs in whole blood measured in five families with mutations in *IFIH1* and 29 healthy controls. RQ is equal to $2^{-\Delta\Delta Ct}$, with -ΔΔCt ± standard deviations (i.e. the normalized fold change relative to a calibrator). Each value is derived from three technical replicates. For ease of presentation, mutation-negative relatives (n=13) have been combined with the control cohort (M-NR+C) in this figure. Horizontal red bars show the median RQ value for each probe in each group. For patients with repeat samples (biological replicates), all measurements are shown. Data analysed by one-way ANOVA using Dunnett's multiple comparison test. *** $p<0.0001$.

Supplementary Tables

Supplementary Table 1. Summary statistics for exome sequencing.

	F102*	F163 HL§
Raw data (Gb)	7.59	4.82
Data mapped to target region (Gb)	5.22	2.70
Percentage of bases with >= 1x coverage	99.9%	97.2%
Percentage of bases >= 10x coverage	99.2%	86.8%
Percentage of bases with >= 20x coverage	97.4%	74.9%
Mean coverage	97x	61x

* HiSeq. § SOLiD 4. F259 sequence data were corrupted before summary data could be compiled.

Supplementary Table 2. Clinical data for *IFIH1* mutation-positive individuals.

Family_Patient	Sex	Consan-guinity	Ancestry	Amino acid alteration	Age at presentation	Clinical phenotype	Interferon score (age in decimalised years at sampling)	CSF white cell count* (age in months at sampling)	CSF IFNα§ (age in months at sampling)	Serum IFNα§ (age in months at sampling)	CSF pterins¶ (age in months at sampling)	Cranial imaging
F102	M	No	European Italian	R720Q	Neonatal	IUGR, congenital microcephaly, hepatosplenomegaly and thrombocytopenia. Profound developmental delay with spastic-dystonic tetraparesis. Seizures and idiopathic hypertension at age 13mo. Died of pneumonia aged 2yr. Phenotype conforms to AGS	na	< 5 (12)	150 (12)	25 (12)	na	CA, C, WMD
F163	M	No	European French	R779H	Neonatal	Neonatal irritability, feeding difficulties, axial hypotonia and abnormal limb posturing. Now, age 13yr, severe developmental delay. Phenotype conforms to AGS	na	< 5 (11)	37 (52); 25 (54)	50 (54); 25 (58); 37 (104)	na	CA, C, WMD
F237	M	No	White American	R337G	15mo	Normal development until age 15mo. Loss of skills over a period of 12mo associated with fluctuating episodes of severe irritability. Now, age 12yr, cannot sit without support, no useful limb function and limited words. Intellect relatively preserved. Phenotype conforms to rapid onset of neuro-regression beginning at age 15mo	12.3 (12.55); 34.2 (12.60)	nr	na	na	Neo x4, Bio x1.5 (24); Neo x3, Bio 1.1 (84)	CA, C, WMD
F259_1 (son of F259_2)	M	No	European Italian	R779H	< 6mo	Axial hypotonia and lower limb spasticity noted at age 6mo. Now, age 8yr, profound physical and intellectual delay, having never acquired the ability to sit, and only smiling and crying when apparently content / upset. Marked	19.2 (8.14); 35.9 (8.21); 26.7 (8.29)	< 5 (31)	50 (31)	<2 (31)	na	CA, C, WMD

						dystonic posturing of the limbs with axial hypotonia. Phenotype conforms to AGS						
F259_2 (father of F259_1)	M	No	European Italian	R779H	Asymptomatic	Asymptomatic at age 48yr	10.8 (48.80); 17.0 (48.87); 29.1 (48.95)	na	na	na	na	na
F259_3 (paternal grandmother of F259_1)	F	No	European Italian	R779H	Asymptomatic	Asymptomatic at age 79yr	12.3 (79.33); 7.02 (79.41)	na	na	na	na	na
F376	M	No	White British	R779C		Neonatal irritability, feeding difficulties, axial hypotonia and abnormal limb tone evolving to severe global delay. Florid presentation of inflammatory process at age 29mo; continued disease despite treatment with high dose immunosuppressives. Died age 3yr. Phenotype conforms to AGS with superadded lupus-like disease	na	< 5 (30)	6 (30)	9 (30)	na	CA, C, WMD
F524_1 (daughter of F524_2)	F	No	White British	G495R	< 7mo	Early-onset spastic paraparesis with episode of sub-acute neurological deterioration accompanied by abnormal immunological profile age 3yr. Now, age 5yr, has significant 4 limb involvement and some intellectual delay. Phenotype is of congenital lower-limb spasticity with acute neurological deterioration with an associated lupus-like disease	20.1 (3.91); 25.4 (5.19)	< 5 (42)	na	na	Neo x15 (43)	CA, WMD, spinal cord involvement
F524_2 (father of F524_1)	M	No	White British	G495R	< 24mo	Diagnosed with 'cerebral palsy' as an infant. Thereafter, very slowly progressive spastic paraplegia involving the lower limbs. Now, age 33yr, has no other features. Phenotype conforms to one of childhood-onset lower-limb spasticity which has been very	15.2 (32.34); 11.5 (32.85); 17.8 (33.13); 29.3 (33.62); 10.0 (33.95)	na	na	na	na	Normal at age 29y

						slowly progressive					
F626	M	No	European Italian	D393V	13mo	Normal development until age 13mo. Rapid loss of skills over a 1mo period resulting in spastic tetraparesis and axial hypotonia. Recent episode of fever, seizures and alveolitis responding to steroids. Now, age 17yr, severe developmental delay. Phenotype conforms to rapid onset of neuro-regression beginning at age 13mo	12.4 (17.14); 15.6 (17.66)	< 5 (72)	na	Neo x5-10, Bio normal (8 times between 72-192)	No CA. C and WMD
F647	M	No	Mixed white Irish / Ukranian	R720Q	Neonatal	IUGR. Severe neonatal thrombocytopenia and anaemia. Developmental delay evident by 5mo. FTT obvious by age 7mo, at which point diagnosed with HOCM and hypertension. Nephrotic syndrome diagnosed at age 10mo. Possible hypothyroidism and atrophic gastritis diagnosed at ages 18mo and 2yr respectively. Now, age 2yr, severe delay. Phenotype conforms to AGS with early, reversible, bone marrow suppression, HOCM, hypothyroidism and nephrotic syndrome	22.0 (1.39); 16.1 (1.96); 18.4 (2.06)	nr	na	na	CA, C, WMD

M, male; F, female; yr, years; mo, months; na, not analysed; nr, not recorded; CSF, cerebrospinal fluid; IFNα, interferon alpha; AGS, Aicardi-Goutières syndrome; IUGR, intrauterine growth retardation; Neo, neopterin; Bio, biopterin; CA, cerebral atrophy; C, calcification; WMD, white matter disease; HOCM, hypertrophic cardiomyopathy; FTT, failure to thrive. * Cells/mm3 (Abnormal>5 cells/mm3). § IU/ml (Abnormal >2 IU/ml). ¶ Values of neopterin (Neo) and biopterin (Bio) given as multiples of upper limit of normal in laboratory where sample(s) tested.

Supplementary Table 3. Summary data of *IFIH1* mutation-negative patients / families demonstrating a positive interferon score.

Family number	Phenotype	Decimalised age at time of sampling, interferon score
F125	AGS	8.39, 25.04
F218	AGS	6.58, 5.61
F306	AGS	7.08, 9.44
F472	Undefined interferonopathy	12.37, 5.50
F473	AGS	5.07, 10.87
F492	Undefined interferonopathy	3.62, 8.13; 4.10, 8.01
F501	Lupus-like	4.86, 11.00
F520_1	AGS	8.69, 22.42; 9.04, 7.40
F520_2	AGS	4.48, 9.80; 4.83, 7.27
F520_3	AGS	1.73, 9.91; 2.08, 10.11
F554	Undefined interferonopathy	1.23, 6.22
F561	Undefined interferonopathy	17.48, 8.40
F571	Lupus-like	7.03, 14.42
F584	AGS	5.33, 8.28
F588	Undefined interferonopathy	2.81, 3.80
F636	Undefined interferonopathy	15.27, 46.52
F670	Undefined interferonopathy	5.31, 39.10
F677	Undefined interferonopathy	14.95, 10.35; 15.33, 6.27
F693	Undefined interferonopathy	2.59, 6.51
F694_1	Undefined interferonopathy	7.77; 36.48
F694_2	Undefined interferonopathy	2.57, 10.24; 2.81, 11.55
F721	Undefined interferonopathy	7.45, 7.76
F722	Undefined interferonopathy	5.77, 7.52

Supplementary Table 4. Results of SIFT, PolyPhen2 and MutationTaster analysis of the effects of the amino acid substitutions observed in *IFIH1* mutation-positive patients.

cDNA	Genomic DNA Chr2 (GRCh37)	Protein	SIFT	PolyPhen2	MutationTaster
c.1009A>G	g.163144731T>C	p.Arg337Gly	Tolerated score 0.12	Probably damaging 1.0	Disease causing (p-value: 1.0)
c.1178A>T	g.163139004T>A	p.Asp393Val	Deleterious score 0.01	Probably damaging 0.998	Disease causing (p-value: 1.0)
c.1483G>A	g.163137879C>T	p.Gly495Arg	Deleterious score 0.01	Probably damaging 0.982	Disease causing (p-value: 0.954)
c.2159G>A	g.163133342C>T	p.Arg720Gln	Deleterious score 0	Probably damaging 0.992	Disease causing (p-value: 1.0)
c.2335C>T	g.163130424G>A	p.Arg779Cys	Deleterious score 0.01	Probably damaging 1.0	Disease causing (p-value: 1.0)
c.2336G>A	g.163130423C>T	p.Arg779His	Tolerated score 0.05	Probably damaging 0.994	Disease causing (p-value: 1.0)

SIFT: http://sift.jcvi.org/www/SIFT_enst_submit.html
PolyPhen2: http://genetics.bwh.harvard.edu/pph2/
Mutation Taster: http://www.mutationtaster.org/
SIFT scores range from 0 to 1. The amino acid change is predicted to be damaging if the score is ≤ 0.05, and tolerated if the score is > 0.05
Polyphen2 qualitative ternary classification appraised at 5%/10% (HumDiv) or 10%/20% (HumVar) FPR thresholds (“Benign”, “Possibly damaging”, “Probably damaging”)
MutationTaster p value is the probability of the prediction, i.e. a value close to 1 indicates a high ‘security’ of the prediction

Supplementary Table 5. Microsatellite genotypes in child-parent trios with *de novo* mutations in *IFIH1*.

Marker	Sample	Allele 1	Allele 2
D3S3640	F102 Mother	132	132
	F102 Father	128	132
	F102 Affected	128	132
	F524_2 Mother	130	134
	F524_2 Father	132	136
	F524_2 Affected	130	132
	F647 Mother	136	138
	F647 Father	132	134
	F647 Affected	132	138
	F626 Mother	132	132
	F626 Father	130	132
	F626 Affected	132	132
	F237 Mother	132	132
	F237 Father	128	132
	F237 Affected	132	132
	F163 Mother	132	134
	F163 Father	132	134
	F163 Affected	132	134
	Control	128	132
D3S3560	F102 Mother	180	180
	F102 Father	180	180
	F102 Affected	180	180
	F524_2 Mother	178	182
	F524_2 Father	180	180
	F524_2 Affected	178	180
	F647 Mother	180	180
	F647 Father	180	180
	F647 Affected	180	180
	F626 Mother	180	180
	F626 Father	182	182
	F626 Affected	180	182
	F237 Mother	180	180
	F237 Father	178	180
	F237 Affected	180	180
	F163 Mother	180	180
	F163 Father	180	182
	F163 Affected	180	182
	Control	180	182
D11S4205	F102 Mother	194	194
	F102 Father	194	194
	F102 Affected	194	194
	F524_2 Mother	194	198
	F524_2 Father	194	194
	F524_2 Affected	194	198
	F647 Mother	194	194
	F647 Father	194	194
	F647 Affected	194	194

	F626 Mother	194	194
	F626 Father	194	198
	F626 Affected	194	194
	F237 Mother	192	198
	F237 Father	194	196
	F237 Affected	196	198
	F163 Mother	194	194
	F163 Father	194	194
	F163 Affected	194	194
	Control	194	196
D11S913	F102 Mother	216	216
	F102 Father	220	220
	F102 Affected	216	220
	F524_2 Mother	216	218
	F524_2 Father	216	220
	F524_2 Affected	216	216
	F647 Mother	218	218
	F647 Father	216	216
	F647 Affected	216	218
	F626 Mother	218	218
	F626 Father	216	218
	F626 Affected	218	218
	F237 Mother	220	220
	F237 Father	216	220
	F237 Affected	216	220
	F163 Mother	218	220
	F163 Father	218	220
	F163 Affected	218	220
	Control	216	218
D11S987	F102 Mother	192	198
	F102 Father	196	196
	F102 Affected	196	198
	F524_2 Mother	198	200
	F524_2 Father	190	198
	F524_2 Affected	190	200
	F647 Mother	196	196
	F647 Father	180	196
	F647 Affected	180	196
	F626 Mother	194	196
	F626 Father	194	196
	F626 Affected	196	196
	F237 Mother	194	196
	F237 Father	192	200
	F237 Affected	192	196
	F163 Mother	192	198
	F163 Father	196	202
	F163 Affected	196	198
	Control	186	196
D11S1889	F102 Mother	233	235
	F102 Father	233	235
	F102 Affected	233	233

	F524_2 Mother	229	235
	F524_2 Father	227	233
	F524_2 Affected	227	229
	F647 Mother	235	237
	F647 Father	225	233
	F647 Affected	225	237
	F626 Mother	229	237
	F626 Father	229	245
	F626 Affected	229	245
	F237 Mother	237	237
	F237 Father	229	235
	F237 Affected	229	237
	F163 Mother	235	237
	F163 Father	227	233
	F163 Affected	233	235
	Control	233	235
D20S847	F102 Mother	129	129
	F102 Father	129	129
	F102 Affected	129	129
	F524_2 Mother	133	137
	F524_2 Father	133	137
	F524_2 Affected	137	137
	F647 Mother	129	129
	F647 Father	129	137
	F647 Affected	129	129
	F626 Mother	129	129
	F626 Father	129	133
	F626 Affected	129	129
	F237 Mother	129	137
	F237 Father	129	135
	F237 Affected	129	129
	F163 Mother	127	129
	F163 Father	137	137
	F163 Affected	129	137
	Control	129	131
D20S896	F102 Mother	187	187
	F102 Father	189	189
	F102 Affected	187	189
	F524_2 Mother	187	189
	F524_2 Father	187	193
	F524_2 Affected	189	193
	F647 Mother	185	197
	F647 Father	187	189
	F647 Affected	185	187
	F626 Mother	187	187
	F626 Father	187	195
	F626 Affected	187	187
	F237 Mother	189	193
	F237 Father	185	189
	F237 Affected	189	193
	F163 Mother	189	193

	F163 Father	189	189
	F163 Affected	189	189
	Control	187	189
D20S843	F102 Mother	256	260
	F102 Father	248	252
	F102 Affected	252	256
	F524_2 Mother	252	256
	F524_2 Father	252	256
	F524_2 Affected	256	256
	F647 Mother	256	256
	F647 Father	254	264
	F647 Affected	256	264
	F626 Mother	254	256
	F626 Father	250	252
	F626 Affected	252	256
	F237 Mother	252	262
	F237 Father	254	256
	F237 Affected	252	256
	F163 Mother	252	256
	F163 Father	250	252
	F163 Affected	250	256
	Control	250	256

Supplementary Table 6. *IFIH1* variants protective against the development of type I diabetes as described by Nejentsev *et al*. [1]

rs ID	Variant	EVS* allele frequency
rs35337543	c.1641+1G>C	106/12900
rs35732034	c.2807+1G>A	70/12936
rs35744605	c.1879G>T p.E627*	52/12954
rs35667974	c.2767A>G p.I923V	151/12855

***** Exome Variant Server (http://evs.gs.washington.edu/EVS/)

1. Nejentsev, S. et al. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. *Science* **324**, 387-9 (2009).

Supplementary Table 7. Primers used to amplify *IFIH1* exons (including intron / exon boundaries).

Exon	Primer name	Sequence
Exon 1	IFIH1_Ex1F	ACAACAGCACCATCTGCTTG
	IFIH1_Ex1R	GCCTAAAAGGCTAGCTCCA
Exon 2	IFIH1_Ex2F	AAAGGGTATTTCCTGTTTAAGGTT
	IFIH1_Ex2R	TGAATTCTCAATCACTAGGCAGA
Exon 3	IFIH1_Ex3F	TGGCACTATGATTTGCATTCT
	IFIH1_Ex3R	TCTGCCCAGTTGGTTTTTCT
Exon 4	IFIH1_Ex4F	TGTGCTGTAGAGGTGTGCAGT
	IFIH1_Ex4R	TGCTTCCACTATATGGCGTCT
Exon 5	IFIH1_Ex5F	GGCCTACGTTCAGTTTCAGG
	IFIH1_Ex5R	CAATGACACAAATGCCATCA
Exon 6	IFIH1_Ex6F	TTCATGCTGGATGCCAAAC
	IFIH1_Ex6R	TTTCCTCCAGGAAGTAGAAGGA
Exon 7	IFIH1_Ex7F	CCCAAGGCAGCTCAATTACT
	IFIH1_Ex7R	CCAAGAAGTCCTGGCATTTG
Exon 8	IFIH1_Ex8F	CGTTGAATAAAGTGAAAGGGAAA
	IFIH1_Ex8R	AGCCTTTGCCATCTTTCTACTG
Exon 9	IFIH1_Ex9F	GCTTGATGGCAGGCTTAAAA
	IFIH1_Ex9R	TGGGGAATCTGTGATATAGTCATCT
Exon 10	IFIH1_Ex10F	GGCACAATTTTAGGGGGTTT
	IFIH1_Ex10R	TGAAAAGGTAAATGAATGACACCA
Exon 11	IFIH1_Ex11F	AACTGTATATTTTTGGTGTACAAAATG
	IFIH1_Ex11R	TGATCATGCCACTGCTCTTC
Exon 12	IFIH1_Ex12F	GATTTTAATGTGTTTAGCATCACAAA
	IFIH1_Ex12R	GCAATTAAAATAGGAACACAACAAA
Exon 13	IFIH1_Ex13F	TGAAGACTGGCATGCTGAAC
	IFIH1_Ex13R	TCAGCACAATTTTTGTCTGGAG
Exon 14	IFIH1_Ex14F	CAGGAGATGATTATATACCAAATTCTT
	IFIH1_Ex14R	TGAGAGGCTAAAGGAGAGGAA
Exon 15_16	IFIH1_Ex15_16F	GGAAGGAATGCCGTGTAGAA
	IFIH1_Ex15_16R	TCCTTACCTCTGCCCAACAA