**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

[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)

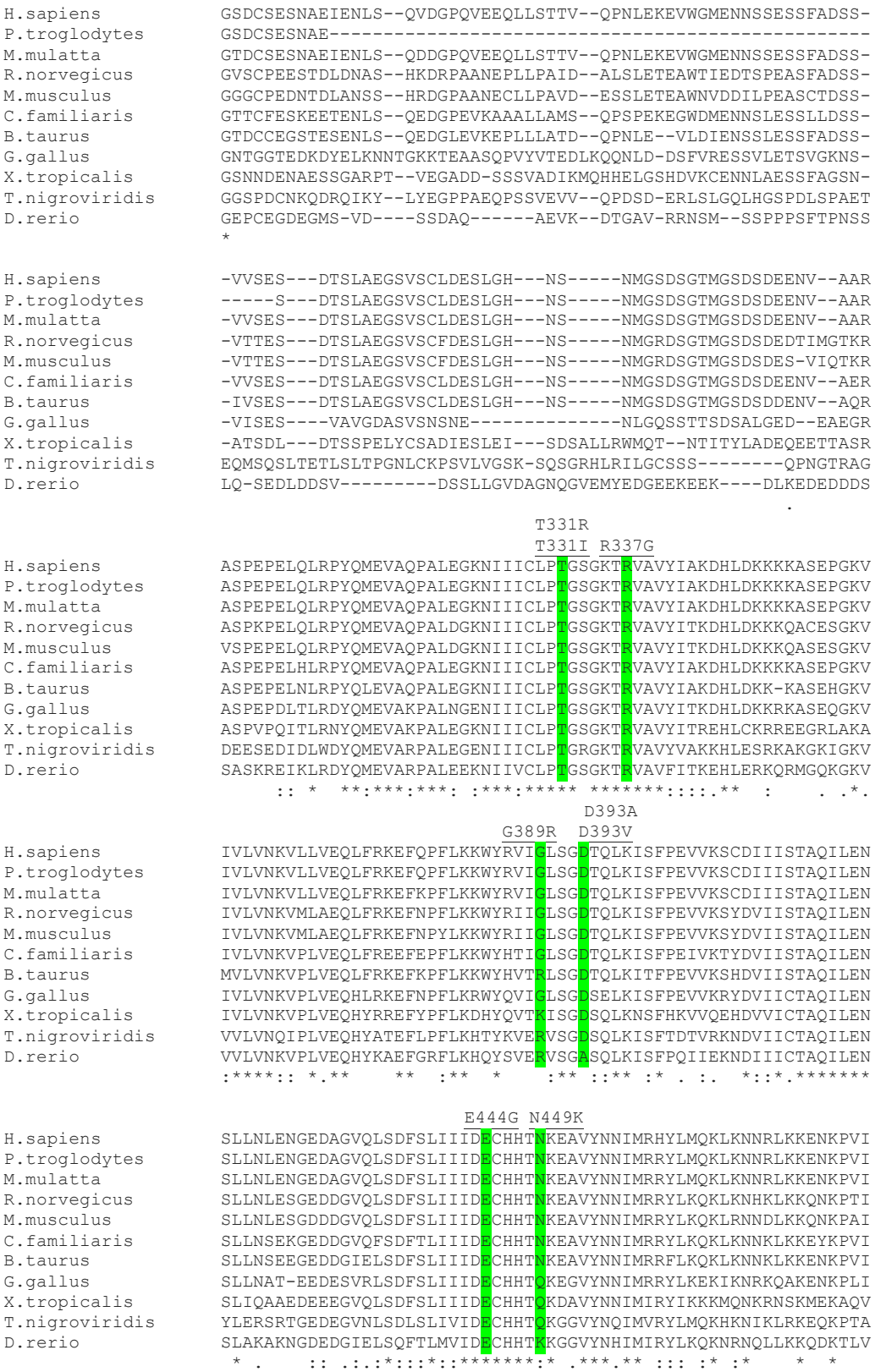
Centre for Genomic and Experimental Medicine, MRC Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, United Kingdom

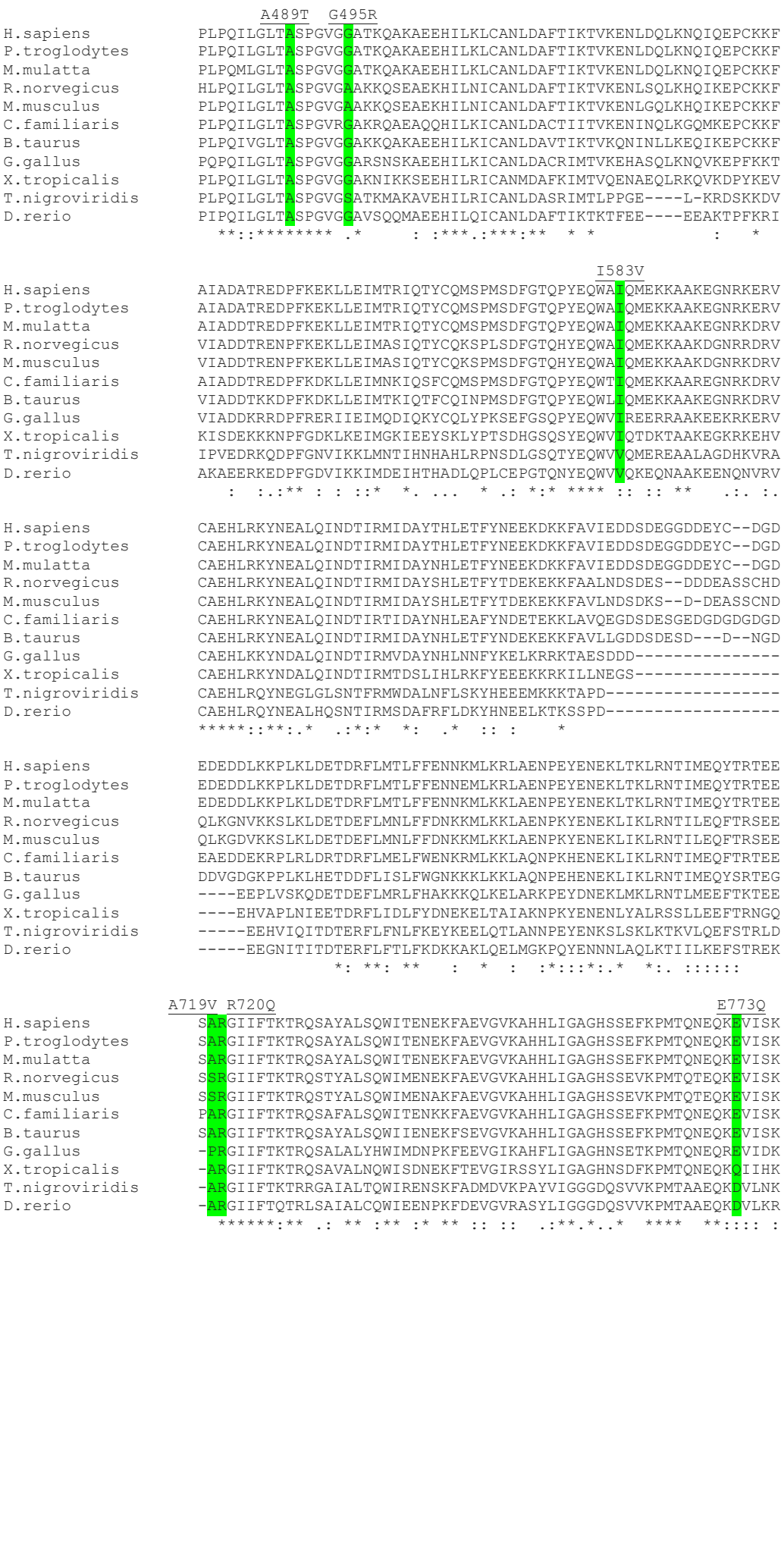
Sun Hur (Sun.Hur@childrens.harvard.edu)

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

## Supp. Figure S1. CLUSTAL Omega alignment of IFIH1 homologues

```
H.sapiens           ------------------------------------------------------------
P.troglodytes       ------------------------------------------------------------
M.mulatta           ------------------------------------------------------------
R.norvegicus        ------------------------------------------------------------
M.musculus          ------------------------------------------------------------
C.familiaris        ------------------------------------------------------------
B.taurus            ------------------------------------------------------------
G.gallus            MVHVSFLFELRVLGATFQLFGIGHIGISPCPALPSRGWARLPLRSKQQAKPKAAAGFMLS
X.tropicalis        ------------------------------------------------------------
T.nigroviridis      ------------------------------------------------------------
D.rerio             ------------------------------------------------------------


H.sapiens           ----------------------------------------MSNGYSTDENFRYLISCFRA
P.troglodytes       ----------------------------------------MSNGCSTDENFRYLISCFRA
M.mulatta           ----------------------------------------MSNGYSTDENFRYLISCFRA
R.norvegicus        ----------------------------------------MSTVCSAEDSFRNLISIFRP
M.musculus          ----------------------------------------MSIVCSAEDSFRNLILFFRP
C.familiaris        ---------------------------------------MWSGRPSADQSFRHLLSCFRA
B.taurus            ---------------------------------------MSSDGSSTDKNFCYLISCFRA
G.gallus            ARNGGSLRTADAVPATPTPSAATAAPAPGSSRAGPGAASAAMSEECRDERFLYMISCFRP
X.tropicalis        ----------------------------------------MPQNDSEDARGIYLIECFRA
T.nigroviridis      ----------------------------------------FIMAADSDEIHERLIEYFMP
D.rerio             ----------------------------------------MDPNMSSDQDTETRHILDCFRD
                                                             :    ::  *

H.sapiens           RVKMYIQVEPVLDYLTFLPAEVKEQIQRTVATSGNMQAVELLLSTLEKGVWHLGWTREFV
P.troglodytes       RVKMYIQVEPVLDYLTFLPAEVKEQIQRTVATSGNMQAVELLLSTLEKGVWHLGWTREFV
M.mulatta           RVKMYIQVEPVLDYLTFLPAEVKEQIQRTVATSGNMQAVELLLSTLEKGVWHLGWTREFV
R.norvegicus        RVKMNIQVEPVLDYLVFLPAETKEQILRKVTTCGNTSAAELLLSTLEQGQWPLGWTQMFV
M.musculus          RLKMYIQVEPVLDHLIFLSAETKEQILKKINTCGNTSAAELLLSTLEQGQWPLGWTQMFV
C.familiaris        RVKTYIQVEPVLDYLNFLPEEVKEQIQRKAANAGNLQAAELLLSTLEKGAWPPGWTRQVL
B.taurus            RVKRYIQVEPVLDYLTFLPPEVKEHIQRTAATTGDIQAADLLLNTLERGNWPLGWARMFV
G.gallus            RLKRCIRVQPVLDWLPSLSAEEKDKVRAAALQRGEVEGAEELLCAVERGRRDPGWFTEFL
X.tropicalis        RLVRYIQAVPVLDHLTWLGRDIREQVVSKAQNQGEQDAARLLLDRIVRGPREPGWFEAFV
T.nigroviridis      RLRNLIVVQQVLDFLDFIEPDQKERIRQRNISRGNLAAADALITAVTQKPHQAGWFRAFV
D.rerio             RLKRLIQVEPLLDLLDFLEPDKKERIKAKVASEGNINAVSLLINEIMRAELQQGWSRTLV
                    *:   * .  :** *  :  : ::::       *:  ..  *:  : :     **   .:

H.sapiens           EALRRTGSPLAARYMNPELTDLPSPSFENAHDEYLQLLNLLQPTLVDKLLVRDVLDKCME
P.troglodytes       EALRRTGSPLAARYMNPELTDLPSPSFENAHDEYLQLLNLLQPTLVDKLLVRDVLDKCME
M.mulatta           EALRRAGSPLAARYMNPELTDLPSPSFENAHDECLQLLNLLQPTLVDKLLVRDVLDKCME
R.norvegicus        EALEHSGNPLAARYVKPSLTDLPSPSSETAHDECLHLLNLLQPTLVGKLLINDVLDTCSE
M.musculus          EALEHSGNPLAARYVKPTLTDLPSPSSETAHDECLHLLTLLQPTLVDKLLINDVLDTCFE
C.familiaris        VALQSAGSVLASRYLNPELADLPSPSAENAHDQCLQLLHLLQPTLVDRLLVKDVLDKCVE
B.taurus            EALRQAGNPLAARYVNPELTDLPSPSSENAHDECLQLLNLLQPTLVDKLLVADVLDKCVE
G.gallus            LALKKGGCDLAACYVNPS--QLPSPQEEDDHDLCVHLVQLLHGTLVDNMQTRQVAEKCLE
X.tropicalis        RALKDSHCTQAAAYLSE---GRPTPSLEATWDYYEQLLIVLYPELIAKIDPKETAPLCRR
T.nigroviridis      DALEQSGCGHAADYIQN---KIPEPEVEAENDYCIRLIQLLSPSLVD-MKTSAVCVQCLS
D.rerio             TALETVGCGHAARYVRN---RPPEPDEEAEHDSCVRLIDLLHLSLVN-MKTRDVCVQCVT
                     **.      *: *:       * *. *   *   :*: :*   *:  :    .   *

H.sapiens           EELLTIEDRNRIA-AAENNGNESGVRELLKRIVQK-ENWFSAFLNVLRQTGNNELVQELT
P.troglodytes       EELLTIEDRNRIA-AAENNGNESGVRELLKRIVQK-ENWFSAFLNVLRQTGNNELVQELT
M.mulatta           EELLTIEDRNRIA-AAENNGNESGVRELLKRIVQK-ENWFSAFLDVLRQTGNDELVQELT
R.norvegicus        KGLVTVEDRNRIS-AAGNSGNESGVRELLRRIVQK-ENWFSTFLDVLRQTGNDALVQELT
M.musculus          KGLLTVEDRNRIS-AAGNSGNESGVRELLRRIVQK-ENWFSTFLDVLRQTGNDALFQELT
C.familiaris        KKLLTDEDRDRIS-AAENNGNQSGVRELLKRIVQK-ENWFSLFLTVLNQTENYALVQELT
B.taurus            EKLLTIEDRNRVS-AAENNGNEAGVRELLKRIVQK-ENWFSTFLTILRQTGNDALAREFT
G.gallus            LGIFQEEDLVGIETVIESRGNRDGARELLSRIVQK-KDWFSQFLVALRETQHESLADDLS
X.tropicalis        EEICSDEDVNVISNVTDQHGNQQGSRELLNRIIKK-QDWFSKFLTVLRTMGQNSLADYLT
T.nigroviridis      QDLITEDDSENIEAATRNQGAKAGARELLGRIVRGRPGWFSKFLDILLKTEHKNLYSELT
D.rerio             LGLLTQEDQENIMTATGNNGNINGARLLLKRLVKNEAGWFSKFLQALEDTEHRDLMRELR
                      :   :*   :  .  . *   * * ** *:::    *** **  *    .  *   :
```

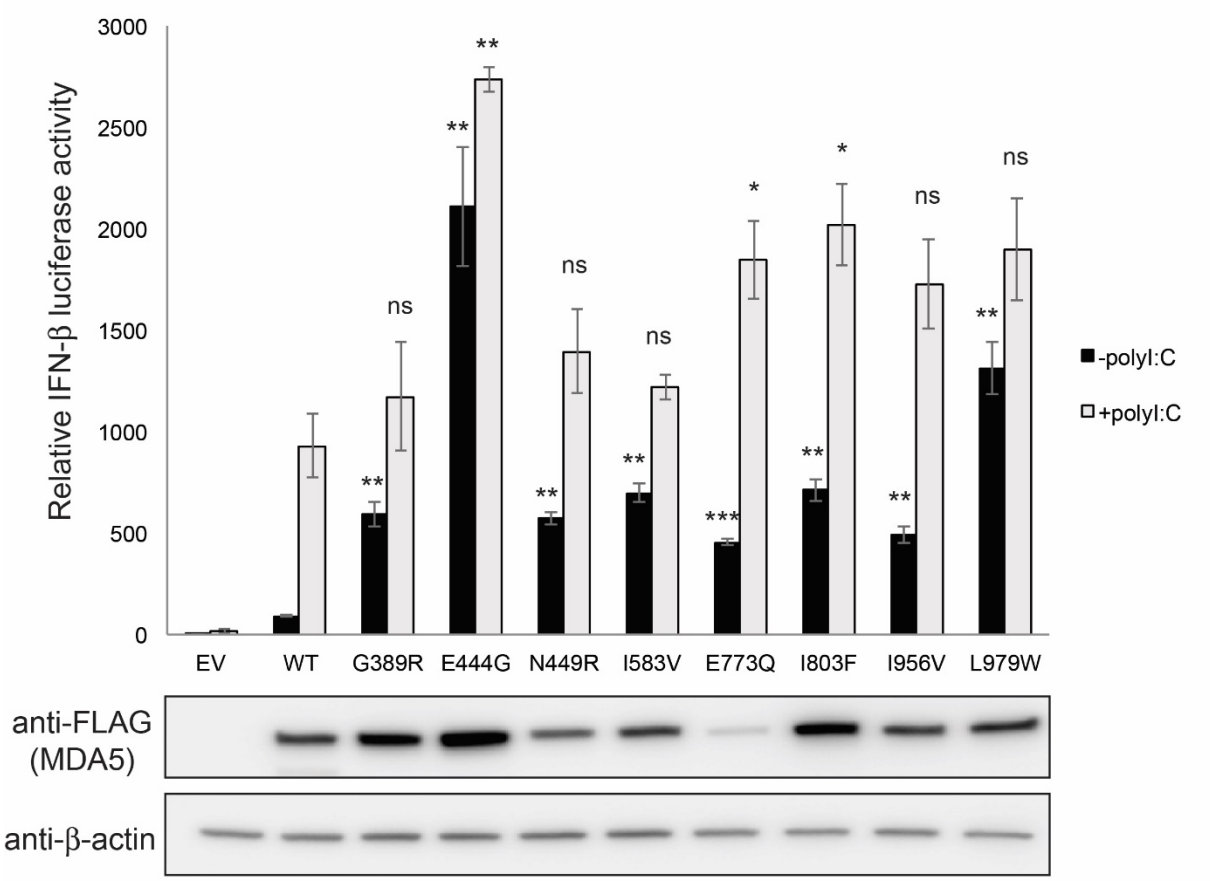

H.sapiens GSDCSESNAEIENLS--QVDGPQVEEQLLSTTV--QPNLEKEVWGMENNSSESSFADSS-
P.troglodytes GSDCSESNAE----------
M.mulatta GTDCSESNAEIENLS--QDDGPQVEEQLLSTTV--QPNLEKEVWGMENNSSESSFADSS-
R.norvegicus GVSCPEESTDLDNAS--HKDRPAANEPLLPAID--ALSLETEAWTIEDTSPEASFADSS-
M.musculus GGGCPEDNTDLANSS--HRDGPAANECLLPAVD--ESSLETEAWNVDDILPEASCTDSS-
C.familiaris GTTCFESKEETENLS--QEDGPEVKAAALLAMS--QPSPEKEGWDMENNSLESSLLDSS-
B.taurus GTDCCEGSTESENLS--QEDGLEVKEPLLLATD--QPNLE--VLDIENSSLESSFADSS-
G.gallus GNTGGTEDKDYELKNNTGKKTEAASQPVYVTEDLKQQNLD-DSFVRESSVLETSVGKNS-
X.tropicalis GSNNDENAESSGARPT--VEGADD-SSSVADIKMQHHELGSHDVKCENNLAESSFAGSN-
T.nigroviridis GGSPDCNKQDRQIKY--LYEGPPAEQPSSVEVV--QPDSD-ERLSLGQLHGSPDLSPAET
D.rerio GEPCEGDEGMS-VD----SSDAQ------AEVK--DTGAV-RRNSM--SSPPPSFTPNSS
H.sapiens -VVSES---DTSLAEGSVSCLDESLGH---NS-----NMGSDSGTMGSDSDEENV--AAR
P.troglodytes -----S---DTSLAEGSVSCLDESLGH---NS-----NMGSDSGTMGSDSDEENV--AAR
M.mulatta -VVSES---DTSLAEGSVSCLDESLGH---NS-----NMGSDSGTMGSDSDEENV--AAR
R.norvegicus -VTTES---DTSLAEGSVSCFDESLGH---NS-----NMGRDSGTMGSDSDEDTIMGTKR
M.musculus -VTTES---DTSLAEGSVSCFDESLGH---NS-----NMGRDSGTMGSDSDES-VIQTKR
C.familiaris -VVSES---DTSLAEGSVSCLDESLGH---NS-----NMGSDSGTMGSDSDEENV--AER
B.taurus -IVSES---DTSLAEGSVSCLDESLGH---NS-----NMGSDSGTMGSDSDDENV--AQR
G.gallus -VISES----VAVGDASVSNSNE--------------NLGQSSTTSDSALGED--EAEGR
X.tropicalis -ATSDL---DTSSPELYCSADIESLEI---SDSALLRWMQT--NTITYLADEQEETTASR
T.nigroviridis EQMSQSLTETLSLTPGNLCKPSVLVGSK-SQSGRHLRILGCSSS--------QPNGTRAG
D.rerio LQ-SEDLDDSV---------DSSLLGVDAGNQGVEMYEDGEEKEEK----DLKEDEDDDS
T331R
T331I R337G
H.sapiens ASPEPELQLRPYQMEVAQPALEGKNIIICLPTGSGKTRVAVYIAKDHLDKKKKASEPGKV
P.troglodytes ASPEPELQLRPYQMEVAQPALEGKNIIICLPTGSGKTRVAVYIAKDHLDKKKKASEPGKV
M.mulatta ASPEPELQLRPYQMEVAQPALEGKNIIICLPTGSGKTRVAVYIAKDHLDKKKKASEPGKV
R.norvegicus ASPKPELQLRPYQMEVAQPALDGKNIIICLPTGSGKTRVAVYITKDHLDKKKQACESGKV
M.musculus VSPEPELQLRPYQMEVAQPALDGKNIIICLPTGSGKTRVAVYITKDHLDKKKQASESGKV
C.familiaris ASPEPELHLRPYQMEVAQPALEGKNIIICLPTGSGKTRVAVYIAKDHLDKKKKASEPGKV
B.taurus ASPEPELNLRPYQLEVAQPALEGKNIIICLPTGSGKTRVAVYIAKDHLDKK-KASEHGKV
G.gallus ASPEPDLTLRDYQMEVAKPALNGENIIICLPTGSGKTRVAVYITKDHLDKKRKASEQGKV
X.tropicalis ASPVPQITLRNYQMEVAKPALEGKNIIICLPTGSGKTRVAVYITREHLCKRREEGRLAKA
T.nigroviridis DEESEDIDLWDYQMEVARPALEGENIIICLPTGRGKTRVAVYVAKKHLESRKAKGKIGKV
D.rerio SASKREIKLRDYQMEVARPALEEKNIIVCLPTGSGKTRVAVFITKEHLERKQRMGQKGKV
D393A
G389R D393V
H.sapiens IVLVNKVLLVEQLFRKEFQPFLKKWYRVIGLSGDTQLKISFPEVVKSCDIIISTAQILEN
P.troglodytes IVLVNKVLLVEQLFRKEFQPFLKKWYRVIGLSGDTQLKISFPEVVKSCDIIISTAQILEN
M.mulatta IVLVNKVLLVEQLFRKEFKPFLKKWYRVIGLSGDTQLKISFPEVVKSCDIIISTAQILEN
R.norvegicus IVLVNKVMLAEQLFRKEFNPFLKKWYRIIGLSGDTQLKISFPEVVKSYDVIISTAQILEN
M.musculus IVLVNKVMLAEQLFRKEFNPYLKKWYRIIGLSGDTQLKISFPEVVKSYDVIISTAQILEN
C.familiaris IVLVNKVPLVEQLFREEFEPFLKKWYHTIGLSGDTQLKISFPEIVKTYDVIISTAQILEN
B.taurus MVLVNKVPLVEQLFRKEFKPFLKKWYHVTRLSGDTQLKITFPEVVKSHDVIISTAQILEN
G.gallus IVLVNKVPLVEQHLRKEFNPFLKRWYQVIGLSGDSELKISFPEVVKRYDVIICTAQILEN
X.tropicalis IVLVNKVPLVEQHYRREFYPFLKDHYQVTKISGDSQLKNSFHKVVQEHDVVICTAQILEN
T.nigroviridis VVLVNQIPLVEQHYATEFLPFLKHTYKVERVSGDSQLKISFTDTVRKNDVIICTAQILEN
D.rerio VVLVNKVPLVEQHYKAEFGRFLKHQYSVERVSGASQLKISFPQIIEKNDIIICTAQILEN
E444G N449K
H.sapiens SLLNLENGEDAGVQLSDFSLIIIDECHHTNKEAVYNNIMRHYLMQKLKNNRLKKENKPVI
P.troglodytes SLLNLENGEDAGVQLSDFSLIIIDECHHTNKEAVYNNIMRRYLMQKLKNNRLKKENKPVI
M.mulatta SLLNLENGEDAGVQLSDFSLIIIDECHHTNKEAVYNNIMRRYLMQKLKNNRLKKENKPVI
R.norvegicus SLLNLESGEDDGVQLSDFSLIIIDECHHTNKEAVYNNIMRRYLKQKLKNHKLKKQNKPTI
M.musculus SLLNLESGDDDGVQLSDFSLIIIDECHHTNKEAVYNNIMRRYLKQKLRNNDLKKQNKPAI
C.familiaris SLLNSEKGEDDGVQFSDFTLIIIDECHHTNKEAVYNNIMRRYLKQKLKNNKLKKEYKPVI
B.taurus SLLNSEEGEDDGIELSDFSLIIIDECHHTNKEAVYNNIMRRFLKQKLKNNKLKKENKPVI
G.gallus SLLNAT-EEDESVRLSDFSLIIIDECHHTQKEGVYNNIMRRYLKEKIKNRKQAKENKPLI
X.tropicalis SLIQAAEDEEEGVQLSDFSLIIIDECHHTQKDAVYNNIMIRYIKKKMQNKRNSKMEKAQV
T.nigroviridis YLERSRTGEDEGVNLSDLSLIVIDECHHTQKGGVYNQIMVRYLMQKHKNIKLRKEQKPTA
D.rerio SLAKAKNGDEDGIELSQFTLMVIDECHHTKKGGVYNHIMIRYLKQKNRNQLLKKQDKTLV


A489T G495R
H.sapiens PLPQILGLTASPGVGGATKQAKAEEHILKLCANLDAFTIKTVKENLDQLKNQIQEPCKKF
P.troglodytes PLPQILGLTASPGVGGATKQAKAEEHILKLCANLDAFTIKTVKENLDQLKNQIQEPCKKF
M.mulatta PLPQMLGLTASPGVGGATKQAKAEEHILKLCANLDAFTIKTVKENLDQLKNQIQEPCKKF
R.norvegicus HLPQILGLTASPGVGAAKKQSEAEKHILNICANLDAFTIKTVKENLSQLKHQIKEPCKKF
M.musculus PLPQILGLTASPGVGAAKKQSEAEKHILNICANLDAFTIKTVKENLGQLKHQIKEPCKKF
C.familiaris PLPQILGLTASPGVRGAKRQAEAQQHILKICANLDACTIITVKENINQLKGQMKEPCKKF
B.taurus PLPQIVGLTASPGVGGAKKQAKAEEHILKICANLDAVTIKTVKQNINLLKEQIKEPCKKF
G.gallus PQPQILGLTASPGVGGARSNSKAEEHILKICANLDACRIMTVKEHASQLKNQVKEPFKKT
X.tropicalis PLPQILGLTASPGVGGAKNIKKSEEHILRICANMDAFKIMTVQENAEQLRKQVKDPYKEV
T.nigroviridis PLPQILGLTASPGVGSATKMAKAVEHILRICANLDASRIMTLPPGE----L-KRDSKKDV
D.rerio PIPQILGLTASPGVGGAVSQQMAEEHILQICANLDAFTIKTKTFEE----EEAKTPFKRI
I583V
H.sapiens AIADATREDPFKEKLLEIMTRIQTYCQMSPMSDFGTQPYEQWAIQMEKKAAKEGNRKERV
P.troglodytes AIADATREDPFKEKLLEIMTRIQTYCQMSPMSDFGTQPYEQWAIQMEKKAAKEGNRKERV
M.mulatta AIADDTREDPFKEKLLEIMTRIQTYCQMSPMSDFGTQPYEQWAIQMEKKAAKEGNRKDRV
R.norvegicus VIADDTRENPFKEKLLEIMASIQTYCQKSPLSDFGTQHYEQWAIQMEKKAAKDGNRRDRV
M.musculus VIADDTRENPFKEKLLEIMASIQTYCQKSPMSDFGTQHYEQWAIQMEKKAAKDGNRKDRV
C.familiaris AIADDTREDPFKDKLLEIMNKIQSFCQMSPMSDFGTQPYEQWTIQMEKKAAREGNRKDRV
B.taurus VIADDTKKDPFKDKLLEIMTKIQTFCQINPMSDFGTQPYEQWLIQMEKKAAKEGNRKDRV
G.gallus VIADDKRRDPFRERIIEIMQDIQKYCQLYPKSEFGSQPYEQWVIREERRAAKEEKRKERV
X.tropicalis KISDEKKKNPFGDKLKEIMGKIEEYSKLYPTSDHGSQSYEQWVIQTDKTAAKEGKRKEHV
T.nigroviridis IPVEDRKQDPFGNVIKKLMNTIHNHAHLRPNSDLGSQTYEQWVQMEREAALAGDHKVRA
D.rerio AKAEERKEDPFGDVIKKIMDEIHTHADLQPLCEPGTQNYEQWVQKEQNAAKEENQNVRV
H.sapiens CAEHLRKYNEALQINDTIRMIDAYTHLETFYNEEKDKKFAVIEDDSDEGGDDEYC--DGD
P.troglodytes CAEHLRKYNEALQINDTIRMIDAYTHLETFYNEEKDKKFAVIEDDSDEGGDDEYC--DGD
M.mulatta CAEHLRKYNEALQINDTIRMIDAYNHLETFYNEEKDKKFAVIEDDSDEGGDDEYC--DGD
R.norvegicus CAEHLRKYNEALQINDTIRMIDAYSHLETFYTDEKEKKFAALNDSDES--DDDEASSCHD
M.musculus CAEHLRKYNEALQINDTIRMIDAYSHLETFYTDEKEKKFAVLNDSDKS--D-DEASSCND
C.familiaris CAEHLRKYNEALQINDTIRTIDAYNHLEAFYNDETEKKLAVQEGDSDESGEDGDGDGDGD
B.taurus CAEHLRKYNEALQINDTIRMIDAYNHLETFYNDEKEKKFAVLLGDDSDESD---D--NGD
G.gallus CAEHLKKYNDALQINDTIRMVDAYNHLNNFYKELKRRKTAESDDD----------
X.tropicalis CAEHLRKYNDALQINDTIRMTDSLIHLRKFYEEEKKRKILLNEGS----------
T.nigroviridis CAEHLRQYNEGLGLSNTFRMWDALNFLSKYHEEEMKKKTAPD----------
D.rerio CAEHLRQYNEALHQSNTIRMSDAFRFLDKYHNEELKTKSSPD----------
H.sapiens EDEDDLKKPLKLDETDRFLMTLFFENNKMLKRLAENPEYENEKLTKLRNTIMEQYTRTEE
P.troglodytes EDEDDLKKPLKLDETDRFLMTLFFENNEMLKRLAENPEYENEKLTKLRNTIMEQYTRTEE
M.mulatta EDEDDLKKPLKLDETDRFLMTLFFENNKMLKKLAENPEYENEKLTKLRNTIMEQYTRTEE
R.norvegicus QLKGNVKKSLKLDETDEFLMNLFFDNKKMLKKLAENPKYENEKLIKLRNTILEQFTRSEE
M.musculus QLKGDVKKSLKLDETDEFLMNLFFDNKKMLKKLAENPKYENEKLIKLRNTILEQFTRSEE
C.familiaris EAEDDEKRPLRLDRTDRFLMELFWENKRMLKKLAQNPKHENEKLIKLRNTIMEQFTRTEE
B.taurus DDVGDGKPPLKLHETDDFLISLFWGNKKKLKKLAQNPEHENEKLIKLRNTIMEQYSRTEG
G.gallus ----EEPLVSKQDETDEFLMRLFHAKKKQLKELARKPEYDNEKLMKLRNTLMEEFTKTEE
X.tropicalis ----EHVAPLNIEETDRFLIDLFYDNEKELTAIAKNPKYENENLYALRSSLLEEFTRNGQ
T.nigroviridis -----EEHVIQITDTERFLFNLFKEYKEELQTLANNPEYENKSLSKLKTKVLQEFSTRLD
D.rerio -----EEGNITITDTERFLFTLFKDKKAKLQELMGKPQYENNNLAQLKTIILKEFSTREK
A719V R720Q E773Q
H.sapiens SARGIIFTKTRQSAYALSQWITENEKFAEVGVKAHHLIGAGHSSEFKPMTQNEQKEVISK
P.troglodytes SARGIIFTKTRQSAYALSQWITENEKFAEVGVKAHHLIGAGHSSEFKPMTQNEQKEVISK
M.mulatta SARGIIFTKTRQSAYALSQWITENEKFAEVGVKAHHLIGAGHSSEFKPMTQNEQKEVISK
R.norvegicus SSRGIIFTKTRQSTYALSQWIMENEKFAEVGVKAHHLIGAGHSSEVKPMTQTEQKEVISK
M.musculus SSRGIIFTKTRQSTYALSQWIMENAKFAEVGVKAHHLIGAGHSSEVKPMTQTEQKEVISK
C.familiaris PARGIIFTKTRQSAFALSQWITENKKFAEVGVKAHHLIGAGHSSEFKPMTQNEQKEVISK
B.taurus SARGIIFTKTRQSAYALSQWIIENEKFSEVGVKAHHLIGAGHSSEFKPMTQNEQKEVISK
G.gallus -PRGIIFTKTRQSALALYHWIMDNPKFEEVGIKAHFLIGAGHNSETKPMTQNEQREVIDK
X.tropicalis -ARGIIFTKTRQSAVALNQWISDNEKFTEVGIRSSYLIGAGHNSDFKPMTQNEQKQIIHK
T.nigroviridis -ARGIIFTKTRRGAIALTQWIRENSKFADMDVKPAYVIGGGDQSVVKPMTAAEQKDVLNK
D.rerio -ARGIIFTQTRLSAIALCQWIEENPKFDEVGVRASYLIGGGDQSVVKPMTAAEQKDVLKR

```
                  R779L                         N802D
                  R779C G781E                   I803F                                 T829S
                  R779H                                                 R822Q R824K
H.sapiens             FRTGKINLLIATTVAEEGLDIKECNIVIRYGLVTNEIAMVQARGRARADESTYVLVAHSG
P.troglodytes         FRTGKINLLIATTVAEEGLDIKECNIVIRYGLVTNEIAMVQARGRARADESTYVLVAHSG
M.mulatta             FRTGKINLLIATTVAEEGLDIKECNIVIRYGLVTNEIAMVQARGRARADESTYVLVAHSG
R.norvegicus          FRTGEINLLIATTVAEEGLDIKECNIVIRYGLVTNEIAMVQARGRARADESTYVLVTSSG
M.musculus            FRTGEINLLIATTVAEEGLDIKECNIVIRYGLVTNEIAMVQARGRARADESTYVLVTSSG
C.familiaris          FRTGKINLLIATTVAEEGLDIKECNIVIRYGLVTNEIAMVQARGRARADESTYVLVAHSN
B.taurus              FRTGKINLLIATTVAEEGLDIKECNIVIRYGLVTNEIAMVQARGRARADESTYVLVAQSG
G.gallus              FRGGSINLLIATTVAEEGLDIKECNIVIRYGLVTNEIAMVQARGRARADESTYALVASSG
X.tropicalis          FSTGELNLLVATSVAEEGLDIKECNVVIRYGLVTNEIAMVQARGRARADDSSYVLVAPSS
T.nigroviridis        FRNGEVNLLIATSVAEEGLDIPKCNFVIRYGHVANEISMIQTEGRGRAEDSSYTVVDVKN
D.rerio               FRTGEINLLIATTVAEEGLDIAECNVVIRYCLVTNEVAMIQARGRGRAEDSSYTLVAEAG
                      *  *.:***:**:******** :**.****  *:**::*:*:.**.**::*:*.:*

                            D848E  M854K
H.sapiens             SGVIEHETVNDFREKMMYKAIHCVQNMKPEEYAHKILELQMQSIMEKKMKTKRNIAK-HY
P.troglodytes         SGVIERETVNDFREKMMYKAIHCVQNMKPEEYAHKILELQMQSIMEKKMKTKRNIAK-HY
M.mulatta             SGVIERETVNDFREKMMYKAIHCVQNMKPEEYAHKILELQMQSIMEKKMKTKRSIAK-HY
R.norvegicus          SGVTEREIVNDFREKMMYKAINRVQNMKPEEYAHKILELQVQSILEKKMKVKRSIAR-QY
M.musculus            SGVTEREIVNDFREKMMYKAINRVQNMKPEEYAHKILELQVQSILEKKMKVKRSIAK-QY
C.familiaris          SGVIEREIVNDFREKMMYKAIDHVQNMNPEEYAHKILELQMQSIMEKKMKIKRSAAK-CY
B.taurus              SGVVERETVNDFREKMMYKAIDRVQNMKPEEYAHKILELQMQSIMEKKMKTKRSIAK-QF
G.gallus              SGAVEREDVNIFRENMMYKAIRRVQEMPPEEYLNKIQDFQLQSIVEKQMKAKRDQRK-TY
X.tropicalis          SGAIERDSVNVYKEEMMHKAIAKVQKMDRATYIDKIEEFQAQTIMEKKVKAKKAIHK-VY
T.nigroviridis        SGVAEKETVNEYRKNMMDKAIEKIGALKHADYDKQIQEFQMQAIMEYMLRMKAKKQEDIK
D.rerio               SGVAERESVNEYREKMMSKAIAKVCKMNRADYEKKIMEFQIQAIMEEKVKTKKKQQKGMM
                      **. *:: ** ::::** ***  :  :    * .:* ::* *:*:*  :: *    .

                                                                          I956V
H.sapiens             KNNPSLITFLCKNCSVLACSGEDIHVIEKMHHVNMTPEFKELYIVRENKALQKKCADYQI
P.troglodytes         KNNPSLITFLCKNCSVLACSGEDIHVIEKMHHVNMTPEFKELYIVRENKALQKKCADYQI
M.mulatta             KNNPSLITFLCKNCSVLACSGEDIHVIEKMHHVNMTPEFKELYIVRENKALQKKCADYQT
R.norvegicus          NSDPSLITLLCKNCSTLVCSGENIHVIEKMHHVNMTPEFKGLYIVRENKALQMKFADYQT
M.musculus            NDNPSLITLLCKNCSMLVCSGENIHVIEKMHHVNMTPEFKGLYIVRENKALQKKFADYQT
C.familiaris          KENPSLINFLCKNCSVLACSGEDIHVIEKMHHVNMTPVFKELYIVRENKALRKKFIDYQT
B.taurus              KGKPSLINFLCKNCGVPACSGEDIHVIEKMHHVNMTPEFKKLYLVRGNKALQTMCVDYQT
G.gallus              KKNPSLITFLCKNCHKLICSGEDIQVIENMHHVSVKKDFQHLYHKRENRTLQDKHADYQT
X.tropicalis          QSNPSLVTFHCKKCSKQACCGTDIQVIATAHHVNTTPKFKTLYSKGPNKTLQEKFADYQI
T.nigroviridis        NENPSNVKFSCRSCSQEVCTGRDIEIMANIHRVNVTPQFRELFILKENTKLKNSLLDYET
D.rerio               KEPPSKISLSCRQCSVFVCSGEDIEIIEKMHHVNVTKQFRKLFIVRENASLQERLLDYET
                      :  ** :.: *:.*    * * :*.:: . *:*. .  *: *:    *  *:    **:

                                  L979W
H.sapiens             NGEIIC-KCGQAWGTMMVHKGLDLPCLKIRNFVVVFKNNS-TKKQYKKWVELPITFPNLD
P.troglodytes         NGEIIC-KCGQAWGTMMVHKGLDLPCLKIRNFVVVFKNNS-TKKQYKKWVELPITFPNLD
M.mulatta             NGEIIC-KCGQAWGTMMVHKGLDLPCLKIRNFVVVFKNNS-TKKQYKKWVELPITFPNLD
R.norvegicus          NGEIIC-KCGQAWGTMMVHKGLDLPCLKIRNFVVNFKNNS-SKKQYKKWVELPIRFPDLD
M.musculus            NGEIIC-KCGQAWGTMMVHKGLDLPCLKIRNFVVNFKNNS-PKKQYKKWVELPIRFPDLD
C.familiaris          NGEIICKMCGQAWGTMMVHKGLDLPCLKIKNFVVIFKNNT-SKKQYKKWVELPIAFPDLD
B.taurus              NGEIICNKCGQAWGTMMVHKGLDLPCLKIKNFVVVFQNNL-PKKQYKKWVELPITFPDLN
G.gallus              NVEIICKDCGQVWGNMMVYRGLDLPCLKIRNFVVAFEDKKTTKEIFKKWGELPIIFPDFD
X.tropicalis          NGDIICKECGKTWGTTMVHKGIEVPCLQIRNFVVKYDDKKMTKDTYDKWSELPIKFPSFT
T.nigroviridis        NGYIACGKCGQKWGSMMHFRGIQCPCLHVKHFVVTINGKK--ISKCQQWMDLPVRLSEFD
D.rerio               NGVIACKKCGQQWGSMMLYRSTECPCLHIKNFVVTYGSK---KKTFSKWRELSISFPAFD
                      *  * *  **: **. * .:. : ***:::.***    :    .  .:* :* : :  :

H.sapiens             YSECCLFSDED---------
P.troglodytes         YSECCLFSDED---------
M.mulatta             YSECCLFSDED---------
R.norvegicus          CSEYCLYSDED---------
M.musculus            YSEYCLYSDED---------
C.familiaris          YSEYCLFSDED---------
B.taurus              YSEYCLFSDED---------
G.gallus              YASHCPSSDED---------
X.tropicalis          YSLP--DSDED---------
T.nigroviridis        YAEHCLFKYSY---------
D.rerio               YTQHADLLVEDSEDEDMDTN
                       :       .
```

IFIH1 homologs 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%.

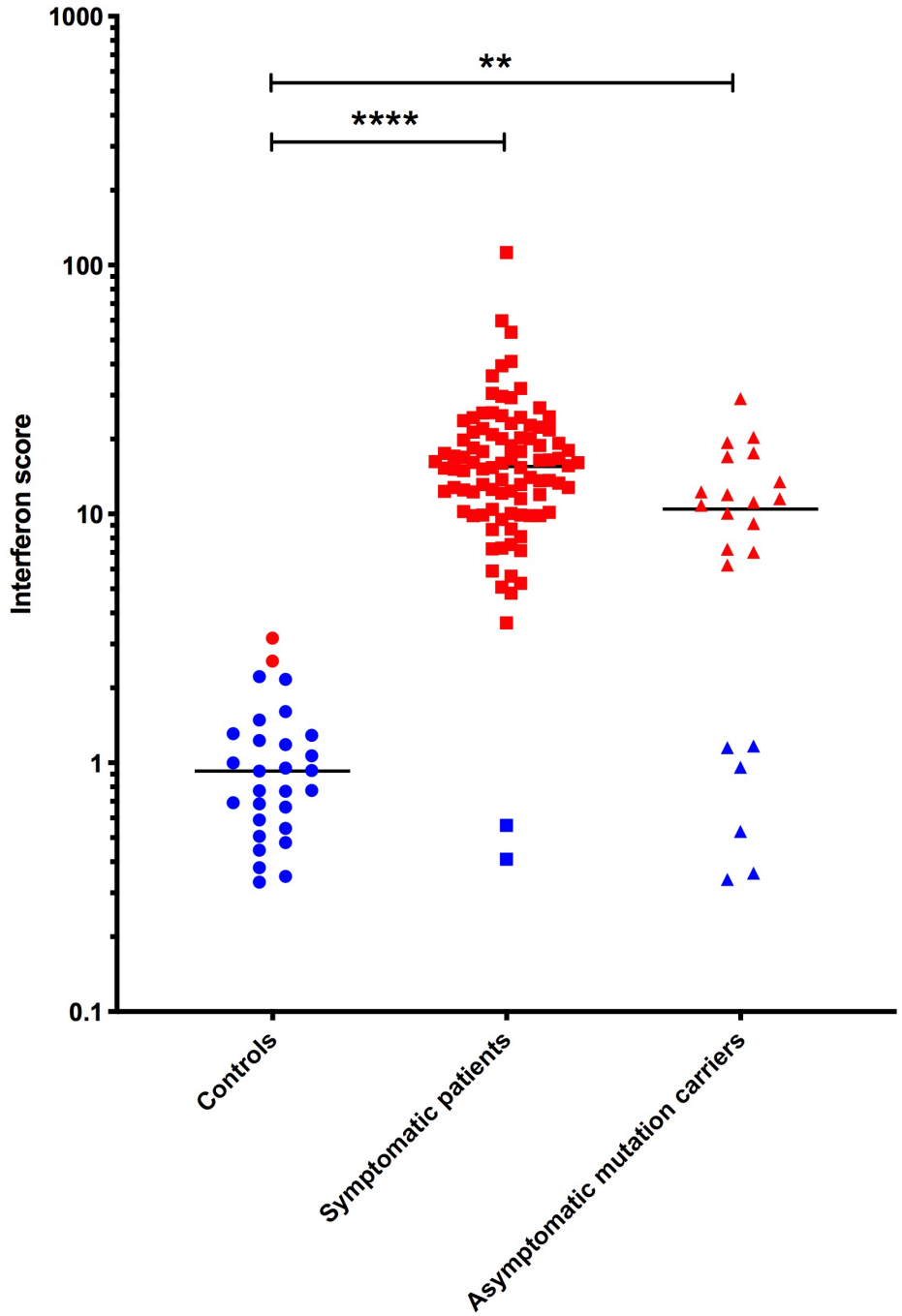
**Supp. Figure S2. Interferon beta (IFN-ß) reporter activity of Flag-tagged wild-type (WT) and mutant *IFIH1* with and without stimulation with polyI:C in HEK 293T cells.**




Error bars represent the standard deviation of three independent experiments. The IFIH1 variants p.Gly389Arg, p.Asn449Lys, p.Ile583Val, p.Ile803Phe and p.Ile956Val are predicted as benign using *in silico* programs. The other three variants (p.Glu444Gly; p.Glu773Gln; p.Leu979Trp) were tested in view of the novelty of the mutation and / or an absence of interferon stimulated gene testing in a variant carrier. EV indicates empty vector. 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. ns indicates not significant. All variants showed significantly higher basal signaling activity compared to WT in the absence of polyI:C. Expression levels of individual constructs were tested by western blotting. While the level of E773Q (p.Glu773Gln) is significantly lower than WT IFIH1 or other variants, this

does not affect our interpretation that E3773Q (p.Glu773Gln) is a gain-of-function mutation. Annotated p values are in comparison to the wild type (WT) value.

**Supp. Figure S3**. Interferon scores in *IFIH1*-mutation positive individuals as measured in the Crow laboratory.




Red denotes elevated score (> 2.44), blue denotes a result within the normal range of 29 controls as previously published (Rice et al. Lancet Neurol 12;1159-69).

**Supp. Table S1**. Details of six putative mutations predicted as benign by *in silico* analysis

| cDNA change | Protein change | Mutated families (de novo inheritance; or, number of symptomatic and non-penetrant individuals where familial) | Associated phenotypes ('/' within family)(';' between families) | Upregulated interferon stimulated gene expression | *In vitro* enhancement of interferon signalling | gnomAD frequency | SIFT | Polyphen2 | CADD score | Varcards |
|---|---|---|---|---|---|---|---|---|---|---|
| c.1165G>A | p.Gly389Arg | AGS848 (2; 1) | AGS/SP/CNP | Yes (four variant-positive individuals, all demonstrating a positive interferon signature on at least two occasions: Crow laboratory) | Yes (this paper) | Novel | Tolerated 0.88 | Benign 0.108 | 5.325 | 01:23 |
| c.1347C>G | p.Asn449Lys | AGS1001 (de novo) | SP | Yes (interferon score positive on both occasions tested: Crow laboratory) | Yes (this paper) | Novel | Tolerated 0.64 | Benign 0.163 | 13.91 | 03:23 |
| c.1747A>G | p.Ile583Val | AGS2369 (de novo) | AGS | Yes (interferon score positive on both occasions tested: Crow laboratory) | Yes (this paper) | Novel | Tolerated 0.48 | Benign 0.00 | 0.573 | 5.23 |
| c.2407A>T | p.Ile803Phe | LD_1488.0 (de novo) | AGS | Yes (interferon score positive on three occasions tested: Vanderver laboratory) | Yes (this paper) | Novel | Tolerated 0.24 | Benign 0.043 | 11.8 | 04:23 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| c.2544T>G | p.Asp848Glu | AGS531 (3; 2) | SP-ICC/CNP | Yes (four variant-positive individuals, all demonstrating a positive interferon signature on at least two occasions: Crow laboratory) | Yes (Ruaud et al. 2018) | Novel | Tolerated 0.4 | Benign 0.004 | 10.08 | 02:23 |
| c.2866A>G | p.Ile956Val | AGS1430 (2; 1) | SP-ICC/CNP | Yes (two variant-positive individuals demonstrating a positive interferon on every occasion (four and three times) tested; one variant-positive individual, an asymptomatic grandfather to the proband, with a normal interferon score on two occasions tested: at the age of 69 years) | Yes (this paper) | Novel | Tolerated 0.77 | Benign 0.004 | 3.576 | 06:23 |

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

AGS: Aicardi-Goutières syndrome; CLL: Chilblain-like lesions; CNP: Clinical non-penetrance; ICC: Intracranial calcification; SP: Spastic paraparesis

**Supp. Table S2**. Molecular and clinical data by family

| Family | Individual | Sex | cDNA | Protein | Clinical details (by family) |
|---|---|---|---|---|---|
| AGS102 | P1 | M | c.2159G>A | p.Arg720Gln | Pre-natal onset; congenital infection-like presentation (low platelets; hepatosplenomegaly; abnormal neurology). Severe DD and SD. Hypertension of undefined cause. Died age 2 years |
| AGS163 | P1 | M | c.2336G>A | p.Arg779His | Presented < 1 month of age with irritability, feeding difficulties, axial hypotonia and abnormal limb posturing. At age 13 years he demonstrated a severe quadriplegia, with no head control, no speech, abnormal ocular movements and microcephaly |
| AGS237 (LD_0762) | P1 | M | c.1009A>G | p.Arg337Gly | Neuro-regression with spasticity and dystonia after normal development to age 15 months. At age 12 years he had no useful hand function, could not sit independently and had limited words, although his understanding was relatively preserved. Died age 16 years of cardiopulmonary arrest secondary to pulmonary hypertension |
| AGS259 | P1 | M | c.2336G>A | p.Arg779His | Apparently normal neonatal history but at age 6 months demonstrated axial hypotonia and lower limb spasticity. At 8 years of age he was severely delayed, never having acquired the ability to sit, with minimal hand function, gastrostomy feeding and no effective communication. Now, at 13 years of age he demonstrates an increasingly severe dystonic movement disorder. Father and paternal grandmother without disease features at 54 and 84 years of age respectively (the latter dying of coronary artery disease at that time) |
| | P2 (father of P1) | M | | | |
| | P3 (mother of P2) | F | | | |

| | | | | | |
|---|---|---|---|---|---|
| AGS376 | P1 | M | c.2335C>T | p.Arg779Cys | Likely prenatal onset with IUGR, poor feeding and developmental delay (obvious by age 4 months). At 29 months of age he developed a lupus-like illness with recurring fevers, weight loss, diarrhea, an ulcerative photosensitive vasculitic rash, hepatosplenomegaly, generalized lymphadenopathy, arthritis of the knees and ankles, serositis with pericardial effusion and a pronounced inflammatory response. Significant gastrointestinal vasculitis with rectal bleeds and liver dysfunction was a major problem and he died aged 3 years despite intensive immunosuppressive therapy |
| AGS524 | P1 | F | c.1483G>A | p.Gly495Arg | Delayed motor development with spasticity, then developing a transverse myelitis at age 21 months in association with a severe lupus-like illness and positive AQP4 antibodies. At age 10 years she exhibits 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. She has had surgery for scoliosis. Her father experienced the childhood-onset of lower-limb spasticity which has been very slowly progressive (to current age of 39 years). His upper limb function and intellect are fully preserved in the absence of any systemic features and with completely normal neuroimaging (brain and spine) |
| | P2 (father of P1) | M | | | |
| AGS531 | P1 | F | c.2544T>G | p.Asp848Glu | The proband developed walking difficulties with moderate spasticity of the lower limbs from 3 years of age, and at age 14 years was unable to walk on tip-toes. Cerebral CT scan demonstrated ICC. Her twin brother is asymptomatic, with unremarkable clinical examination and normal CT brain. The father of the proband experienced walking difficulties from 2 years of age, with a slowly progressive course. He could run when aged 24 years, but by 41 years of age he demonstrated severe spastic weakness with normal intellect. Brain CT at age 24 years revealed extensive ICC. The father's brother experienced progressive lower limb motor impairment since age 13 years. At 38 years of age, he demonstrated spastic weakness with normal cognition. Again, ICC was seen on CT. The proband's paternal grandfather was asymptomatic and without abnormal clinical signs at age 65 years |
| | P2 (brother of | M | | | |

| | | | | | |
|---|---|---|---|---|---|
| | P1) | | | | |
| | P3 (father of P1 and P2) | M | | | |
| | P4 (brother of P3) | M | | | |
| | P5 (father of P3 and P4) | M | | | |
| AGS626 | P1 | M | c.1178A>T | p.Asp393Val | Mild transaminitis noted at age 3 months and microvesicular steatosis seen on liver biopsy at 1 year of age. Transaminase values gradually normalized by age 4 years. After exhibiting normal motor development to 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. At age 17 years he demonstrated a spastic tetraplegia with mild dystonic movements in all 4 limbs, communication was limited to eye movements and he had no useful hand function |
| AGS647 | P1 | M | c.2159G>A | p.Arg720Gln | Neonatal infection-like presentation (low platelets; anaemia). Developmental delay obvious by age 3 months with SD of the limbs and axial hypotonia. Concentric hypertrophy of the left cardiac ventricle diagnosed age 9 months, and acute nephrotic syndrome with focal glomerular sclerosis and tubuloreticular inclusions on renal biopsy at 10 months of age. Hypothyroidism identified at age 18 months and atrophic gastritis at 2 years of age. At this time he was severely developmentally delayed with poor head control, no useful hand function and limited social interaction |
| AGS674 | P1 | M | c.992C>G | p.Thr331Arg | Normal development to age 12 months when he developed a spastic tetraparesis with dystonia, dysarthria, mild intellectual deficit and a mixed sensory and motor neuropathy. White matter lesions and calcification on neuroimaging. He also exhibited growth failure, dental abnormalities with absence of many permanent teeth, and psoriatic-like skin lesions with hypochromic lentigines |

| | | | | | |
|---|---|---|---|---|---|
| AGS723 | P1 | F | c.2335C>T | p.Arg779Cys | Severe psoriatic-like lesions noted in the first months of life, with a progressive spastic paraparesis becoming evident during childhood and preserved intellect. Striatal calcification present on cerebral CT scanning |
| AGS735 | P1 | M | c.2471G>A | p.Arg824Lys | Normal development until age 12 months when he experienced psychomotor regression, with abnormal white matter on cerebral MRI. At 13 years of age he exhibited a spastic dystonic tetraparesis with normal head circumference. Verbal expressive language was absent but non-verbal intelligence quotient was within the normal range |
| AGS755 | P1 | M | c.1465G>A | p.Ala489Thr | Proband presented with chilblain-like lesions of the ears and erythematous cheeks at age 1 year. His father had exhibited similar ear lesions at 8 months of age and subsequently developed psoriatic-like lesions, lentigines, deformities of the hands and feet with tendon retractions and loss of secondary dentition after adolescence. The younger brother to the proband demonstrated early chilblain-like lesions and multiple lentigines, with delayed motor development and then a period of motor regression at age 2.5 years so that he was unable to walk or stand, having only a few spoken words at this age. Glaucoma identified soon thereafter |
| | P2 (brother of P1) | M | | | |
| | P3 (father of P1 and P2) | M | | | |
| AGS848 | P1 | M | c.1165G>A | p.Gly389Arg | The proband demonstrated a phenotype characteristic of AGS presenting in early infancy. His father exhibited a lower limb spasticity without other features. The father's maternal grandmother, his mother being deceased with unknown history, was reported to be asymptomatic at age 84 years (but was not formally examined) |
| | P2 (father of P1) | M | | | |
| | P3 (maternal grandmother of P2) | F | | | |

| | | | | | |
|---|---|---|---|---|---|
| AGS1001 | P1 | M | c.1347C>G | p.Asn449Lys | Development considered normal until age 18 months when concerns were raised about the need for continued support whilst walking. Over the following year he developed progressive spasticity in his lower limbs, and he demonstrated speech delay - not speaking until age 4 years. He developed autoimmune hypothyroidism at age 6 years. ICC identified at age 11 years. At age 18 years he was able to walk with two sticks, exhibited a marked startle response and had mild learning difficulties. He was normocephalic, had lower limb spasticity with normal upper limb examination, Raynaud phenomenon and psoriasis |
| AGS1004 | P1 | F | c.2335C>T | p.Arg779Cys | Perinatal thrombocytopenia, followed by pneumocystis carinii and CMV positive pneumonia requiring ventilation at age 3 months. Development considered normal until age 8 months when he lost skills and developed a SD, with widespread hyperintense white matter lesions on T2 MRI. Now, no motor skills, no speech and minimal comprehension, spastic dystonic tetraparesis, microcephaly, short stature, severe visual impairment, psoriatic-like lesions, glaucoma and nystagmus |
| AGS1156 | P1 | M | c.2335C>T | p.Arg779Cys | Normal development to age 8 months at which time he experienced a period of regression. The following 8 months were characterised by sterile pyrexias, irritability, increasing spasticity and exaggerated startle responses to sounds. At 16 months of age he was microcephalic, small, and centrally hypotonic with peripheral spasticity. As reported in Kothur et al. he has shown an apparently positive response to treatment with ruxolitinib, so that at age 5 years, having taken ruxolitinib 5mg bd for 3.5 years, he is making developmental gains, although he continues to demonstrate a spastic-dystonic tetraparesis. He crawls at speed independently, and has good functional use of his hands with finger feeding, and drives a motorised wheelchair with skill and good visuo-spatial accuracy. He uses his iPad to communicate, and can make requests and jokes. His repeat MRI shows no atrophy and cardiac examination remains normal |
| AGS1290 | P1 | M | c.2486C>G | p.Thr829Ser | The older sibling demonstrated apparently normal development in the first 5 months of life and then experienced rapid neurological decline, whilst his brother was always delayed. Both children are fully dependent for all activities, gastrostomy fed and exhibit a severe SD. Abnormal white matter and calcification seen on brain imaging, with a raised level of neopterin in the CSF. The proband developed seizures at age 7 years |

| | | | | | |
|---|---|---|---|---|---|
| | P2 (brother of P1) | M | | | |
| AGS1351 | P1 | F | c.2336G>A | p.Arg779His | Severe, early onset developmental delay with peripheral spasticity and truncal hypotonia. Died age 2 years |
| AGS1430 | P1 | M | c.2866A>G | p.Ile956Val | The proband experienced an episode of encephalitis at the age of 6 years, before which time his development was completely normal. He subsequently exhibited a progressive spastic paraparesis with abnormal white matter and cerebral calcification. His father experienced a similar onset of disease at age 2 years. Both individuals are of normal intellect with no other features. The putative mutation was inherited from the clinically asymptomatic paternal grandfather of the proband |
| | P2 (father of P1) | M | | | |
| | P3 (father of P2) | M | | | |
| AGS1504 (LD_1175) | P1 | F | c.2159G>A | p.Arg720Gln | Early development possibly normal but after 1st DPT vaccination aged 2 months she experienced fevers with prolonged crying and change in feeding pattern. Persistent elevation of TSH noted at 3 months of age requiring long-term thyroxine replacement. Persistent feeding difficulties and failure to thrive led to gastrostomy placement at 16 months of age. Head control achieved by age 7 months, and sitting with support at 13 months of age. However, at age 16 months she developed fevers and experienced developmental regression with loss of skills. Now, she demonstrates profound intellectual disability with SD, remains hypothyroid, has a persistent thrombocytopenia and has undergone orthopaedic surgery for contractures and hip dislocations. She is under gynaecological review for premature adrenarche, and is small (height -4.7 SD; weight -2.46 SD) and microcephalic (OFC -4.5 SD). Progressive neurodegeneration with abnormal white matter and calcification on brain imaging |
| AGS1509 | P1 | M | c.2336G>A | p.Arg779His | Global developmental delay obvious by age 9 months, progressing to spastic quadriplegia with axial hypotonia and distal hypertonia. CSF pterins elevated. Cranial CT and brain MRI at 2 years of age showed basal ganglia and frontal / parietal lobe calcification with delayed myelination |

| | | | | | |
|---|---|---|---|---|---|
| AGS1514 | P1 | M | c.2465G>A | p.Arg822Gln | Developmental delay clear by age 6 months. At 12 months of age he was able to pull and stand, but these milestones were then lost and he subsequently developed spasticity with contractures of the knee joints and elbows. Brain MRI at age 2 years was normal, but calcification was seen on cerebral CT at 6 years of age. A chronic transaminitis was noted |
| AGS1938 | P1 | F | c.992C>T | p.Thr331Ile | All three affected family members exhibited a phenotype consistent with a diagnosis of SMS, variably demonstrating a deforming non-destructive arthropathy, abnormal dentition, aortic valve stenosis and psoriatic like lesions with multiple lentigenes |
| | P2 (mother of P1) | F | | | |
| | P3 (sister of P2) | F | | | |
| AGS1972 | P1 | F | c.992C>G | p.Thr331Arg | Father and daughter with a phenotype consistent with a diagnosis of SMS, variably demonstrating a deforming non-destructive arthropathy, abnormal dentition, psoriatic like lesions and glaucoma |
| | P2 (father of P1) | M | | | |
| AGS2081 | P1 | M | c.2561T>A | p.Met854Lys | Normal milestones until age 14 months when he developed a progressive spastic paraparesis, losing the ability to walk by age 4 years. Demyelinating sensory polyneuropathy diagnosed at 3 years of age. Wide-spread erythema and hyperkeratotic lesions on elbows and knees from an early age, with delayed tooth eruption and then precocious loss of permanent teeth. Glaucoma diagnosed at age 5 years. Brain MRIs demonstrating non-specific hypersignal of periventricular white matter initially, with dense and wide-spread calcification noted at age 9 years. Cognitive and language skills normal at 12 years of age |

| | | | | | |
|---|---|---|---|---|---|
| AGS2154_1 (LD_1240) | P1 | M | c.2335C>T | p.Arg779Cys | No past medical history and normal development until onset of headache, dizziness and repeated falls at age 8 years. Brain MRI remarkable for extensive T2 high signal of white matter limited to the right hemisphere. Brain biopsy of right frontal cortex showed vasculopathy of unknown aetiology and myelin loss. Headaches have worsened, often associated with limb pain. Motor development, intellect and neurological examination considered normal. His mother, aged 38 years, demonstrates no clinical features (with normal interferon signature on 3 occasions) |
| AGS2154_2 (LD_1240.1) | P2 (mother of P1) | F | | | |
| AGS2177 | P1 | M | c.2336G>A | p.Arg779His | Psychomotor development was normal until age 12 months, at which time he had started to walk and babble. There then followed a sudden regression with loss of skills and the onset of a progressive spastic tetraparesis. At age 29 years he is spastic and wheelchair bound without useful hand function or recognisable words. The same mutation was identified in the proband's healthy sister and mother (aged 32 and 67 years respectively) |
| | P2 (mother of P1) | F | | | |
| | P3 (sister of P1) | F | | | |
| AGS2180 | P1 | F | c.2335C>T | p.Arg779Cys | Likely never normal development; head control at age 3 months, sitting at 9 months of age but never cruising, and there were significant parental concerns before her first birthday. Now, age 4 years, she demonstrates a significant SD of all four limbs, with no speech but good communication skills |
| AGS2222 | P1 | M | c.2471G>A | p.Arg824Lys | Isolated liver disease (neonatal hepatitis, bile duct paucity, liver fibrosis). Normal neurology; Now 7 years of age |

| | | | | | |
|---|---|---|---|---|---|
| AGS2369 | P1 | M | c.1747A>G | p.Ile583Val | Presented with delayed walking and expressive language. Found to have white matter abnormalities on brain MRI. Now, aged 9 years, has some truncal hypotonia and peripheral spasticity. He is wheelchair bound but is considered bright, using sign language and a communication aid |
| AGS2399 | P1 | M | c.2317G>C | p.Glu773Gln | Normal development until age 16 months, at which time he was walking. He then demonstrated psychomotor regression, with his condition stabilising by age 20 months. He is now left with a spastic diplegia and no expressive language |
| AGS2422 | P1 | F | c.2159G>A | p.Arg720Gln | Bilateral glaucoma at age 3 years. At 4 years of age she experienced walking difficulties and demonstrated a pyramidal syndrome and demyelinating neuropathy. Two episodes of neurological deterioration at age 17 years (post-trauma) and 22 years (post-partum), so that she became wheelchair bound. She demonstrates livedo reticularis, psoriasis, xerosis and chilblains. On brain MRI there are features of an occipital pseudo-stroke or vasculitis, associated with calcification of the basal ganglia. Vision has deteriorated over time with neovascularisation and cataract. She demonstrates a mild neutropenia and renal impairment. Head circumference is reduced (52 cm), but her intellect is normal |
| AGS2507 | P1 | F | c.2335C>T | p.Arg779Cys | This girl was microcephalic at birth, and then experienced a sudden onset of irritability and crying at three weeks of age, subsequently demonstrating severe psychomotor retardation (no eye contact or other communication), spastic tetraparesis and dystonia. Mild liver and spleen enlargement with elevated liver transaminases noted soon after presentation were still present at 10 months of age. MRI of the brain at age 6 months showed hypomyelination and calcifications |
| AGS2548 | P1 | M | c.2159G>A | p.Arg720Gln | Born at term weighing 1.67 kg (-3.76 SD) with features of sepsis and autoimmune thrombocytopenia. Developmental delay obvious by age 3 months, with white matter disease and intracranial calcification. Now at age 3 years he demonstrates severe psychomotor delay and a spastic dystonic tetraparesis |

| | | | | | |
|---|---|---|---|---|---|
| AGS2586 | P1 | M | c.1178A>C | p.Asp393Ala | Born at 38 weeks gestation in good condition but symmetrically small. Fed poorly and presented with fever at age 6 weeks. Anaemic, with thrombocytopenia, leucocytosis and hepatosplenomegaly which resolved although he had a persistent mild hepatic transaminitis. He was delayed in his development but made steady progress so that at age 18 months he had reduced truncal tone but could commando crawl, use pincer grasp bilaterally and had many purposeful words. At 21 months of age he began to regress, becoming irritable, losing the ability to commando crawl or lift his head when prone, becoming stiff, and developing hand fisting. He remained visually bright, and understood language but lost his expressed words. Cerebral MRI showed mild delayed myelination only, and CSF was acellular but with a raised neopterin of 307 nmol/l (normal <30) |
| AGS2662 (LD_1640) | P1 | F | c.2404A>G | p.Asn802Asp | Development considered normal until 11 months of age when she became irritable and febrile, associated with a loss of skills. She had not achieved walking, but lost the ability to pull herself up and sit unassisted, and began to exhibit features of a SD with apparently normal cognition |
| AGS2669 | P1 | M | c.1331A>G | p.Glu444Gly | Presented in early infancy with severe irritability and developmental delay. MRI showed symmetrical white matter abnormalities, and intracranial calcification was observed on CT. He was very irritable and dystonic - needing to be carried much of the time. Pulmonary insufficiency became significant and he died at age 6 months |
| Hm_1 | P1 | F | c.2156C>T | p.Ala719Val | Neonatal presentation with growth retardation, microcephaly, abnormal neurology, anaemia and low platelets. She experienced a CMV pneumonia at age 3 months. At the age of 7 months she experienced further neurological deterioration coinciding with immunisation. Liver biopsy undertaken because of a persistent transaminitis suggested an autoimmune hepatitis (mild chronic hepatitis with mild interface activity, mild portal and perisinusoidal fibrosis) treated with steroids. Now, at age 27 months, she is severely developmentally impaired with no head control, a spastic tetraparesis and truncal hypotonia, no words, no reactive smiling and increased blood pressure |

| | | | | | |
|---|---|---|---|---|---|
| Berg_1 | P1 | F | c.2336G>A | p.Arg779His | Development normal until age 9 months when she became irritable and developed a progressive encephalopathy with abnormal white matter on cerebral MRI. At the age of 20 months a neuroblastoma was detected, leading to a diagnosis of opsoclonus-myoclonus syndrome in the absence of opsoclonus. By this time she demonstrated severe psychomotor retardation with no speech and a SD. At age 6 years she developed a lupus-like illness with skin rash and vasculitic lesions, raised ESR and normal CRP. Autoantibody titres were elevated and skin biopsy demonstrated findings compatible with SLE |
| Orc_0098 | P1 | M | c.2336G>A | p.Arg779His | Clinical onset at 8 months with irritability, disturbed sleep-wake pattern and fevers and frank psychomotor regression from 11 months with gradual appearance of microcephaly, spastic-dystonic tetraplegia. Abnormal white matter and calcification on cerebral imaging |
| LD_0940.0 | P1 | M | c.2342G>A | p.Gly781Glu | Mild developmental delay with frank regression at age 15 months, losing the ability to hold a bottle, sit and roll. He developed a diffuse panniculitis at this time. At 17 months of age cerebral MRI and CT documented intracranial calcification and leukoencephalopathy. Now he has a spastic-dystonic tetraparesis, is able to smile, vocalise and recognize familiar faces |
| LD_0943.0 | P1 | F | c.2342G>A | p.Gly781Glu | Presentation in the second year of life with toe-walking and lower limb spasticity which has been progressive. Brain Imaging showed calcification and leukodystrophy. Current neurologic evaluation demonstrates spastic diplegia with apparently intact cognition |
| LD_0982.0 | P1 | M | c.2159G>A | p.Arg720Gln | Neonatal thrombocytopenia and hepatosplenomegaly with failure to thrive. Persistent thrombocytopenia until 2 years of age despite IVIG and steroids. At age 9 months he was not sitting. Liver biopsy at 1 year of age was complicated by haemorrhage and associated with a loss of skills and the ability to feed. Cerebral MRI and CT demonstrated severe atrophy and leukodystrophy. Pulmonary hypertension diagnosed prior to his fourth birthday in the setting of acute illness, and confirmed by cardiac catheterization at the age of 7 years. Now demonstrates developmental delay with spastic quadriparesis and microcephaly |

| | | | | | |
|---|---|---|---|---|---|
| LD_1030.0 | P1 | F | c.2335C>T | p.Arg779Cys | Seen at age 4 months with developmental delay, petechiae, leucocytosis, thrombocytopenia, hepatosplenomegaly and elevated LFTs. Liver biopsy consistent with autoimmune hepatitis. Brain MRI at age 10 months revealed delayed myelination and diffuse calcifications. Now demonstrates developmental delay with spastic quadriparesis and microcephaly |
| LD_1067.0 | P1 | M | c.2336G>T | p.Arg779Leu | Developmental delay obvious by age 5 months. At age 1 year he could hold his head, babble and could use his hands to hold objects and feed himself with a spoon. Brain MRI showed some areas of high signal white matter on T2 sequences. At age 24 months he could use a walker with good trunk control. Repeat imaging at this time showed new white matter signal abnormalities and basal ganglia calcification. After a viral infection he experienced psychomotor regression, losing the ability to walk, sit with support and hand use, with evolving spastic dystonic tetraparesis. He now uses an eye gaze device for augmentative communication, a head switch for wheelchair control and has a gastrostomy |
| LD_1199.0 | P1 | F | c.2336G>A | p.Arg779His | Delayed motor milestones obvious by age 6 months. She learned to crawl, pull to stand, cruise and use up to 10 words. At 18 months of age, after a viral infection with a high fever, she experienced rapid neurological regression, developing lower limb spastic diplegia. She now crawls fisted, though she can still scribble and use a fork or spoon, with expressive speech and language delay. White matter T2 high signal on brain MRI at 2 years of age |
| LD_1346.0 | P1 | M | c.2936T>G | p.Leu979Trp | Respiratory distress with cardiomegaly and pulmonary hypertension recognised shortly after birth requiring intubation. He also demonstrated severe anaemia and thrombocytopenia requiring multiple transfusions. Brain imaging revealed calcifications and CSF neopterin was elevated. Gastrostomy tube placed at 2 months of age for poor feeding and growth. He died of respiratory problems at age 4 months |

| | | | | | |
|---|---|---|---|---|---|
| LD_1381.0 A (Hart) | P1 | F | c.2336G>A | p.Arg779His | Development normal until 30 months when experienced regression with loss of walking in the absence of any cognitive deficit. Her younger brother demonstrated spasticity and mild motor deficit noted in first year of life, with apparently normal cognition despite abnormal white matter and calcification on brain imaging. The father of these two children was diagnosed with cerebral palsy as an infant. Toe walking and mild, spastic gait with basal ganglia calcification but otherwise well with no cognitive deficit. The paternal grandfather of the proband was clinically asymptomatic at age 68 years |
| LD_1381.0 B (Hart) | P2 (brother of P1) | M | | | |
| LD_1381.2 (Hart) | P3 (father of P1 and P2) | M | | | |
| LD_1381.3 (Hart) | P4 (father of P3) | M | | | |
| LD_1488.0 | P1 | F | c.2407A>T | p.Ile803Phe | Irritability and poor feeding noted by 6 weeks of age, with obviously delayed development by age 4 months. She acquired a social smile at 2 months of age, but later milestone have never been acquired. Neurologic exam is remarkable for truncal hypotonia and a SD |
| LD_1585.0 | P1 | F | c.2336G>A | p.Arg779His | Early history unavailable but increased leg tone noted by age 1 year treated with baclofen. She was able to cruise around furniture at age 20 months. Global developmental delay clear at 3 years of age. Currently has spastic tetraplegia |

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

AGS: Aicardi-Goutières syndrome; CLL: Chilblain-like lesions; CMV: Cytomegalovirus; CRP: C-reactive protein; CSF: Cerebrospinal fluid; CT: Computed tomography; DD: Developmental delay; DPT: Diphtheria, pertussis, tetanus; ESR: Erythrocyte sedimentation rate; F: Female; LLD: Lupus-like disease; ICC: Intracranial calcification; IUGR: Intrauterine growth retardation; IVIG: Intravenous immunoglobulins; LFTs: Liver

function tests; M: Male; MRI: Magnetic resonance imaging; SD: Spastic dystonia; SLE: Systemic lupus erythematosus; SMS: Singleton-Merten syndrome; SP: Spastic paraparesis; TM: Transverse myelitis; TSH: Thyroid stimulating hormone

**Supp. Table S3**. Summary data of interferon stimulated gene expression testing in the Crow laboratory

| Family | Individual | ISG scores (age) |
|---|---|---|
| AGS102 | P1 | Not available |
| AGS163 | P1 | Not available |
| AGS237 (LD_0762) | P1 | 12.26 (12.55); 22.70 (12.60) |
| AGS259 | P1 | 19.22 (8.14); 35.92 (8.21); 26.72 (8.29) |
| | P2 (father of P1) | 10.83 (48.80); 17.00 (48.87); 29.08 (48.95); 20.35 (49.73) |
| | P3 (mother of P2) | 12.28 (79.33); 7.02 (79.41); 19.38 (80.19) |
| AGS376 | P1 | Not available |
| AGS524 | P1 | 20.08 (3.91); 25.45 (5.19); 41.01 (5.83); 13.14 (7.36) |
| | P2 (father of P1) | 15.17 (32.34); 11.51 (32.85); 17.85 (33.13); 29.34 (33.62); 9.90 (33.95); 15.99 (36.11); 22.33 (36.33); 24.39 (36.61) |
| AGS531 | P1 | 53.82 (13.33); 9.51 (13.65) |
| | P2 (brother of P1) | 17.59 (13.33); 13.46 (13.65) |
| | P3 (father of P1 and P2) | 24.83 (39.87); 30.52 (40.15) |
| | P4 (brother of P3) | 9.89 (36.97); 8.66 (37.24) |
| | P5 (father of P3 and P4) | 9.16 (65.65); 6.23 (65.93) |
| AGS626 | P1 | 12.36 (17.14); 15.63 (17.66) |
| AGS647 | P1 | 22.03 (1.39); 16.12 (1.96); 18.41 (2.06) |
| AGS674 | P1 | 21.77 (9.44) |
| AGS723 | P1 | 39.45 (15.20); 16.08 (18.91) |
| AGS735 | P1 | 18.86 (14.57); 20.10 (14.75) |
| AGS755 | P1 | 7.54 (2.16); 21.33 (2.47); 59.77 (3.04) |
| | P2 (brother of P1) | 7.29 (3.82); 31.92 (4.70) |
| | P3 (father of P1 and P2) | 9.84 (41.00); 16.72 (41.88) |
| AGS848 | P1 | 20.84 (3.52); 112.49 (4.30); 16.67 (6.80) |
| | P2 (father of P1) | 23.7 (37.88); 11.95 (38.44); 5.90 (38.66); 9.86 (39.63); 17.54 (41.16) |
| | P3 (maternal grandmother of | 11.96 (78.67); 11.14 (78.89); 10.08 (81.39) |

| | | |
|---|---|---|
| | P2) | |
| AGS1001 | P1 | 24.47 (14.72); 13.61 (17.46) |
| AGS1004 | P1 | 20.21 (4.97) |
| AGS1156 | P1 | 12.54 (1.66); 12.78 (1.81);12.78 (2.03); 10.22 (2.11); 5.08 (2.18); 8.71 (2.31) |
| AGS1290 | P1 | 17.81 (3.79) |
| | P2 (brother of P1) | 25.49 (5.30) |
| AGS1351 | P1 | 12.49 (1.47); 10.43 (1.81) |
| AGS1430 | P1 | 15.28 (10.60); 13.66 (10.67); 16.43 (10.86); 13.74 (11.29) |
| | P2 (father of P1) | 19.82 (46.72); 24.55 (46.91); 16.24 (47.33) |
| | P3 (father of P2) | 0.96 (69.10); 1.15 (69.52); 1.17 (69.66) |
| AGS1504 (LD_1175) | P1 | Not available |
| AGS1509 | P1 | 10.06 (5.06) |
| AGS1514 | P1 | 7.23 (6.32) |
| AGS1938 | P1 | 29.72 (18.31) |
| | P2 (mother of P1) | 15.14 (45.35) |
| | P3 (sister of P2) | 16.89 (27.34) |
| AGS1972 | P1 | 14.03 (9.65) |
| | P2 (father of P1) | 18.82 (47.53) |
| AGS2081 | P1 | 15.38 (10.34) |
| AGS2154_1 (LD_1240) | P1 | 5.27 (10.56); 0.41 (10.65); 0.56 (10.75) |
| AGS2154_2 (LD_1240.1) | P2 (mother of P1) | 0.53 (38.89); 0.36 (38.98); 0.34 (39.08) |
| AGS2177 | P1 | 13.14 (29.97) |
| | P2 (mother of P1) | 7.22 (62.69) |
| | P3 (sister of P1) | 11.52 (33.19) |
| AGS2180 | P1 | 12.11 (2.68); 23.14 (3.51); 14.94 (3.70); 5.62 (3.79); 9.85 (3.87); 13.30 (4.02); 7.13 (4.19) |
| AGS2222 | P1 | 3.65 (5.26); 4.81 (5.51); 10.151 (7.14) |

| | | |
|---|---|---|
| AGS2369 | P1 | 16.49 (7.76); 12.32 (8.27) |
| AGS2399 | P1 | Not available |
| AGS2422 | P1 | 18.03 (38.08) |
| AGS2507 | P1 | 8.10 (0.82) |
| AGS2548 | P1 | Not available |
| AGS2586 | P1 | 15.36 (3.04) |
| AGS2662 (LD_1640) | P1 | Not available |
| AGS2669 | P1 | 17.1 (0.42) |
| AGS2685 | P1 | Not available |

Boxes coloured blue represent clinically asymptomatic individuals. Boxes coloured pale green define individuals with normal interferon scores (<2.466) on at least one occasion

**Supp. Table S4**. Details of previously published cases with *IFIH1* mutations that were not ascertained as part of the present series

| cDNA change | Protein change | Report(s) | Associated phenotypes | Documented upregulation of interferon signalling | gnomAD frequency | Seen in the present study |
|---|---|---|---|---|---|---|
| c.1114C>T | p.Leu372Phe | Oda et al. (de novo) | AGS | Yes | Novel | No |
| c.1354G>A | p.Ala452Thr | Oda et al. (de novo) | AGS | Yes | Novel | No |
| c.2336G>A | p.Arg779His | Oda et al. (de novo); Van Eyck et al. (de novo) | AGS; SP-LLD | Yes | 1/244230 | Yes |
| c.2335C>T | p.Arg779Cys | Marguet et al. (de novo) | AGS | No | Novel | Yes |
| c.2439A>T | p.Glu813Asp | Amari et al. (de novo) | AGS | No | Novel | No |
| c.2465G>A | p.Arg822Gln | Rutsch et al. (2 familial; 1 de novo); Pettersson et al. (1 familial) | SMS | Yes | 6/244096 | Yes |
| c.2561T>A | p.Met854Lys | Takeichi et al. (de novo) | AGS-SMS | No | Novel | Yes |

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

AGS: Aicardi-Goutières syndrome; LLD: Lupus-like disease; Spastic paraparesis; SMS: Singleton-Merten syndrome

* Note that we consider it unlikely that the c.961G>T / p.Glu321* variant described by Al Mutairi et al (Pediatr Neurol 2018;78:35-40) is responsible for the putative Aicardi-Goutières syndrome phenotype reported in that paper, given that all other such variants are gain-of-function due to missense substitutions, and noting that *IFIH1* is not highly constrained (i.e. multiple nonsense variants are recorded on gnomAD).

Amari, S., Tsukamoto, K., Ishiguro, A., Yanagi, K., Kaname, T., & Ito, Y. (2019). An extremely severe case of Aicardi-Goutieres syndrome 7 with a novel variant in IFIH1. *Eur J Med Genet*. doi:10.1016/j.ejmg.2019.04.003

Marguet, F., Laquerriere, A., Goldenberg, A., Guerrot, A. M., Quenez, O., Flahaut, P., . . . Nicolas, G. (2016). Clinical and pathologic features of Aicardi-Goutieres syndrome due to an IFIH1 mutation: A pediatric case report. *Am J Med Genet A, 170A*(5), 1317-1324.

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.

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.

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.

Takeichi, T., Katayama, C., Tanaka, T., Okuno, Y., Murakami, N., Kono, M., . . . Akiyama, M. (2018). A novel IFIH1 mutation in the pincer domain underlies the clinical features of both Aicardi-Goutieres and Singleton-Merten syndromes in a single patient. *Br J Dermatol, 178*(2), e111-e113.

Van Eyck, L., De Somer, L., Pombal, D., Bornschein, S., Frans, G., Humblet-Baron, S., . . . Liston, A. (2015). IFIH1 mutation causes systemic lupus erythematosus with selective IgA-deficiency. *Arthritis Rheumatol, 67*(6), 1592-1597.