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Assessment of interferon-related biomarkers in Aicardi-Goutières syndrome associated with mutations in *TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1*, and *ADAR*: a case-control study

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Summary

Background—Aicardi-Goutières syndrome (AGS) is an inflammatory disorder caused by mutations in any of six genes (*TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1*, and *ADAR*). The disease is severe and effective treatments are urgently needed. We investigated the status of interferon-related biomarkers in patients with AGS with a view to future use in diagnosis and clinical trials.

Methods—In this case-control study, samples were collected prospectively from patients with mutation-proven AGS. The expression of six interferon-stimulated genes (ISGs) was measured by quantitative PCR, and the median fold change, when compared with the median of healthy controls, was used to create an interferon score for each patient. Scores higher than the mean of controls plus two SD (>2·466) were designated as positive. Additionally, we collated historical data for interferon activity, measured with a viral cytopathic assay, in CSF and serum from mutation-positive patients with AGS. We also undertook neutralisation assays of interferon activity in serum, and looked for the presence of autoantibodies against a panel of interferon proteins.

Findings—74 (90%) of 82 patients had a positive interferon score (median 12·90, IQR 6·14–20·41) compared with two (7%) of 29 controls (median 0·93, IQR 0·57–1·30). Of the eight patients with a negative interferon score, seven had mutations in *RNASEH2B* (seven [27%] of all 26 patients with mutations in this gene). Repeat sampling in 16 patients was consistent for the presence or absence of an interferon signature on 39 of 41 occasions. Interferon activity (tested in 147 patients) was negatively correlated with age (CSF, r=−0·604; serum, r=−0·289), and was higher in CSF than in serum in 104 of 136 paired samples. Neutralisation assays suggested that

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See **Online** for appendix

Contributors

YJC, GIR, and JHL collated and reviewed all the molecular, clinical, and radiological data. GIR performed quantitative PCR analysis with assistance from GMAF. PCR and sequencing was performed by MS with assistance from GMAF. Viral cytopathic interferon activity assays were performed by PL and FR. Autoantibody studies were performed by MH, PP, and KK. AA, MSA-H, SA, RA, KMB, UB, CBa, GB, CBo, MPB, CC, KEC, LD, RCD, CDL, CGELDG, MdT, LE, NNE, EF, BG, J-PS-ML, JHL, CML, WM, PO, MR, AR, JLS, SAS, RSi, RSp, KJS, SAT, GV, CNV, JV, VW, WPW, DS, IO, SO, MSA, MSZ, GMHA-S, and AV provided clinical samples and critically reviewed patient data. GIR and YJC conceived the study, planned, designed, and interpreted experiments, and wrote the initial draft with the assistance of DSC. All authors critically reviewed the manuscript and agreed to its publication.

Conflicts of interest

We declare that we have no conflicts of interest.

measurable antiviral activity was related to interferon α production. We did not record significantly increased concentrations of autoantibodies to interferon subtypes in patients with AGS, or an association between the presence of autoantibodies and interferon score or serum interferon activity.

Interpretation—AGS is consistently associated with an interferon signature, which is apparently sustained over time and can thus be used to differentiate patients with AGS from controls. If future studies show that interferon status is a reactive biomarker, the measurement of an interferon score might prove useful in the assessment of treatment efficacy in clinical trials.

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Introduction

First described in 1984,[1] Aicardi-Goutières syndrome (AGS) is a mendelian inflammatory disease most typically characterised by microcephaly, spasticity, dystonia, psychomotor retardation, and, in about 35% of cases, childhood death.[2] We have shown that AGS is genetically heterogeneous, occurring due to mutations in any one of the genes encoding a 3-repair exonuclease with preferential activity on single-stranded DNA (*TREX1*),[3] the three non-allelic components of the RNASEH2 endonuclease complex acting on ribonucleotides in RNA:DNA hybrids (*RNASEH2A, RNASEH2B, RNASEH2C*),[4] a Sam domain and HD domain containing protein that functions as a deoxynucleoside triphosphate triphosphohydrolase (*SAMHD1*),[5] and an enzyme catalysing the hydrolytic deamination of adenosine to inosine in double-stranded RNA (*ADAR*).[6]

Although some children are affected in utero and display features of illness at birth, most infants with AGS appear to experience the onset of disease in the first 12 months after birth.[2] Moreover, clinical observation suggests that there is frequently an early period of active regression, seemingly occurring over several months, after which the disease apparently stabilises. For these reasons, and because of the occurrence of later-onset features —particularly vasculitic lesions of the skin (chilblains),[7] and an intracranial vasculitis seen in a subset of patients[8,9]—the development of therapies for AGS is fully warranted.

As part of our ongoing efforts to define rational approaches to the treatment of AGS,[10] we wished to identify a reactive biomarker that could be used to monitor treatment efficacy in future clinical trials. Raised concentrations of interferon α in CSF and serum of patients with AGS were first reported by Lebon and colleagues in 1988.[11] This finding not only led to a highly consistent diagnostic marker of early disease, but also presaged fundamental insights into the pathogenesis of AGS, where evidence now suggests that the syndrome results from an accumulation of immunostimulatory nucleic acid species, leading to the induction of an inappropriate type I interferon response.[12,13]

Type I interferon is also thought to be central to the development of the autoimmune disease systemic lupus erythematosus, in which affected patients frequently have increased expression of type I interferon-stimulated genes (ISGs) in peripheral blood—a so-called interferon signature.[14,15] In keeping with this observation, some children with AGS develop an early-onset form of lupus.[16,17] Importantly, a combinatorial panel of ISGs is already

being used for patient stratification and treatment monitoring in a phase 1 clinical trial approved by the US Food and Drug Administration in patients with systemic lupus erythematosus.[18,19]

For these reasons, we aimed to assess interferon-related biomarkers in a large cohort of patients with molecularly defined AGS.

Methods

Participants

For this case-control study, patients were ascertained through direct contact or referral by the physician responsible for the care of the patient, based on our longstanding involvement in studies of AGS and interferon metabolism. The only criterion for inclusion in the study was the presence of either biallelic mutations in one of the six genes known to be mutated in AGS (*TREX1*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *SAMHD1*, *ADAR*) or a recognised dominant disease-causing mutation in TREX1 (Asp18Asn or Asp200Asn) or ADAR (Gly1007Arg).

We tested all available samples, which were collected prospectively, from patients with mutation-proven AGS received up to the time of data analysis for the measurement of ISG expression. Control samples were collected from an ethnically diverse group of individuals who self-reported not to have any medical condition, and who were physically well and not taking any medication at the time of sampling. We also collated historical data for interferon activity in CSF, serum, or both, from a subset of the patients for whom ISG activity had been assessed, for additional mutation-positive individuals, and for controls. We measured autoantibodies to interferon subtypes in patients, and control data relating to antibody concentrations were historically derived.

The study was approved by the Leeds (East) Research Ethics Committee (reference number 10/H1307/132). Clinical data were collected with either verbal consent (1985–2003) or written consent (2003 onwards) from the parents of affected patients at the time of recruitment. Samples for the interferon activity studies were collected with either verbal or written consent from the parents of affected patients at the time of recruitment, or as part of clinical service provision in the virology laboratory of Cochin Hospital (Paris, France) according to French legislation. Clinical data and samples for the ISG and autoantibody studies were collected with written consent between 2011 and 2013. Controls for the ISG studies were recruited with written consent during the same period.

Procedures

Blood for ISG assessment was collected into PAXgene tubes (PreAnalytix, Hombrechtikon, Switzerland) and, after being kept at room temperature for between 2 and 72 h, was frozen at −20°C until extraction. Total RNA was extracted from whole blood with a PAXgene (PreAnalytix) RNA isolation kit. RNA concentration was assessed with a spectrophotometer (FLUOstar Omega, Labtech, Ortenberg, Germany). Quantitative reverse transcription PCR (qPCR) analysis was performed using the TaqMan Universal PCR Master Mix (Applied Biosystems, Paisley, UK), and cDNA derived from 40 ng total RNA. Using TaqMan probes

for *IFI27* (Hs01086370_m1), *IFI44L* (Hs00199115_m1), *IFIT1* (Hs00356631_g1), *ISG15* (Hs00192713_m1), *RSAD2* (Hs01057264_m1), and *SIGLEC1* (Hs00988063_m1), the relative abundance of each target transcript was normalised to the expression level of *HPRT1* (Hs03929096_g1) and *18S* (Hs999999001_s1), and assessed with the Applied Biosystems StepOne software (version 2.1) and DataAssist software (version 3.01). For each of the six probes, individual (patient and control) data were expressed relative to a single calibrator (control C25).

We previously investigated the expression of 15 ISGs in AGS, according to an assessment of published interferon signature data in systemic lupus erythematosus.[18] A subset of six ISGs was then selected on the basis of high expression in a cohort of ten patients with AGS.[6] We also obtained genome-wide RNA-Seq data from a further 12 patients with AGS, confirming that these six genes are among the highest expressed ISGs in the patients sequenced (data not shown). As previously described, the median fold change of the six ISGs, when compared with the median of the combined healthy controls, was used to create an interferon score for each patient.[18] The relative quantification (RQ) for each transcript is equal to $2^{-\Delta\Delta Ct}$—ie, the normalised fold change relative to a control. When a patient was assayed on more than one occasion, the data for repeat measurements were combined to calculate a mean value (with Applied Biosystems DataAssist software, version 3.01).

To determine the threshold of the ISG assay, blood samples from two healthy donors were collected in lithium heparin tubes pre-filled with phosphate buffered saline (PBS), or PBS with a final concentration of 0·1, 0·5, 1, 2, or 5 international units (IU) per mL of interferon (interferon alfa-2b 25 million IU/2·5 mL [Merck Sharp and Dohme, Whitehouse Station, NJ, USA]).[20] Samples were incubated at 37°C for 4 h and then transferred to PAXgene tubes (PreAnalytix). After storage at room temperature overnight, RNA was extracted and quantified as described for samples from patients. qPCR was performed for the six ISGs plus two housekeeping genes *HPRT1* and *18S*, and the fold change of each ISG for each concentration of interferon was recorded.

We measured type I interferon activity in patient CSF or serum by establishing cytopathic reduction—ie, the extent to which samples protect Madin-Darby bovine kidney (MDBK) cells, which are refractory to interferon γ, against cell death after infection with vesicular stomatitis virus.[21–24] A reference of human interferon α, standardised against the National Institutes of Health reference Ga 023-902-530, was included with each titration. Interferon α activity in normal serum is less than 2 IU/mL.[11] As previously described, a neutralisation assay was performed on patient serum.[24,25] Two-fold serial dilutions of serum, containing anti-interferon α or anti-interferon β antibodies, were incubated with patient serum samples. These were then analysed in a standard cytopathic reduction assay, and a neutralisation titre established as a reciprocal of the antibody dilution that suppressed the interferon activity.[25]

For anti-interferon autoantibody detection, coding sequences of interferon α2, interferon α5, interferon α8, interferon α14, interferon ω, and interleukin 29 (interferon λ1) without signal peptides were cloned into a modified pPK-CMV-F4 fusion vector (PromoCell, Heidelberg, Germany) downstream of a naturally secreted *Gaussia* luciferase (Gluc) that was inserted into the plasmid to replace the firefly luciferase. HEK 293 cells were transfected, and

secreted Gluc-antigen fusion protein was collected from the tissue culture supernatant 48 h later. A luciferase immunoprecipitation system (LIPS) assay was modified from Burbelo and colleagues.[26] LIPS was done in 96-well MultiScreenHTS filter plates (Millipore, Bedford, MA, USA) at room temperature with buffer A (50 mmol/L Tris, pH 7·5, 100 mmol/L NaCl, 5 mmol/L $MgCl_2$, 1% Triton X-100) for all dilutions. IgG from test sera (diluted 1:10, tested in duplicate) were captured on to Protein G Agarose beads (25 μL of 4% suspension, Exalpha Biologicals, Shirley, MA, USA), which were then incubated with supernatants containing Gluc-antigen fusion protein at 10^6 luminescence units (LU) per precipitation reaction. After 1 h the plate was washed, Gluc substrate (coelenterazine GAR-2B, Targeting Systems, El Cajon, CA, USA) was added, the plate was shaken, and the luminescence intensity recorded using a Victor X plate reader (PerkinElmer Life Sciences, Waltham, MA, USA). The positive–negative discrimination level was set for each antigen as the mean plus three SD of the LU value of sera previously calculated from 15 healthy controls who were not known to have any medical condition, were physically well at the time of sampling, and were negative for autoantibodies to nuclear, smooth muscle, mitochondrial, and parietal cell antigens. Because of the known association with the presence of high titres of neutralising autoantibodies to type I interferons,[27,28] serum from a patient with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) was run as a positive control with each assay.[28]

Statistical analysis

The mean interferon score of the controls plus two SD above the mean was calculated. Plus two SD was chosen as a conservative approach to the analysis of the data. Scores higher than this value (2·466) were designated as positive. For participants with repeat samples, the mean combined measurement is shown. In the absence of a normal distribution, ISG levels and interferon scores were log-transformed and analysed with parametric testing (*t* tests for two groups, one-way ANOVA for more than two groups). Tests for multiple comparisons, Bonferroni's multiple comparison test or Dunnett's multiple comparison test as appropriate, were applied as detailed in the figure legends. We used Spearman rank correlation to assess the relation between age and CSF and serum interferon activity. GraphPad Prism version 6 for Mac OS X was used for statistical analysis.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

ISG transcript levels were measured in 82 patients from 72 independent families with mutations in one of the known AGS-related genes (appendix p 26). Nine full sibling-pairs and one paternal half-sibling pair were included. Samples were obtained on more than one occasion from 16 patients, giving a total of 107 datapoints (table 1). The median level of each of the six ISGs was significantly higher in patients than in controls (RQ *IFI27*: controls 0·44, IQR 0·32–0·67, patients 48·58, 14·31–118·40; *IFI44L*: controls 0·63, 0·34–1·09,

patients 25·41, 11·55–46·16; *IFIT1*: controls 0·48, 0·28–0·94, patients 6·31, 3·95–10·87; *ISG15*: controls 0·97, 0·68–1·47, patients 11·72, 5·28–17·73; *RSAD2*: controls 0·55, 0·32–0·89, patients 11·25, 5·99–18·93; *SIGLEC1*: controls 1·14, 0·70–2·01, patients 11·20, 5·47–20·73; figure 1A). When the patients were analysed by genotype, the median level of each ISG was also significantly higher in patients compared with controls (figure 1B). There was no obvious pattern to the differential upregulation of individual ISGs according to genotype (appendix p 27).

The median fold change of the six probes combined was calculated to give an interferon score for each individual. The age, sex, ethnicity, mutation status, and interferon score for each patient and control are shown in appendix pp 5–12. Data for recurrent mutations are summarised in appendix p 13. A positive interferon score (>2·446) was seen in 74 (90%) of 82 patients compared with two (7%) of 29 controls (table 2, figure 2A). Of the eight patients with a negative interferon score (appendix p 14), seven had mutations in *RNASEH2B* (seven [27%] of all 26 patients with *RNASEH2B* mutations). The median interferon score in the 26 patients with *RNASEH2B* mutations was lower than in patients with all other genotypes combined (6·55, IQR 2·32–12·94, *vs* 14·76, 10·53–23·38; table 2), in whom 55 (98%) of 56 had a positive score (table 2, figure 2B). Where scores were available for sibling pairs, an analysis of the whole dataset omitting the result for the sibling with the higher interferon score did not affect the p values obtained for any of the analyses (data not shown).

There was no obvious correlation of interferon score with age, either in the patient group as a whole (figure 3) or by individual genotype (appendix p 28). All eight patients who were older than 20 years when tested had a positive interferon score (median 14·14, IQR 7·11–21·09, *vs* 12·30, 6·11–19·79, for patients younger than 20 years; p=0·69). The oldest patient who we were able to assay was 32 years of age at the time of sampling, and had an interferon score of 10·59. 16 patients were sampled on more than one occasion (median time between sampling 0·36 years, IQR 0·30–0·78; table 3, appendix pp 6–12) generating a total of 41 individual datapoints. Positive or negative status for the interferon score was reproducible on all but two occasions.

Interferon activity in CSF or serum, or both, was recorded in 147 patients (table 4), in 32 of whom we derived ISG expression data. Age, sex, ethnicity, mutation status, and interferon activity for each patient are shown in appendix pp 15–23. Recurrent mutations are summarised in appendix p 24. Interferon activity in CSF was detected in 158 (89%) of 178 samples assayed at any age, versus 96 (64%) of 151 serum samples (figure 4A and B, appendix p 29). CSF and serum activity measurements were significantly correlated within individuals (Spearman's rank correlation r=0·50, p<0·0001; appendix p 30). CSF and serum interferon activity were negatively correlated with age (Spearman rank correlation: CSF, r= −0·604, p<0·0001; serum, r=−0·289, p=0·0003; appendix p 31). The level of interferon activity was higher in CSF than in serum in 104 of 136 paired samples, and interferon activity in CSF was higher than in serum across all samples (CSF, median 25 IU/mL, IQR 9–75; serum, 6 IU/mL, 2–25; Mann-Whitney *U* test, p<0·0001; appendix pp 32, 33).

Table 5 shows a comparison of the interferon score and serum type I interferon activity when tested on the same occasion in 13 patients. The positive or negative status of the ISG

score and serum interferon activity correlated in 12 of 14 paired samples (appendix p 34). In two patients, the ISG score was positive (12·9 and 12·3) when no interferon activity was detected. Incubation of control blood samples with interferon alfa-2b showed that the ISG assay could detect less than 1 IU/mL of interferon activity (appendix p 35), suggesting that the measurement of ISG transcripts might be more sensitive than the viral cytopathic assay.

In all 14 patients tested, interferon activity could be neutralised using an antibody against interferon α, but not an antibody against interferon β (all 14 patients were tested for ISGs). Autoantibodies against interferon α2, interferon α5, interferon α8, interferon α14, interferon ω, and interleukin 29 (interferon-λ1) measured in 15 patients with AGS (14 of whom were included in the ISG analysis) are shown in appendix p 25. Autoantibody values were within plus three SD of the LU value of sera previously calculated from 15 healthy controls in all cases apart from one patient with mutations in *RNASEH2B*. In this patient, although the values of antibodies to four of the six antigens were raised compared with controls, the levels were not equivalent to those seen in the patient with APECED, a mendelian disorder consistently associated with the presence of high titres of neutralising autoantibodies against type I interferons.

Discussion

Type I interferon was originally described more than 50 years ago as a soluble factor, produced by cells treated with inactivated non-replicating viruses, that blocked subsequent infection with live virus.[29,30] Over time it became evident that multiple species of type I interferon existed, and this heterogeneity was shown to result from the presence of 17 distinct interferon-encoding genes clustered on human chromosome 9p. Although most often referred to generically as the type I interferons, differences between subtypes might in fact allow for a subtlety of immune function that is currently only poorly appreciated.[31] Type I interferons play a pivotal part in the immune response to infection by inducing an antiviral state through the expression of a large repertoire of ISGs. Hundreds of ISGs have been identified since their description more than 25 years ago. However, for most ISG products, little is known about their antiviral potential, their target specificity, or their mechanisms of action.[32]

The rapid induction and amplification of the type I interferon system is highly adaptive in terms of virus eradication. However, in the mid-1970s, Ion Gresser and colleagues[33–35] drew attention to the possibility that inappropriate exposure to type I interferon might be detrimental in mammals. To our knowledge, besides infection, the first description of an association of raised levels of type I interferon with human disease was in autoimmune phenotypes, most particularly systemic lupus erythematosus,[36,37] and on the basis of subsequent research the associated interferon signature is being used as a biomarker for assessing treatment efficacy in that condition.[18,19,38]

There is no consensus about the precise set of genes to measure when testing for an interferon signature.[18,20,38–42] Nor is there a universally accepted method for calculation of an interferon score based on a composite of multigene transcript upregulation. The genes chosen for this study, and the methods used to calculate the interferon score, are similar to

those used in other studies.[18,20] Despite the rarity of AGS, we were able to prospectively assay ISG transcript data in 82 patients with mutations in one of the genes known to be related to AGS. Of note, the percentage of patients by genotype reflected the proportions seen in our database of 262 mutation-positive families collected over a 14 year period (reference 2 and unpublished data).

Our results illustrate five major features. First, patients with mutations in any of *TREX1*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *SAMHD1*, and *ADAR* consistently showed a type I interferon signature, reflecting a marked induction of the interferon response system in almost all patients assayed.

Second, of the patients with a negative score, seven of the eight had disease due to mutations in *RNASEH2B*—27% of all patients with mutations in this gene. By contrast, 98% of patients with mutations in the other AGS-related genes had a positive interferon score. Furthermore, the median interferon score in patients with *RNASEH2B*-associated disease was lower than in patients with disease due to all other genotypes combined. Of possible relevance, we have previously reported a lower frequency of morbidity and mortality in association with the *RNASEH2B* genotype than with all other genotypes.[2] Circulating neutralising autoantibodies are able to downregulate ISG expression in peripheral blood mononuclear cells of patients with APECED;[27] however, we did not observe significantly increased concentrations of autoantibodies to interferon subtypes in patients with AGS, including four individuals with *RNASEH2B* mutations, or an association between the presence of autoantibodies and ISG score (or serum interferon activity; appendix p 25).

Third, the interferon score in patients with AGS remained high over time, many years beyond the subacute encephalopathic stage of the disease. As a corollary of this observation, repeat sampling in 16 patients showed consistency in the presence or absence of an interferon signature, albeit that the absolute value of the interferon score can vary over time.

Fourth, when serial measurements were available, although CSF and serum interferon activity remained raised in most cases, the absolute level of activity was negatively correlated with age. When paired measurements of CSF and serum were made, interferon activity in CSF was higher than in serum in 104 of 136 paired samples, perhaps indicative of intrathecal production of interferon, as previously suggested.[43] Because of the prospective nature of our collection for ISG analysis, we are not able to comment on the possibility that the interferon score might also be higher earlier in the disease process. In 12 of 14 sample pairs of CSF and serum, there was consistency in positive or negative status across the two tests. However, there was no correlation between the absolute value of the interferon score and the level of interferon activity (table 5). In two cases with a positive interferon score (of >12), no interferon activity was detectable in serum.

Finally, ISGs can be induced by both type I (α, β, ε, κ, ω) and type II (γ) interferons. Importantly, the MDBK cells used in our antiviral cytopathic assay do not respond to interferon γ, and thus interferon γ is not responsible for the activity in this assay.[21–23] Taken together with the results of our neutralisation assays showing that activity can be neutralised

by antibodies to interferon α, but not those to interferon β, these data suggest that the measurable antiviral activity recorded in patients with AGS is related to interferon α.

Our results confirm the association of upregulated type I interferon production with AGS (panel).[11,43,44] The results are remarkably consistent across all six genotypes, each of which can be regarded as a discrete mendelian type I interferonopathy.[45] Clinical observation suggests that a large proportion of patients with AGS have an early period of active disease, after which the disease process apparently attenuates.[2,10] This observation is consistent with the higher levels of interferon activity recorded in infancy, which then apparently fall with age. However, both ISG and interferon activity assays clearly show an ongoing biological disease process, which is probably driven by interferon α, but might also involve an interferon-independent pathway to ISG induction.[46] The latter possibility might account for the absence of correlation between the absolute value of the interferon score and the level of interferon activity seen in our cohort.

An interferon score is fairly easy to derive, needing only 2·5 mL of peripheral blood, and can be reported within 48 h of sampling. Our data suggest that assessment of interferon signature in AGS might be more sensitive at recording of ongoing disease than a viral cytopathic assay designed to directly assess interferon activity.

AGS is a highly debilitating disorder, associated with significant morbidity and mortality. Current management is symptomatic, and there is an urgent need to develop effective treatments. Our results suggest that an assay for the presence or absence of an upregulation of ISG transcripts can be used as a reliable screening method in the diagnosis of AGS. That is, the test shows high sensitivity for the presence of mutations in one of the AGS-related genes, although the specificity of a positive interferon score for AGS versus other disease states is undetermined. Our data emphasise the possible value of the interferon score as a biomarker in future clinical trials.[10] The *RNASEH2B* genotype is associated with lower interferon scores and has been shown to have a lower mortality rate,[2] although phenotypic observation suggests that there is not a simple relation between interferon score and clinical status. Thus, a limitation of our work at this time is uncertainty as to whether the AGS-associated interferon signature represents a reactive biomarker—a point that will become clear only when assessed in the context of effective treatments for AGS.

We have recently discussed the concept of grouping mendelian disorders associated with an upregulation of type I interferon,[45] suggesting—in a manner analogous to autoinflammatory disorders and primary immuno-deficiencies—that such diseases might represent a novel set of inborn errors of immunity due to either inappropriate stimulation of the type I interferon response pathway, or defective negative regulation of the type I interferon system. Our data lead us to suggest that other mendelian type I interferonopathies might also exist, and could be identified both by systematic screening of patients with neurological and immunological phenotypes for the presence of an interferon signature, and by the use of exome sequencing to identify mutations in obvious candidate genes (which could include, for example, molecules involved in nucleic acid metabolism and components of the interferon negative-feedback machinery). The recognition of phenotypes as type I interferonopathies is not only of academic interest, since definition of a disturbance of type I interferon in the pathogenesis

of a disease immediately suggests the possibility of anti-interferon and other anti-inflammatory therapies.

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Panel: Research in context

Systematic review

We searched PubMed and Online Mendelian Inheritance in Man with the terms “Aicardi-Goutières syndrome”, “TREX1”, “RNASEH2A”, “RNASEH2B”, “RNASEH2C”, “SAMHD1”, “ADAR1”, “interferon signature”, “interferon stimulated gene(s)”, and “interferon autoantibodies” for articles in English published between Jan 1, 1984, and May 31, 2013. We also searched reference lists of identified papers. These searches revealed previous knowledge of an association of increased type I interferon activity in the CSF and serum of patients with Aicardi-Goutières syndrome (AGS) in the early stages of the disease.[11,43,44] We also identified one report of an upregulation of interferon-stimulated gene transcripts, an interferon signature, in patients with AGS due to mutations in *ADAR*[6] We found that a similar interferon signature had been described in the autoimmune disease systemic lupus erythematosus,[14,15] and is currently being used for patient stratification and treatment monitoring in that disorder.[18–20]

Interpretation

Our findings extend data from previous studies by showing that almost all patients with AGS have an upregulation of interferon-related biomarkers; in particular, a robust interferon signature was seen in association with mutations in any of the known AGS-related genes. Importantly, this signature was maintained beyond the subacute encephalopathic stage of the disease (almost all participants are beyond this stage), showing no apparent attenuation with patient age.[2] Our data suggest that measurement of the expression of type I interferon-induced gene transcripts can be used to differentiate patients with AGS from controls, and lend support to the idea that the interferon signature might in future serve as a biomarker to assess treatment efficacy in future clinical trials.

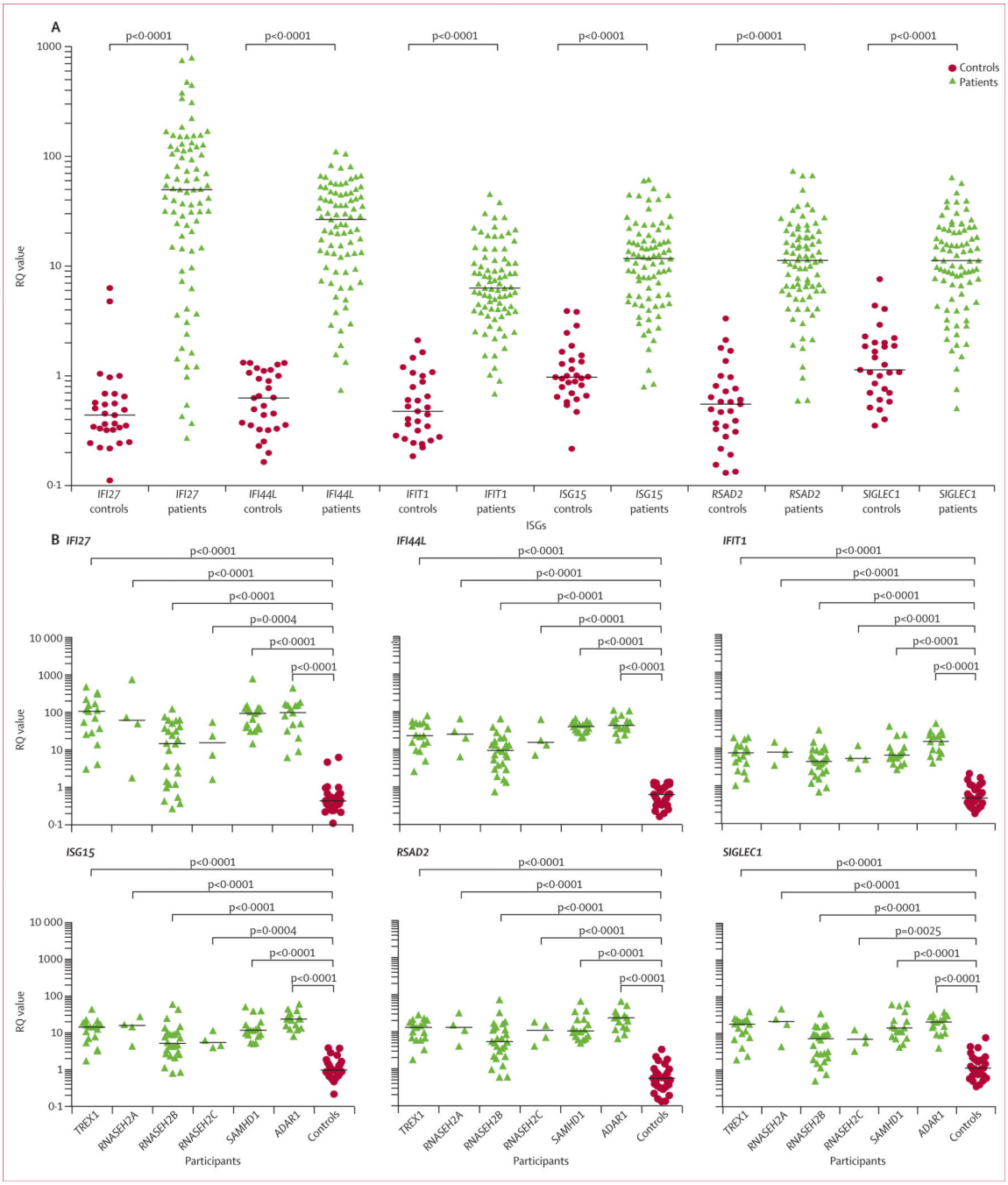

Figure 1. ISG transcript levels in patients and controls

(A) Quantitative reverse transcription PCR of a panel of six ISGs in whole blood measured in 82 patients with Aicardi-Goutières syndrome and 29 controls. Horizontal black bars show the median RQ value for each probe in each group. For participants with repeat samples, the mean combined measurement is shown. Analysed with one-way ANOVA with Bonferroni's multiple comparison test. (B) ISG RQ by genotype compared with controls. Horizontal black bars show the median RQ value for each probe in each group. Analysed with one-way

ANOVA with Dunnett's multiple comparison test. ISG=interferon-stimulated gene. RQ=relative quantification.

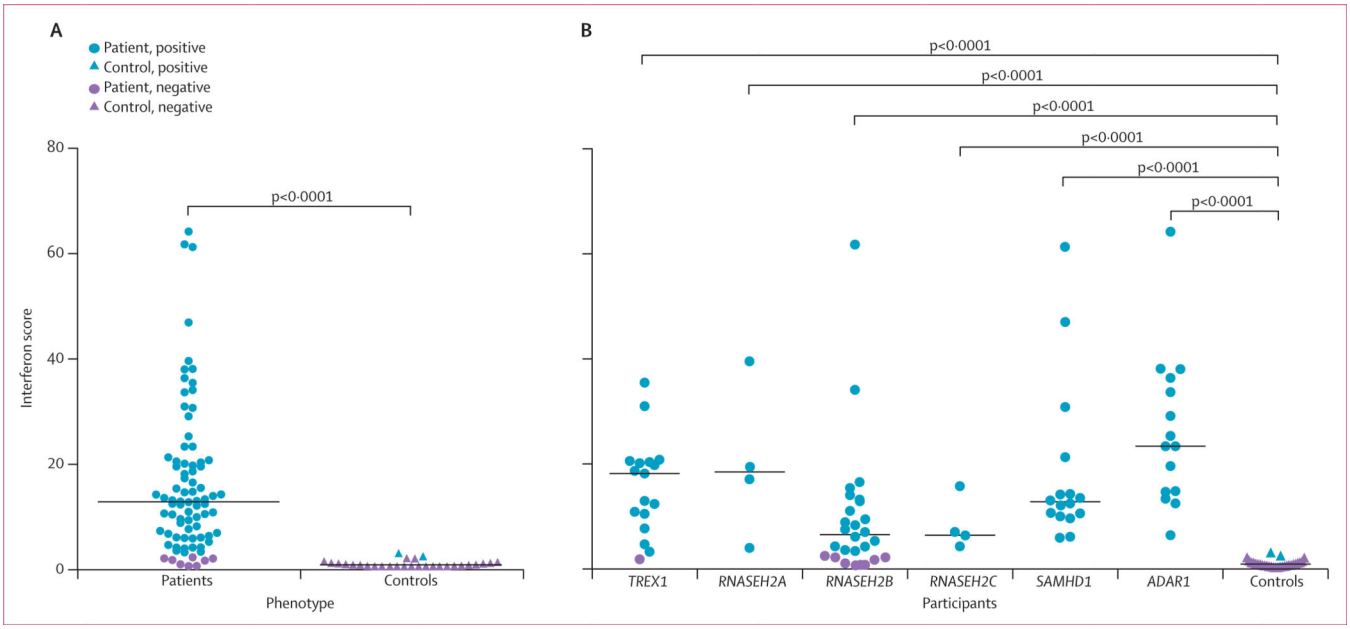

Figure 2. Interferon scores in patients and controls

(A) Interferon score in all patients and controls calculated from the median fold change in relative quantification value for a panel of six interferon-stimulated genes. For participants with repeat samples, the mean combined measurement is shown. Black horizontal bars show the median interferon score in patients and controls. Negative scores are those less than 2·466 (mean of control interferon score plus two SD of mean) and positive scores are 2·466 or greater. Analysed with *t* test. (B) Interferon score for patients with mutations in genes related to Aicardi-Goutières syndrome versus controls. Black horizontal bars show the median interferon score for each gene. Analysed with one-way ANOVA with Dunnett's multiple comparison test.

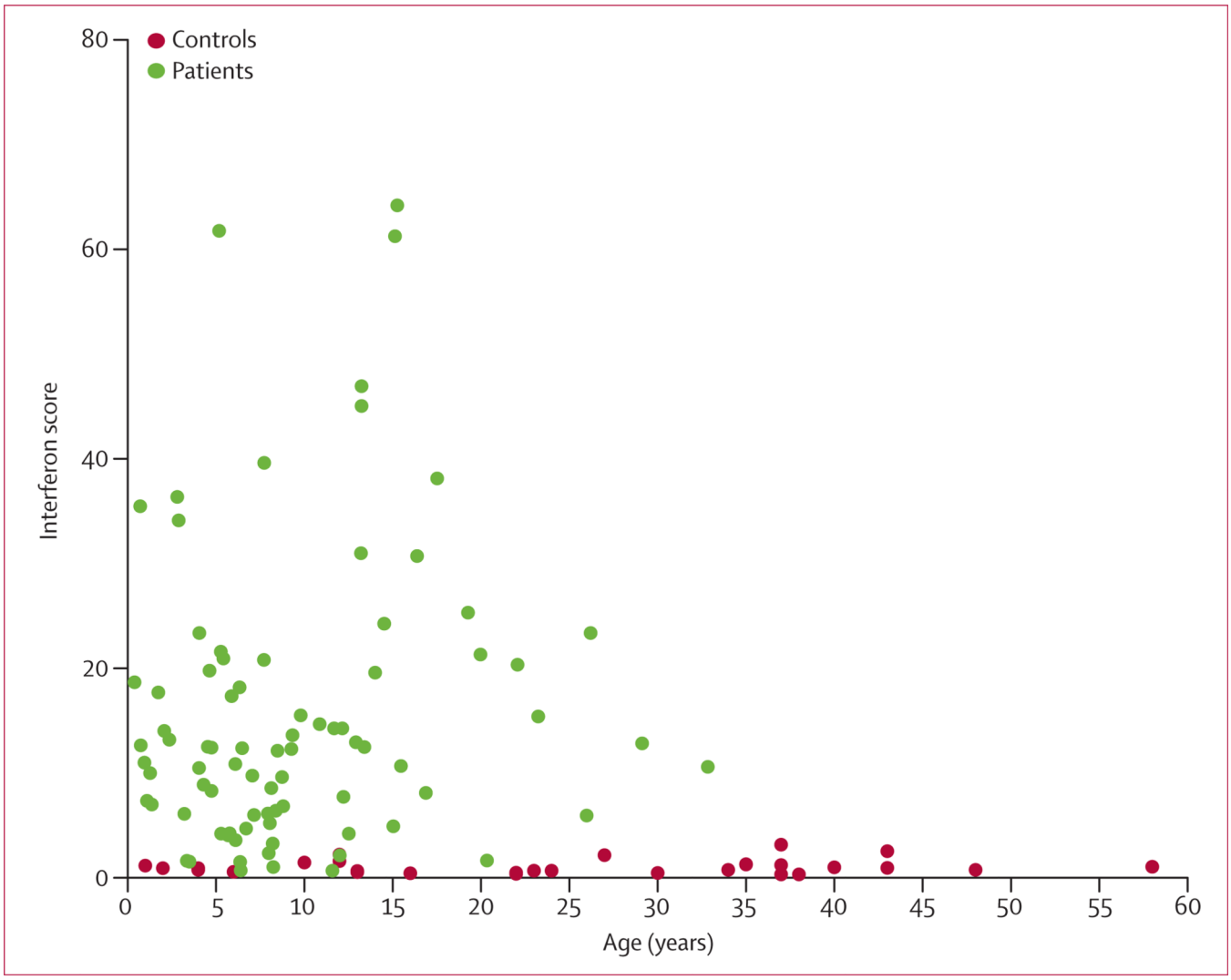

Figure 3. Interferon score plotted against age for patients and controls

Interferon scores calculated from the median fold change in relative quantification values for a panel of six interferon-stimulated genes. Spearman's rank correlation: patients, r=0·121, p=0·28; controls, r=0·062, p=0·75.

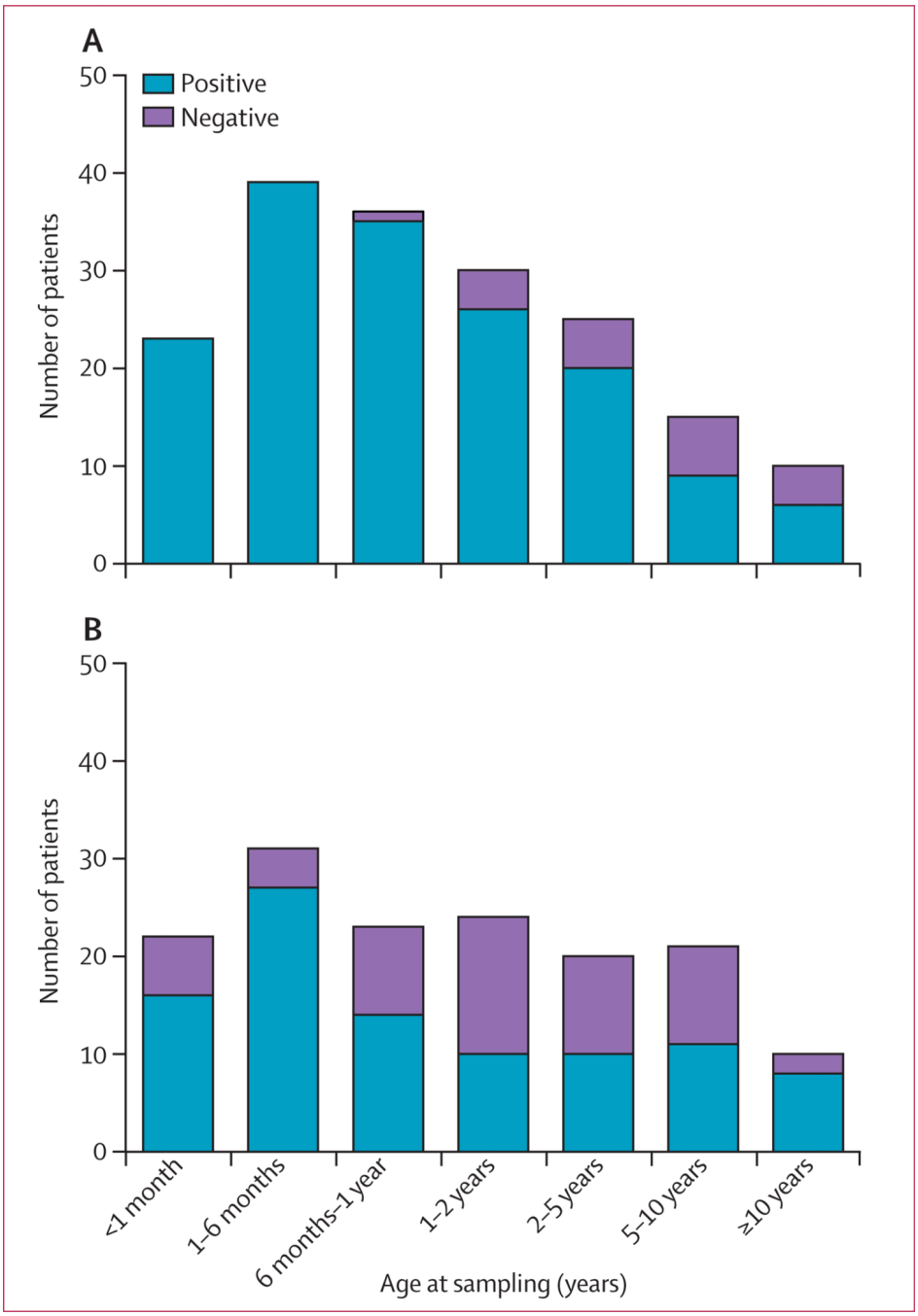

Figure 4.
Patients with positive or negative (below threshold) measurement of (A) CSF interferon activity or (B) serum interferon activity, by age at time of assay

Table 1

Mutation-positive patients assayed for interferon-stimulated gene transcripts

	Number of Patients	Number of Families	Number of sampling points	Age range at time of sampling	Ethnic group
TREX1	17	16	19	8 months–22 years	European, North American, north African, south Asian
RNASEH2A	4	4	4	5 years–14 years	European, African, North American
RNASEH2B	26	22	39	11 months–29 years	European, North American, South American, north African
RNASEH2C	4	4	4	5 years–9 years	South Asian, European
SAMHD1	16	14	18	1 year–32 years	European, south Asian, North American, north African
ADAR	15	12	23	2 years-26 years	European, south Asian, South American, North American, Australasian
Total	82	72	107	··	··

The 29 control individuals ranged in age from 1 to 58 years, and 14 (48%) were male. See appendix for patient characteristics.

Table 2

Median interferon score in patients, and score by genotype

	Number of patients	Median (IQR)	Patients with positive or negative interferon score	
			Positive	Negative
Patients	82	12·90 (6·14–20·41)	74 (90%)	8 (10%)
Controls	29	0·93 (0·57–1·30)	2 (7%)	27 (93%)
TREX1	17	18·19 (9·12–20·46)	16 (94%)	1 (6%)
RNASEH2A	4	18·47 (7·51–34·62)	4 (100%)	0
RNASEH2B	26	6·55 (2·32–12·94)	19 (73%)	7 (27%)
RNASEH2C	4	6·43 (4·54–13·35)	4 (100%)	0
SAMHD1	16	12·81 (10·15–19·57)	16 (100%)	0
ADAR	15	23·39 (14·69–36·39)	15 (100%)	0
RNASEH2B with positive score	19	8·89 (5·32–14·03)	··	··
TREX1, RNASEH2A, RNASEH2C, SAMHD1, ADAR combined	56	1476 (10·53–23·38)	··	··

Patients versus controls, χ^2 test p<0·0001.

Table 3

Results for individual patients with an interferon score sampled at more than one timepoint, by patient number

	Gene	Number of sampling points	Ages at which sampled (years)	Number positive	Number negative	Range
AGS535	*TREX1*	2	0·75, 1·08	2	0	12·648–28·759
AGS635	*TREX1*	2	1·74, 2·01	2	0	17·557–25·086
AGS026_P1	*RNASEH2B*	3	20·35, 20·64, 20·71	2	1	1·668–4·495
AGS026_P2	*RNASEH2B*	4	16·63, 16·88, 16·92, 16·99	4	0	8·109–30·048
AGS068	*RNASEH2B*	3	7·73, 8·05, 8·61	3	0	5·220–10·371
AGS301	*RNASEH2B*	2	6·26, 6·37	0	2	1·518–2·329
AGS539	*RNASEH2B*	2	3·35, 3·66	1	1	1·635–2·638
AGS540	*RNASEH2B*	4	15·04, 15·34, 15·36, 15·37	4	0	4·931–6·318
AGS580_P1	*RNASEH2B*	2	8·14, 8·18	2	0	8·584–12·372
AGS128	*SAMHD1*	3	6·77, 7·02, 7·06	3	0	9·761–15·899
AGS081_P1	*ADAR*	3	14·53, 15·01, 15·78	3	0	24·267–53·356
AGS081_P2	*ADAR*	2	4·82, 5·28	2	0	21·589–37·822
AGS251	*ADAR*	2	8·1, 9·27	2	0	12·301–28·367
AGS430_P1	*ADAR*	2	4·75, 5·53	2	0	8·296–21·538
AGS430_P2	*ADAR*	2	4·75, 5·53	2	0	12·444–14·306
AGS474	*ADAR*	3	5·42, 5·88, 6·02	3	0	20·961–32·319

Table 4

Mutation-positive patients assayed for CSF or serum interferon activity, or both

	Number of patients	Number Of families	Number Of sampling points	Age range at time of sampling	Ethnicity	CSF interferon (number of samples)	Serum interferon (number of samples)
TREX1	42	39	47	In utero-22 years	North American, south Asian, European, Afro-Caribbean, north African	42	35
RNASEH2A	5	5	6	1 month-3 years	European, north African	6	4
RNASEH2B	61	50	85	In utero-19 years	North African, European, North American, south Asian	77	61
RNASEH2C	13	9	17	In utero-12 years	North African, south Asian, European	15	13
SAMHD1	16	12	27	1 day-16 years	European, north African, south Asian, Arabian	26	24
ADAR	10	7	18	In utero-15 years	European, south Asian	12	14
Total	147	122	200	..	..	178	151

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Table 5

Comparison of interferon score and type I interferon activity measured in serum of mutation positive patients, by patient number

	Gene	Age at sampling (years)	Interferon score	Serum interferon activity (IU/mL)
AGS044	*TREX1*	13·21	30·999	50, 9*
AGS060	*TREX1*	12·93	12·954	<3
AGS205	*TREX1*	22·07	20·360	6
AGS635	*TREX1*	174	17·557	100
AGS015	*RNASEH2B*	12·01	2·149	<3
AGS068	*RNASEH2B*	7·73	10·371	12
AGS068	*RNASEH2B*	8·05	8·163	5
AGS266	*RNASEH2B*	5·19	61764	25
AGS534	*RNASEH2B*	0·96	11·020	50
AGS081_P1	*ADAR*	1578	45·676	12
AGS081_P3	*ADAR*	5·28	21·590	12
AGS251	*ADAR*	9·27	12·301	<2, <3*
AGS430_P1	*ADAR*	5·53	14·306	5, 3*
AGS430_P2	*ADAR*	5·53	21·538	6

* Measurements repeated in a separate aliquot of serum taken on the same day of sampling.