Letter to the Editor

A novel kindred with inherited STAT2 deficiency and severe viral illness

To the Editor:

Autosomal recessive signal transducer and activator of transcription 2 (STAT2) deficiency was first reported in 2 siblings. One developed disseminated vaccine-strain measles following routine immunization with measles-mumps-rubella but recovered. The younger sibling died in infancy from a probable viral infection.[1] Pedigree analysis showed incomplete clinical penetrance for the severe viral infection phenotype in this kindred, giving rise to questions regarding the completeness of the STAT2 defect. Two other siblings with autosomal recessive STAT2 deficiency were also reported to suffer from febrile illness following measles-mumps-rubella immunization; one went on to develop opsoclonus-myoclonus syndrome and suffered from severe neurological impairment but was alive at reporting at 2.5 years.[2] His sibling was alive and well as reported at age 2 years, 3 months. STAT2 deficiency was complete and was associated with impaired type I interferon signaling. The 6 reported patients with autosomal recessive STAT1 deficiency suffer from a broader phenotype, including mycobacterial disease due to the additional disruption of type II interferon signaling via STAT1-STAT1 complexes (gamma-activated factor [GAF]).[3,4] Here we describe 2 siblings with compound heterozygous *STAT2* mutations who suffered from severe viral illnesses since infancy. One succumbed at the age of 7 years from an infection with an unidentified agent, whereas the other is well at age 11 years. Detailed clinical history of the 2 described patients can be found in this article's Online Repository and in Tables E1-E3 at www.jacionline.org.

Whole-exome sequencing was performed on patient 2 (P2), the parents, and the younger healthy sibling. After filtering out common polymorphisms, we found that P2 was compound heterozygous for *c.C1528T* and *c.G1576A* variants in *STAT2* (Fig 1, *A* and *B*). PCR analysis showed the specific absence of *STAT2* cDNA transcripts in primary fibroblasts from P2 (see Fig E1 in this article's Online Repository at www.jacionline.org).

STAT1 and STAT2 expression and phosphorylation were examined in primary fibroblasts of P2, the parents, the younger healthy brother, and an unrelated control. Immunoblotting for STAT2 and Y-690-phosphorylated STAT2 after stimulation with IFN-α demonstrated absence of expression of STAT2 protein in P2 fibroblasts, as neither full length (Fig 1, *C*) nor truncated protein (data not shown) was detectable. By contrast, STAT1 expression and phosphorylation after stimulation with IFN-γ was normal (Fig 1, *C*). These results indicate that the *c.C1528T* variant (p.R510X) drives nonsense-mediated decay. The *c.G1576A* variant, by contrast, encodes a variant (p.G526A) at a splice junction. Transfection of a prespliced *c.G1576A* variant into a *STAT2* knockout fibrosarcoma cell line (U6A) resulted in normal STAT2 expression and normal STAT2 phosphorylation after IFN-α stimulation (see Fig E2 in this article's Online Repository at www.jacionline.org), indicating a defect in splicing drove the null expression phenotype. We confirmed that the *c.G1576A* variant in *STAT2* was responsible for aberrant splicing by introduction of exon 16 and its intron boundaries into an exon-trapping plasmid system (Fig E2, *C*). This is supported by the Human Splice Finder (Bioinformatics Team INSERM FO Desmet, D Hamroun, M Lalande, G Collod-Beroud, C Beroud; http://www.umd.be/HSF3) (score 81.98—new acceptor) and by Splice-site analyzer (48.01 dG 1.8) software (ibis.tau.ac.il/ssat/SpliceSiteFrame.htm).

With undetectable protein expression of STAT2 in patient cells, function was compromised. IFN-α-induced IFN-stimulated gene factor 3 complex–mediated binding to IFN-α sequence response element was specifically abolished in P2, whereas IFN-γ and IFN-α induced gamma activating factor-mediated (gamma-activating sequences interaction were not affected; see Fig E3 in this article's Online Repository at www.jacionline.org). Moreover, interferon-inducible genes (*MxA*, *ISG15*, and *OAS1*) were not upregulated following IFN-α stimulation in the patient's primary fibroblasts (Fig 1, *D*; see Fig E4 in this article's Online Repository at www.jacionline.org). We next tested susceptibility to infection with *Vesicular stomatitis* virus *in vitro* by measuring cell survival in primary fibroblasts from P2, her healthy sibling, and parents. Pretreatment with exogenous IFN-α and IFN-γ protected the primary fibroblasts of healthy family members, whereas for P2, pretreatment with IFN-γ was protective but IFN-α had no effect (Fig 1, *E*). Finally, lentiviral transduction of wild-type *STAT2* in primary fibroblasts of the patient restored STAT2 expression, IFN-α-induced STAT2 phosphorylation (Fig 1, *F*), and upregulation of interferon-inducible target genes (*MxA*, *ISG15*, and *OAS1*) (Fig 1, *G-I*).

Together, these results demonstrate that *c.C1528T/c.G1576A STAT2* mutation cripples STAT2 expression and function of the IFN-stimulated gene factor 3–dependent IFN-α pathway, within the limit of detection of these *in vitro* assays, whereas STAT1 function and the GAF-dependent IFN-γ pathway were intact. Febrile illness following measles-mumps-rubella vaccination has been documented in 6 of 6 immunized patients in the 3 unrelated kindreds (see Table E3 in this article's Online Repository), highlighting the importance of type I interferon signaling in the initial immune response against measles infections.[5] Severe complications of natural measles infection include primary measles encephalitis (1-3 cases per 1,000 cases of measles infection) and subacute sclerosing panencephalitis (4-11 cases per 100,000). Whether these cases are driven by STAT2 deficiency remains to be elucidated.

Hambleton et al[1] reported in the first described family that the majority of childhood viral illnesses were remarkably mild. Although some childhood viral illnesses such as respiratory syncytial virus bronchiolitis had a relatively uneventful course in P2, she did, however, suffer from severe recurrent varicella and enterovirus infections requiring multiple hospital admissions. She also suffered from severe course of primary EBV infection with delayed EBV suppression in peripheral blood and cerebrospinal fluid, in line with the important role of type I interferon signaling in the initial immune response against EBV.[6] Overall, STAT2 deficiency may display incomplete penetrance for several viral infections and even for complicated live measles vaccine as evidenced in Table E3. Similar diversity in presentation has been reported in other innate immune disorders such as TLR3 deficiency.[7]

Impaired response to IFN-α and life-threatening viral diseases in STAT2-deficient patients are reminiscent of the phenotype of

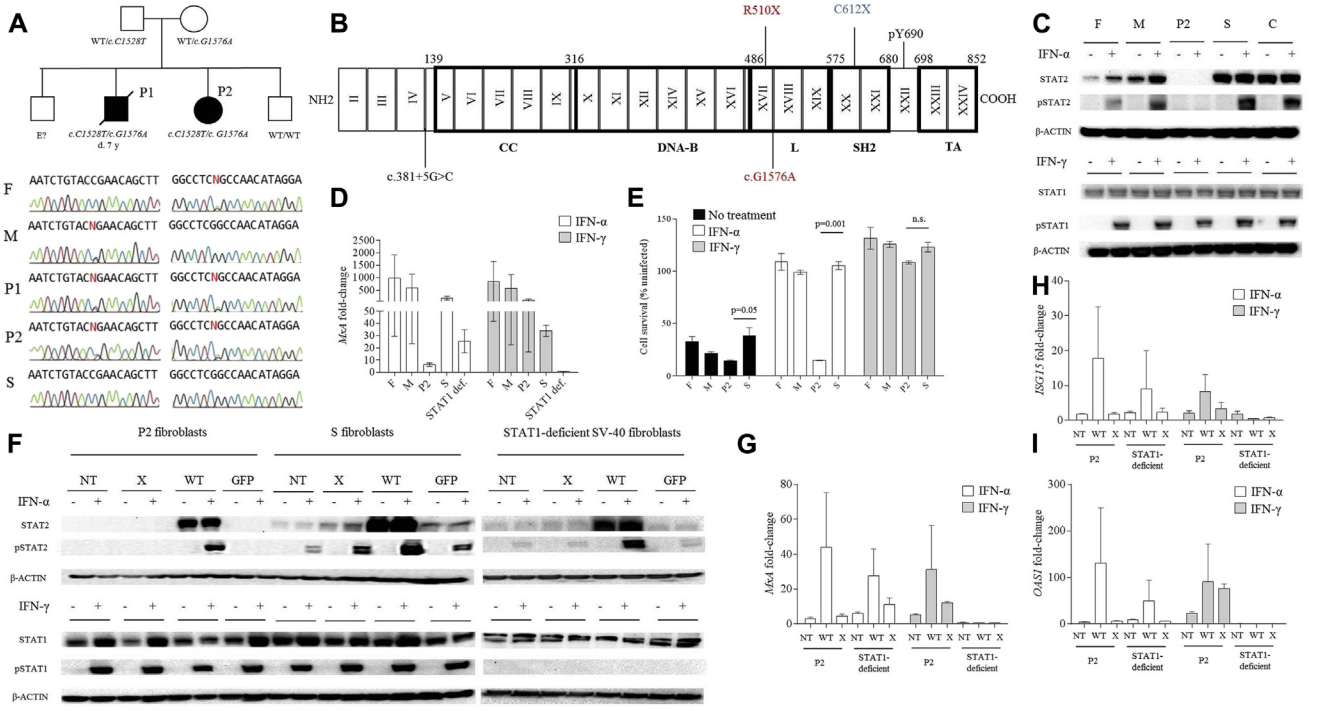

FIG 1. Failure in IFN-α antiviral responses in STAT2-deficient patient cells and restoration of IFN-α responses by *STAT2* complementation. **A,** Clinical phenotype of the investigated family *STAT2* genotype. **B,** Schematic overview of STAT2 protein indicating reported mutations. Annotated in *red* are the mutations described in the current study, in *black* the mutations reported in Hambleton et al,[1] and in *blue* the mutation reported in Shahni et al.[2] Mutations in amino acid nomenclature except for notations starting with c., which are nucleotide nomenclature. **C,** Western blot analysis of phosphorylated STAT1 and STAT2 of fibroblasts following stimulation with IFN-α or IFN-γ. **D,** Relative expression of *MxA* in fibroblasts and STAT1-deficient cell SV40 fibroblast cell lines after stimulation with IFN-α or IFN-γ. **E,** Cell survival at day 3 post *Vesicular stomatitis* virus (*VSV*) infection in fibroblasts with or without pretreatment with IFN-α and IFN-γ. *P* values are shown for sibling (*S*) versus patient 2 (*P2*), 2-way ANOVA with Bonferroni correction. Means ± SEMs calculated from 3 independent experiments are presented in **D** and **E**. **F,** Stable expression of wild-type (*WT*) STAT2 by lentiviral transduction in fibroblasts and STAT1-deficient SV40-fibroblasts. Phosphorylated STAT1 and phosphorylated STAT2 expression following stimulation with IFN-α or IFN-γ as detected by Western blot. β-actin expression is used as loading control and expression is normalized to the untransduced condition in the sibling (sibling/patient) or to untransduced fibroblasts (STAT1-deficient condition). Lentiviral transduction of vector containing the paternal stop codon variant or green fluorescent protein were included as transduction controls (representative immunoblots of n = 2 independent transduction experiments). Relative expression of *MxA* **(G)**, *ISG15* **(H)**, and *OAS1* **(I)** in lentiviral transduced fibroblasts and STAT1-deficient SV40-fibroblasts after stimulation with IFN-α or IFN-γ. Lentiviral transduction of vector containing paternal stop codon variant in both cell lines were included as transduction controls (data pool from 2 independent transfections). Means ± SEMs calculated from 2 independent experiments are represented in **G** and **H**. *C*, Healthy control; *cc*, coiled-coiled domain; *d*, death; *DNA-B*, DNA binding domain; *F*, father; *GFP*, control transduction with lentiviral vector containing green fluorescent protein only; *L*, linker domain; *M*, mother; *MW*, molecular marker; *n.s.*, not significant; *NT*, not transduced; *SH2*, SH2 domain; *STAT1 def.*, STAT1-deficient SV40 fibroblast cell line; *STAT2*$^{-/-}$, *STAT2* knockout fibrosarcoma cell line; *TA*, transactivation domain; *X*, transduction with lentiviral vector containing paternal stop codon variant.

patients with complete autosomal recessive STAT1 deficiency, who additionally suffer from severe infections with weakly virulent *Mycobacteria* and other intracellular bacteria.[3] The STAT1-deficiency viral phenotype, however, seems more severe and broad than that of STAT2 deficiency, as out of the 6 identified patients, 4 died in early infancy due to herpes simplex virus-1, cytomegalovirus, or unknown viruses, and 2 received hematopoietic stem cell transplantation, from which 1 succumbed and the other was well 91 days after.[4] Similar, albeit less severe, presentation has been observed patients with autosomal recessive partial STAT1 deficiency.[8] Comprehensive analysis of STAT1-deficient cells strongly suggests that the corresponding patients' viral susceptibility is due to impaired IFN-α/β response, whereas their susceptibility to intracellular bacteria is due to defective IFN-γ signaling.[9] These effects are separated out in patients with autosomal dominant STAT1 deficiency, where there is no deficiency in IFN-stimulated gene factor 3–dependent signaling to IFN-α and no lethal viral disease. Here, mutant alleles were shown to be dominant for IFN-γ-GAF activity but recessive for IFN-α–IFN-α sequence response element activity,[E1] explaining

the clinical divergence at the molecular level. The differential IFN-stimulated gene signature for type I and II interferons is also a likely culprit. Moreover, IFN-λ or type III interferon, which plays a crucial role at the mucosal surfaces of lung and intestine, has been shown to depend on the same signaling pathways as type I interferons do. Therefore the IFN-λ arm of the innate immune system is likely to be impaired in patients with STAT2 deficiency.[E2,E3]

Finally, we observed a good response to high doses of intravenous immunoglobulin (IVIG) in P2 during infectious episodes. This response may in part be due to the anti-inflammatory effect of high doses of IVIG; however, it is probable that passive immunization may play a predominant role in controlling the ongoing viral infections. Therefore it could be argued that IgG replacement therapy might be beneficial for STAT2-deficient patients during childhood, until their adaptive immune system has sufficiently developed, as shown in patients with defects in the Toll-like receptor pathways.[E4] The history of P1 indeed demonstrates that patients may still be at risk for overwhelming viral illness beyond early childhood. Based on our limited experience, we recommend monthly IVIG substitution to prevent infections and high dose IVIG treatment in the course of severe (viral) infectious episodes with signs of emerging coagulopathy and/or immune dysregulation. The protective effect of *in vitro* infection with *Vesicular stomatitis* virus in P2's fibroblast culture by preincubation with IFN-γ invites speculation on the prophylactic or therapeutic use of IFN-γ. However, the long-term use of IFN-γ is not without side effects, and at the time of severe infection, its use may enhance macrophage activation. Therefore at present, IVIG seems to be the safer alternative. Our findings also add to the growing consensus that any fatal or life-threatening viral infection should not be seen simply as a case of "bad luck," but should instead trigger comprehensive genetic analysis even in the absence of identifiable immunological (blood) anomalies.[E5] This will allow for genetic counseling of the family, individualized directed treatment, and a better understanding of the immune system.

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Patients

Patient 1 (P1) was the second child of unrelated parents of Belgian European descent. The first 3 years of his life were characterized by frequent viral infections, each time necessitating admission to pediatric infectious diseases ward. At the time of adenovirus, enterovirus, and respiratory syncytial virus infection, he presented with high fever, malaise, pneumonitis, hepatitis, and pancytopenia and required in-patient supportive therapy. At the age of 24 months, he developed high fever 1 week after administration of the live measles-mumps-rubella vaccine, with a morbilliform rash, tonsillitis, conjunctivitis, lymphadenopathies, hepatosplenomegaly, and arthritis. Blood analysis showed pancytopenia and elevated liver enzymes suggestive of hepatitis. He recovered with supportive care. Basic immunological screening was normal. Physical and mental development was normal. From the age of 4 years, he had a relatively infection-free period. At the age 7 years, he succumbed to an infection with an unidentified agent. Clinically he suffered from a mild febrile illness, but after 48 hours, his condition suddenly deteriorated and he died of multiple organ failure in the context of disseminated intravascular coagulation. All cultures remained negative and no causal pathogen could be identified—viral pathogens were not sought.

His younger sister P2 also suffered from frequent and severe viral infections in her first years of life, including severe *Varicella zoster* virus infection (6 months), Influenza A virus infection (age 7 months), rotavirus (age 8 months), recurrent Coxsackievirus, and adenovirus infections. Infections were accompanied by moderate thrombocytopenia, lymphopenia, neutropenia, monocytopenia, elevated levels of serum transaminases, and elevated percentage of T cells expressing HLA-DR (up to 50% of CD4(+) T cells) as well as hypergammaglobulinemia. Between infectious bouts, immunological screening could not demonstrate abnormalities in adaptive or innate immunity, with normal numbers of leucocyte subsets, normal lymphocyte proliferation tests, restoration of normal HLA-DR expression levels, normal immunoglobulin levels, and vaccine responses and normal activation of Toll-like receptor pathways (Table E1). Physical and mental development was normal. At age 18 months, she developed disseminated measles following routine immunization, complicated by hepatitis and pneumonitis. At this time, she received high-dose intravenous immunoglobulin (2 g/kg body weight) due to her poor condition and the emergence of coagulopathy, after which she recovered and became afebrile within 24 hours.

At age 2.5 years, P2 suffered from severe EBV infection with high fever, malaise, tonsillitis, lymphadenopathy, and splenomegaly that again responded well to high-dose intravenous immunoglobulin treatment. There were no signs of macrophage activation. Over the next 3 years, repeated measurements by PCR showed persistent EBV presence in both blood and cerebrospinal fluid despite the presence of anti-EBV IgG (Table E2). At age 5 years she was admitted with a protracted course of Coxsackie B meningitis. From age 5 years until 11 years, she suffered from cutaneous plantar warts (not responding to acetyl salicylic acid and cryotherapy) and *Molluscum contagiosum*. From the age of 5 years, the frequency and severity of viral infections decreased. However, she suffered from recurrent chest infection requiring chronic antibiotic treatment with azithromycine, inhalation steroids, and daily chest physiotherapy. Computed tomography scan of the chest did not show irreversible lung damage. At age 11 years, P2 still experiences several mild viral infections per year, mostly upper respiratory infections. She has been off all medication since the age of 10 years.

METHODS

The study was performed in accordance with the modified version of the Helsinki declaration. The study was approved by the Ethics Committee of University Hospitals Leuven. Written informed consent was obtained prior to DNA isolation from blood of all family members and from bone marrow of the deceased patient.

Whole-exome sequencing

We performed whole-exome sequencing on the surviving patient, the unaffected parents, and the younger healthy sibling. Genomic DNA samples for whole-exome sequencing were prepared from heparinized peripheral blood using the QIAamp DNA Blood Midi Kit (QIAGEN, Hilden, Germany). Exome sequence libraries were prepared using a SeqCap EZ Human Exome Library v3.0 kit (Roche NimbleGen, Madison, Wis). Paired-end sequencing was performed on the Illumina HiSeq2000 (Genomics Core Facility, University of Leuven, Belgium). BWA software was used to align the sequence reads to the Human Reference Genome Build hg19 (Heng Li, http://bio-bwa.sourceforge.net). GATK Unified Genotyper (Broad Institute, https://software.broadinstitute.org/gatks/bestpractices) was used to identify single nucleotide variants and insertions/deletions. ANNOVAR (http://annovar.openbioinformatics.org) was used for annotation.

Sanger sequencing

A DNA sample of the deceased patient P1 was obtained from a bone marrow sample using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich, St Louis, Mo). The regions of interest in exon 17 of *STAT2* were sequenced using the primers 5′-GTTCTGCCCTGTGGGACAGATAG-3′ and 5′-CTCAATTGCCTGGGCTTCAGTTC-3′. Sanger sequencing was performed on an ABI 3730 XL Genetic Analyzer (Applied Biosystems, Foster City, Calif) at the LGC Genomics Facility in Berlin, Germany. Sequencing data were analyzed using CLC Main Workbench 6.9.1 (CLC Bio, Aarhus, Denmark). No DNA sample was available from the older healthy brother.

Determination of *STAT2* transcripts by endpoint PCR, relative quantitative RT-PCR, and exon trapping analysis

Total RNA was extracted with Trizol reagent (Life Technologies, Carlsbad, Calif) from primary fibroblasts and/or SV40 fibroblasts left unstimulated or stimulated with 10^3 IU/mL IFN-α (Thermo Fisher Scientific, Waltham, Mass) or IFN-γ (R&D Systems, Abingdon, United Kingdom) for 6 hours. mRNA was then reverse transcribed directly with Superscript Vilo cDNA synthesis kit (Life Technologies). *STAT1*, *STAT2*, and *STAT3* transcripts were evaluated by PCR. *STAT2* qPCR (Taqman gene expression array) was performed for distinct cDNA fragments: ex8-9: HS01013120-g1;ex16-17: HS01013132-m1; ex19-20: HS01013123-m1. For the determination of *MxA* (Hs00895608), *ISG15* (Hs00192713), and *OAS1* (Hs00973635) transcript levels, Taqman gene expression assays (Applied Biosystems) was used. The results were normalized to values from the endogenous *GUSB* (Hs00939627; Applied Biosystems).

Exon trapping analysis

COS-7 cells (ATTC) were transfected with pSPL3 plasmids containing *STAT2* exon16 (wild-type or G1576A mutation) with a part of the introns at 5′ and 3′ end. RT-PCR amplified products were analyzed by agarose electrophoresis and visualized with GelRed staining (Biotium, Inc).

Functional assays

Dermal primary fibroblasts were obtained from skin biopsies of the patient, family members, and an unrelated control and cultured in Dulbecco modified Eagle medium with 10% FBS and supplemental penicillin and streptomycin.

Western blot analysis

Primary fibroblast cultures were stimulated with IFN-α2 (10^4 U/mL) or IFN-γ (10^3 U/mL) for 30 minutes or left unstimulated. Cells were lysed in radioimmunoprecipitation assay buffer after stimulation, and immune blotting was performed using anti-STAT1 p84/p91 (M-22, sc-592; Santa Cruz Biotechnology, Dallas, Tex), anti-STAT2 (22/Stat2, 610187; BD Biosciences, San Jose, Calif), anti-phosphoSTAT1 (Tyr701, sc-7988; Santa Cruz Biotechnology), anti-phosphoSTAT2 (Tyr690 AF2890; R&D Systems, Minneapolis, Minn), and anti-β actin (A5441; Sigma-Aldrich) antibodies. All Western blot images were captured and quantified with a ChemiDoc MP imager and Image Lab software (Bio-Rad Laboratories, Hercules, Calif) after adding Pierce ECL Western blotting substrate (Thermo Fisher Scientific).

Electrophoretic mobility shift assay and supershift assays

SV40 fibroblasts and fibrosarcoma cell cultures were stimulated with IFN-α (10^5 U/mL) or IFN-γ (10^4 U/mL) for 30 minutes or left unstimulated. Nuclear extracts, electrophoretic mobility shift assay, and supershift assays were performed as previously described.[E1]

Virus assays

Primary fibroblasts were seeded in 96-well plates at 2×10^4 cells per well in Dulbecco modified Eagle medium with 2.5% FBS and supplemental penicillin and streptomycin, and they were stimulated with IFN-α (10^3 U/mL) or IFN-γ (10^3 U/mL) for 24 hours or left unstimulated. Cells were infected with *Vesicular stomatitis* virus (strain Indiana, MOI 0.005). Percentage of cell survival was evaluated by measuring the mitochondrial dehydrogenase activity (fluorometric cell cytotoxicity assay kit ab112119; Abcam, Cambridge, United Kingdom) 3 days after viral infection.

Production of STAT2 lentiviral vectors

The codon optimized coding sequence for human *STAT2* isoform 1 (NP_005410.1. or NM_005419.3) was ordered in 2 parts as gBlocks, with or without the p.R510X mutation engineered in. Sequences were cloned into the HIV-based lentiviral vector transfer plasmid, pCHMWS_eGFP_T2A_Fluc-IRES-Puro cl.3, in frame with the peptide2A sequence. HIV-based viral vectors were produced by triple transient transfection of 293T producer cells. A Vesicular Stomatitis Virus Glycoprotein G envelope encoding plasmid, a packaging plasmid together with the respective transfer plasmids were transfected using polyethylenimine (Polysciences, Amsterdam, The Netherlands). After collecting the supernatant, the medium was filtered using a 0.45-μm filter (Corning Inc, Seneffe, Belgium) and concentrated using a Vivaspin 50,000 MW column (Vivascience, Bornem, Belgium). The vector containing concentrate was aliquoted and stored at −80°C.

Generation of stable cell lines

HIV-based vectors were used to transduce primary fibroblasts, in a serial dilution series, and subjected to puromycin selection (0.5 μg/mL). Vector dilutions that resulted in <30% eGFP (ehanced green fluorescent protein) positive cells (equivalent to 1 integrated copy/cell) were subjected to puromycin from day 5 onward and selected until all cells were green fluorescent protein positive. STAT2 expression was corroborated by Western blot analysis.

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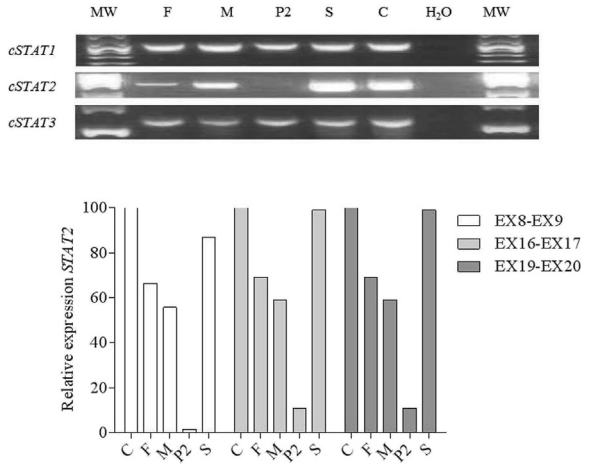

FIG E1. Agarose gel electrophoresis of full-length *STAT2* transcript and fragments of *STAT1* and *STAT3* transcripts levels (*top*), and quantitative real-time PCR *STAT2* expression (*bottom*). Fragments are located around exon 8-9, exon 16-17, and exon 19-20. *C*, Healthy control; *EX*, exon; *F*, father; *M*, mother; *MW*, molecular weight marker; *S*, sibling.

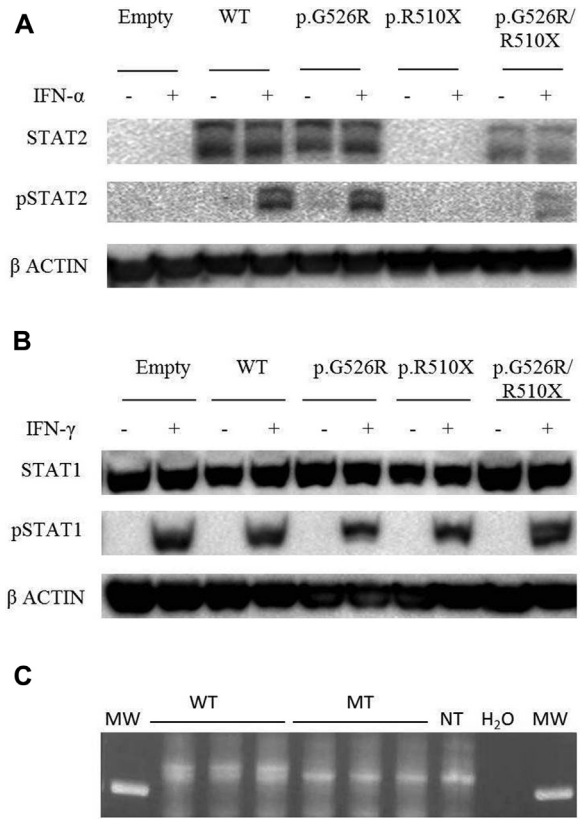

FIG E2. STAT2 knockout fibrosarcoma (U6A) cells were transfected with pMCSV empty vector or pMCSV vector containing wild-type *STAT2*, c.G1576A, c.C1528T, or cotransfection of both *STAT2* variants. Expression of *STAT2* **(A)** and *STAT1* **(B)**, and phosphorylated *STAT2* or *STAT1* following stimulation with or without IFN-α or IFN-γ respectively (30 minutes, 103 IU/mL) in whole cell extracts. β-ACTIN expression was used as loading control. **(C)** Exon trapping analysis: agarose gel electrophoresis of RT-PCR after transfection of COS-7 cells with pSPL3 plasmids containing wild-type (*WT*) or G1576A mutant (*MT*) *STAT2* exon 16 and intron boundaries in triple. *MW*, Molecular weight marker; *NT*, not transfected.

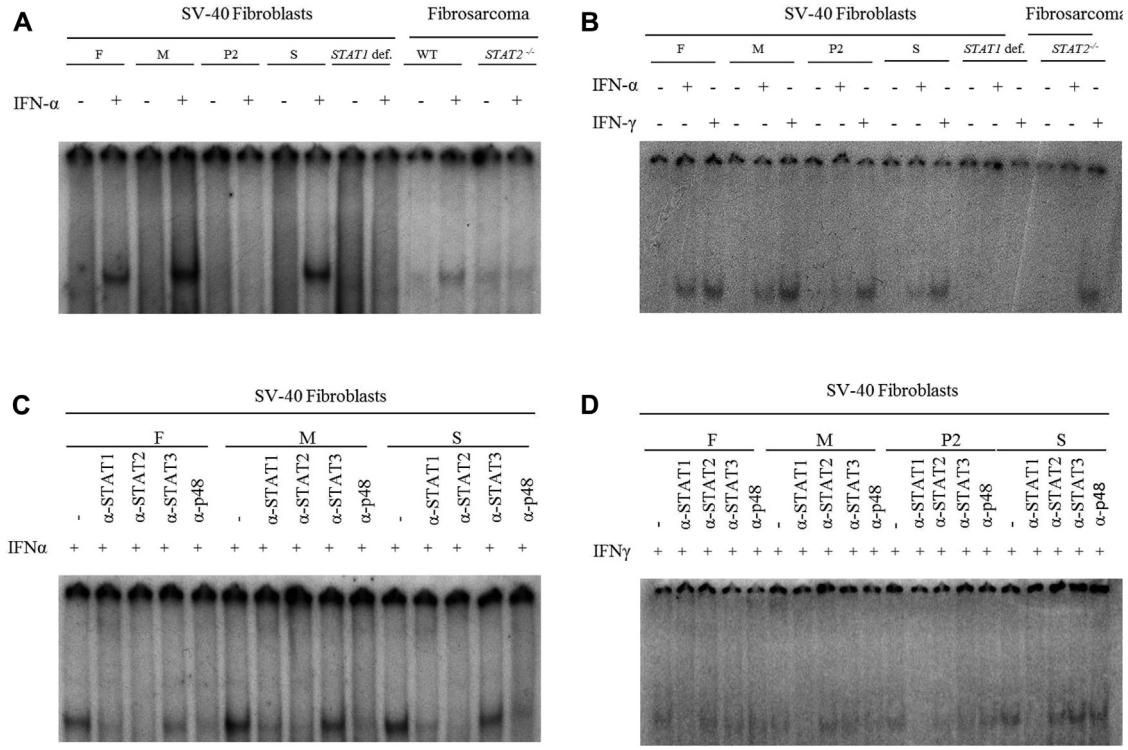

FIG E3. Electrophoretic mobility shift assay (**A** and **B**) and electrophoretic mobility shift assay supershift (**C** and **D**) of nuclear extracts of SV40 fibroblasts and fibrosarcoma cell lines. The cells were left unstimulated or were stimulated with the indicated doses of IFN-α or IFN-γ. The radiolabeled interferon senstive response element probe (**A** and **C**) or gamma-activating sequences (**B** and **D**) probe were used, respectively. *C*, Healthy control; *F*, father; *M*, mother; *S*, sibling; *STAT1 def.*, *STAT1*-deficient SV40 fibroblast cell line; *STAT2*$^{-/-}$, *STAT2* knockout fibrosarcoma cell line.

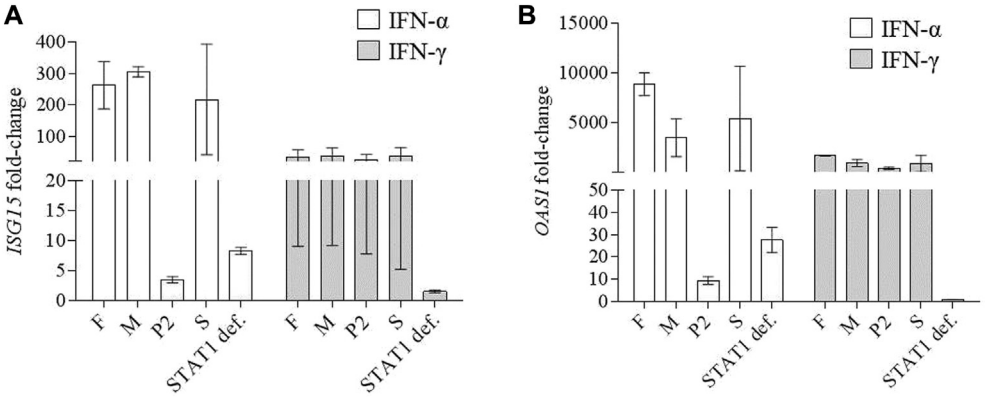

FIG E4. Relative expression of *ISG15* **(A)** and *OAS1* **(B)** in primary fibroblasts and *STAT1*-deficient cell SV40 fibroblast cell lines after stimulation with IFN-α or IFN-γ. *C*, Healthy control; *F*, father; *M*, mother; *S*, sibling; *STAT1 def.*, *STAT1*-deficient SV40 fibroblast cell line.

TABLE E1. Lymphocyte subsets and immunoglobulin measurements in P2

Leucocyte subset or immunoglobulin	2 y	5 y	10 y
Neutrophils, per μL	6,400 (1,500-8,500)	2,600 (1,500-8,500)	4,700 (1,800-8,000)
Lymphocytes, per μL	4,338 (2,700-11,900)	2,880 (1,700-6,900)	2,490 (1,500-6,500)
T cells, per μL	2,239 (1,400-8,000)	1,668 (900-4,500)	1,444 (700-4,200)
$CD4^+$ T cells, per μL	1,384 (900-5,500)	895 (500-2,400)	822 (500-2,400)
$CD8^+$ T cells, per μL	738 (400-2,300)	637 (300-1,600)	511 (300-1,600)
NK cells, per μL	152 (100-1,400)	681 (100-1,000)	586 (100-1,000)
B cells, per μL	1,048 (600-3,100)	506 (200-2,100)	647 (200-2,000)
Switched memory B cells, %	NA	13.4	13.5
IgG (g/L)	16.2 (3.02-9.85)	11.7 (4.78-11.29)	11.8 (5.30-13.06)
IgA (g/L)	0.78 (0.13-1.08)	0.89 (0.35-1.90)	1.06 (0.60-2.70)
IgM (g/L)	1.32 (0.26-1.60)	0.54 (0.34-1.34)	0.49 (0.43-1.73)
IgE (kU/L)	47 (<91)	1,177 (<224)	1,127 (<331)
Measles Ab	Protective	Protective	NA
Poliomyelitis Ab	Protective	NA	NA
Hepatitis B Ab	Protective	Absent	NA

NA, Not applicable; *NK*, natural killer cell.

TABLE E2. EBV measurement by PCR in P2

Age (y)	2.5	3	4	4.5	5.5	10
EBV, log copies/mL	4.73	2.99	4.25	3.07	4.33 (blood) 2.71 (CSF)	Undetectable

CSF, Cerebrospinal fluid.

TABLE E3. Overview of mutations, clinical characteristics, viral illnesses, and outcome of reported STAT2-deficient patients

Patient	Mutation	Phenotype	Virus	Treatment	Outcome at latest follow-up reported
1[E7]	c.381+5G>C	18 mo: disseminated vaccine strain measles	Blood and BAL vaccine strain measles (+)		6 y, A&W
	c.381+5G>C	HSV gingivostomatitis, Influenza A pneumonia			
2[E7]	c.381+5G>C	10 wk: febrile illness	Unknown		Deceased at 10 wk
	c.381+5G>C				
3[E7]	c.381+5G>C	—	NA	—	Adult, A&W
	c.381+5G>C				
4[E7]	c.381+5G>C	3 mo: bronchiolitis post-MMR FS	ND	—	6 y, A&W
	c.381+5G>C				
5[E7]	c.381+5G>C	Admitted for febrile viral illness	No MMR	—	4 y, A&W
	c.381+5G>C				
6[E8]	c.1836C>A	12 mo: post-MMR FS	CSF viral PCR (–)	IVIG	2.5 y: spasticity, chorea, cortical visual impairment
	c.1836C>A	13 mo: opsoclonus-myoclonus		AB	
		2.5 y: fever, diarrhea, metabolic acidosis, meningoencephalitis, thrombocytopenia		AED	
7[E8]	c.1836C>A	13 mo: post-MMR FS	CSF PCR mumps (+)	IVIG	27 mo?: A&W
	c.1836C>A	15 mo: fever, malaise, septic shock, metabolic acidosis	CSF PCR HSV, VZV enterovirus (–)		
8 (P1)	c.C1528T	0-3 y: severe febrile episodes	Adenovirus, enterovirus, RSV		Deceased at 7 y
	c.G1576A	24 mo: post-MMR FS			
		7 y: febrile illness			
9 (P2)	c.C1528T	<2 y: recurrent severe viral infection	VZV, Coxsackie virus, HPV, enterovirus, Influenza A, adenovirus, rotavirus, *Molluscum contagiosum*	—	11 y: A&W
	c.G1576A	18 mo: post-MMR FS	EBV		
		2.5 y: fever, lymphoproliferation	Coxsackie B		
		5 y: meningitis			

AB, Antibiotics; *AED*, antiepileptic drugs; *A&W*, alive and well; *BAL*, broncho-alveolar lavage; *CSF*, cerebrospinal fluid; *FS*, febrile syndrome; *HPV*, human papilloma virus; *HSV*, herpes simplex virus; *IVIG*, intravenous immunoglobulin substitution; *MMR*, measles-mumps-rubella vaccine; *NA*, not applicable; *ND*, not done; *RSV*, respiratory syncytial virus; *VZV*, *Varicella zoster* virus.