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BRIEF REPORT

IFIH1 Mutation Causes Systemic Lupus Erythematosus With Selective IgA Deficiency

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Objective. **To identify the underlying genetic defect in a 16-year-old girl with severe early-onset and refractory systemic lupus erythematosus (SLE), IgA deficiency, and mild lower limb spasticity without neuroradiologic manifestations.**

Methods. **Whole-exome sequencing and extensive immunologic analysis were performed on samples from the index patient.**

Results. **We identified a de novo p.R779H *IFIH1* gain-of-function mutation in a patient with severe early-onset SLE, selective IgA deficiency, and mild lower limb spasticity. The same mutation in *IFIH1* was recently identified in patients with Aicardi-Goutières syndrome, a rare neuroimmunologic disorder associated with elevated levels of type I interferon (IFN). IFN induced with helicase C domain 1 functions as an intracellular innate immune receptor that senses viral nucleic acids and leads to the induction of type I IFN and proinflammatory cytokines. Despite systemic immunosuppressive treatment, disease activity persisted in the patient and was associated with elevated serum levels of IFNα and up-regulation of *IFIH1* itself.**

Conclusion. **This finding adds a new genetic causation for Mendelian lupus and greatly extends the disease spectrum associated with mutations in *IFIH1* (ranging from inflammatory encephalopathy to prototypic systemic autoimmune disease). This marked phenotypic heterogeneity, despite an identical mutation, demonstrates the importance of modifying factors in type I IFN–dependent pathologies caused by mutations in *IFIH1*.**

Interferon induced with helicase C domain 1, a member of the retinoic acid–inducible gene 1–like family of cytoplasmic RNA receptors, senses double-stranded RNA and mediates an antiviral response by activating type I interferon (IFN) signaling (1). Recently, gain-of-function mutations in *IFIH1* were identified in patients with Aicardi-Goutières syndrome (AGS) (2), a rare neuroimmunologic disorder associated with elevated levels of type I IFN and characterized by leukoencephalopathy, brain atrophy, and intracranial calcifications leading to profound intellectual disability, spasticity, and dystonia (3). The same *IFIH1* mutation was also identified in a patient with spastic paraplegia with normal results on neuroimaging and normal cognitive function (4).

Systemic lupus erythematosus (SLE) is a systemic multiorgan autoimmune disease that is associated with elevated levels of type I IFN (5). Patients with monogenic causes of SLE are thought to comprise ~1% of the adult SLE cohort, with greater prevalence among patients with severe early-onset disease (6). Known genetic defects associated with SLE include mutations in *DNASE1L3, DNASE1, PRKCD, TREX1,* and *SAMHD1* (6). Mutations in *TREX1* and *SAMHD1* are also found in AGS, demonstrating genetic associations between AGS and SLE (6). In genome-wide association studies, common polymorphisms in *IFIH1* have been identified as risk factors for SLE (7), type 1 diabetes mellitus (8), and selective IgA deficiency (9).

PATIENT AND METHODS

The study was approved by the Ethics Committee of UZ Leuven, Belgium, and written informed consent was obtained from the parents of the patient and the age-

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Table 1. Results of blood analysis in an SLE patient with a gain-of-function mutation in *IFIH1**

	Age			
	8 years	15.3 years	16.2 years	16.8 years
WBC count, $\times 10^9$ units/liter (normal 4.5–13.5)	5.8	5.01	7.44	7.86
Neutrophil count, $\times 10^9$ units/liter (normal 2.0–8.0)	3.6	2.8	6.4	6.0
Lymphocyte count, $\times 10^9$ units/liter (normal 1.5–6.5)	1.4	1.7	0.9	1.1
Hemoglobin, gm/dl (normal 12–16)	10.6	11.9	11.7	9.9
Thrombocyte count, $\times 10^9$ units/liter (normal 150–450)	108	243	297	294
Erythrocyte sedimentation rate, mm/hour	74	29	11	8
C-reactive protein, mg/liter	NA	<0.3	<0.3	<0.3
C3, gm/liter (normal 0.79–1.52)	0.87	0.60	0.93	0.81
C3d, % (normal ≤2.4)	4.6	12.2	3.0	2.3
C4, gm/liter (normal 0.16–0.38)	0.1	NA	0.11	0.13
IgG, gm/liter (normal 5.58–12.54)	16.9	18.9	10.3	9.36
IgA, gm/liter (normal 0.13–1.08)	<0.07	NA	NA	NA
IgM, gm/liter (normal 0.34–1.42)	1.44	NA	NA	NA
ANA titer	>1:640	1:320	1:640	1:160
Double-stranded DNA, IU/ml†	>100	>100	35.8	17.4
pANCA titer	1:640	NA	NA	NA
IgM anticardiolipin antibodies, MPL units/ml (normal <20)	78	NA	NA	NA
IgG anticardiolipin antibodies, GPL units/ml (normal <20)	80	267	44	NA
Lupus anticoagulant	Strongly positive	Strongly positive	Strongly positive	NA
Antithyroglobulin antibodies, kU/liter (normal ≤200)	272	NA	NA	NA
Antithyroid peroxidase antibodies, kU/liter (normal ≤100)	853	NA	NA	NA

* SLE = systemic lupus erythematosus; WBC = white blood cell; NA = not available; ANA = antinuclear antibody; pANCA = perinuclear antineutrophil cytoplasmic antibody.
† By Farr immunoassay.

matched healthy individuals. The study was performed in accordance with the modified version of the Declaration of Helsinki.

Clinical features of the index patient. The patient, a 16-year-old girl of European Belgian ancestry, initially presented with frequent respiratory infections before the age of 1 year. Immunologic testing revealed selective IgA deficiency. By the age of 2.5 years she developed lower limb spasticity, without cognitive or developmental impairment. Magnetic resonance imaging (MRI) of the brain and spine showed no abnormalities. At 8 years old, the patient was diagnosed as having SLE with secondary antiphospholipid syndrome. Clinically, her disease manifested as arthritis, livedo rash, necrotizing cutaneous vasculitis, and deep venous thrombosis. Blood analysis demonstrated a marked inflammatory response, complement activation, and an abnormal autoantibody profile, including highly increased levels of anti–double-stranded DNA, anticardiolipin, and antithyroid antibodies (Table 1). Single photon–emission computed tomography, computed tomography, and MRI of the brain showed no abnormalities. Despite systemic immunosuppressive treatment, persistently increased levels of circulating autoantibodies and complement activation remained, and attempts at decreasing immunosuppressive medication were associated with disease flares. Spasticity remained confined to the lower limbs, cognitive functioning remained excellent, and results of all neuroradiologic examinations remained normal.

Whole-exome sequencing. We performed whole-exome sequencing on genomic DNA from the patient and the patient's unaffected mother. Genomic DNA samples were prepared from heparinized peripheral blood using a QIAamp DNA Blood Midi kit (Qiagen). Exome sequence libraries were prepared using a SeqCap EZ Human Exome Library version 3.0 kit (Roche NimbleGen). Paired-end sequencing was performed on an Illumina HiSeq2000 (Genomics Core Facility). Burrows-Wheeler Aligner software was used to align the sequence reads to Human Reference Genome Build hg19. GATK Unified Genotyper was used to identify single nucleotide variants and insertions/deletions. ANNOVAR software was used for annotation.

Sanger method of sequencing. The region of interest in exon 12 of *IFIH1* was sequenced using the primers 5′-CTTTCTTCCTCTGGAGTCACCCATC-3′ and 5′-CAGACCTTCTTCTGCCACTGTGG-3′. Sanger sequencing was performed on an ABI 3730xl Genetic Analyzer (Applied Biosystems) at the LGC Genomics Facility in Berlin, Germany. Sequencing data were analyzed using CLC Main Workbench 6.9.1 (CLC Bio).

Flow cytometry. Peripheral blood mononuclear cells (PBMCs) were isolated from the heparinized blood of subjects using a lymphocyte separation medium (MP Biomedicals) and frozen in 10% DMSO (Sigma). Thawed cells were stained with antibodies against CD11c (3.9), CD3 (SK7), CD4 (RPA-T4), CD8α (RPA-T8), CD19 (HIB19), CD45RA (HI100), CD56 (MEM188), HLA–DR (LN3), IFNγ (4S.B3), interleukin-17 (IL-17) (eBio64DEC17), IL-2 (MQ1-17H12), CD31 (WM-59), CCR7 (3D12), CD27 (O323), IgE (IgE21), CD24 (eBioSN3, SN3 A5-2 H10), CD38 (HIT2), γ/δ T cell receptor (B1.1), CD56 (MEM188), CD14 (61D3), CD123 (6H6), and IL-4 (8D4-8) (all from eBioscience), FoxP3 (206D) and IgM (MHM-88) (both from BioLegend), and CXCR5 (IgG23) (R&D Systems). For cytokine staining, T cells were stimulated ex vivo for 5 hours in 50 ng/ml phorbol 12-myristate 13-acetate (PMA) and 500 ng/ml ionomycin (both from Sigma) in the presence of GolgiStop (BD

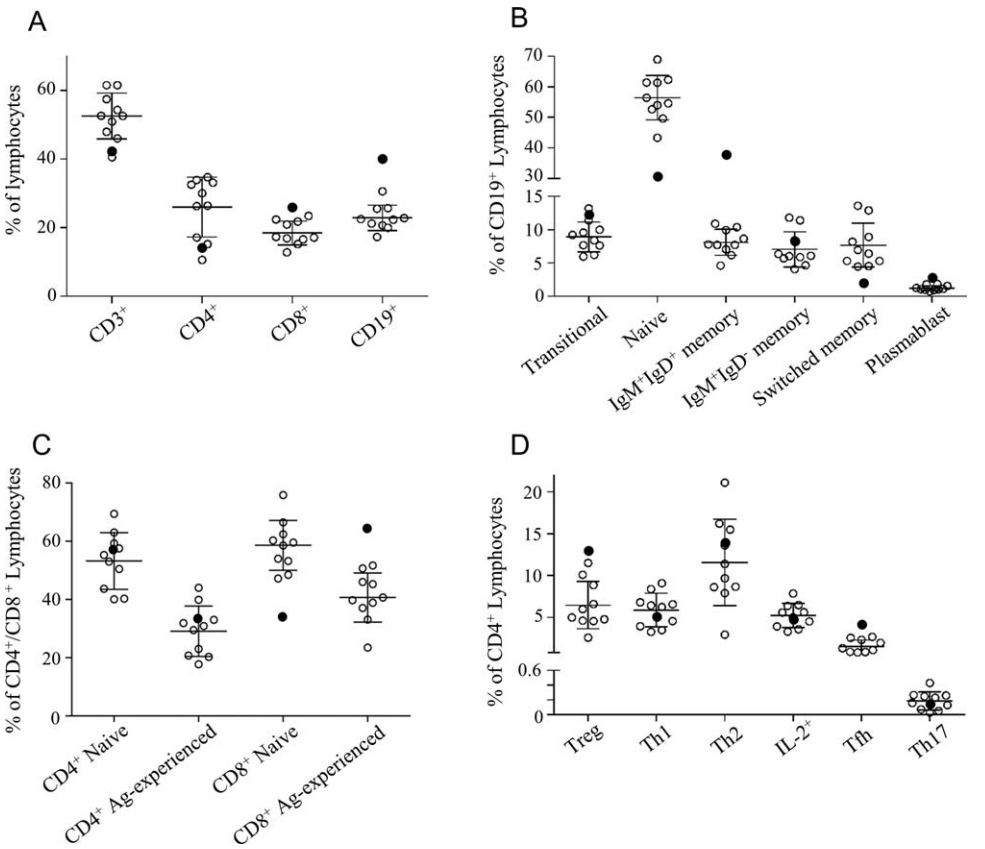

Figure 1. Immunologic profile of peripheral blood mononuclear cells from a systemic lupus erythematosus patient with a gain-of-function mutation in *IFIH1* (solid circles) as compared to healthy age-matched controls (open circles). Percentages of the major leukocyte subsets (**A**), CD19+ B lymphocyte subsets (**B**), CD4+ and CD8+ T lymphocyte subsets (**C**), and T helper cell lineages (**D**) were determined. Bars show the mean ± SD in the healthy controls. Ag = antigen; IL-2 = interleukin-2; Tfh = follicular helper T cell.

Biosciences) before staining. Prior to intracellular staining, cells were surface stained as described, fixed, and permeabilized using a fixation/permeabilization buffer (eBioscience) for FoxP3 staining or using Cytofix/Cytoperm (BD Biosciences) for other intracellular staining. All data were acquired on a BD FACSCanto II and analyzed with FlowJo (Tree Star).

Real-time quantitative polymerase chain reaction (PCR). Total RNA was isolated from PBMCs using an RNeasy Mini kit (Qiagen). Complementary DNA (cDNA) was synthesized by reverse transcription using a SuperScript III First-Strand Synthesis System (Life Technologies). The cDNA was analyzed by real-time quantitative PCR. An appropriate amount of the cDNA was mixed with Fast SYBR Green Master Mix (Applied Biosystems) supplemented with gene-specific primers: for IFIH-1, 5′-CATATGCGCTTTCCCAGTG-3′ (forward) and 5′-TGAGCATACTCCTCTGGTTTCA-3′ (reverse); for β-actin, 5′-CTGGGACGACATGGAGA AAA-3′ (forward) and 5′-AAGGAAGGCTGGAAGAGTGC-3′ (reverse); for GAPDH, 5′-AGAAGGCTGGG GCTCATTTG-3′ (forward) and 5′-GCATCAGCAGAG GGGGCAGA-3′ (reverse); and for hypoxanthine guanine phosphoribosyltransferase (HPRT), 5′-GTAGCCCTCTGTGTGCTCAAGG-3′ (forward) and 5′-GGCTTATATCCAACACTTCGTGGGG-3′ (reverse). Real-time quantitative PCR analysis was performed on a StepOnePlus real-time PCR system (ABI). The thermal cycling protocol was 1 cycle of 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The resultant PCR products were analyzed with ABI 7500 software (Applied Biosystems). Gene expression was analyzed with the $2^{-\Delta\Delta C_t}$ method (10), and all quantifications were normalized to the average of the level of β-actin, GAPDH, and HPRT. Experiments were performed in triplicate and repeated twice.

RESULTS

Alterations in the peripheral immune system. Extensive profiling of the patient's peripheral immune cells was performed at age 15 years, when the patient's disease was clinically stable and treatment was limited to mycophenolate mofetil. Of the major mononuclear leukocyte cell types surveyed, natural killer cell percentages were decreased (data not shown), percentages of T cells were reduced (especially among CD4+ T cells), and CD19+ B cell percentages were increased compared to healthy age-matched individuals (Figure 1A). Within the CD19+ B lymphocyte population, plasmablast levels were elevated and an excess of CD27+

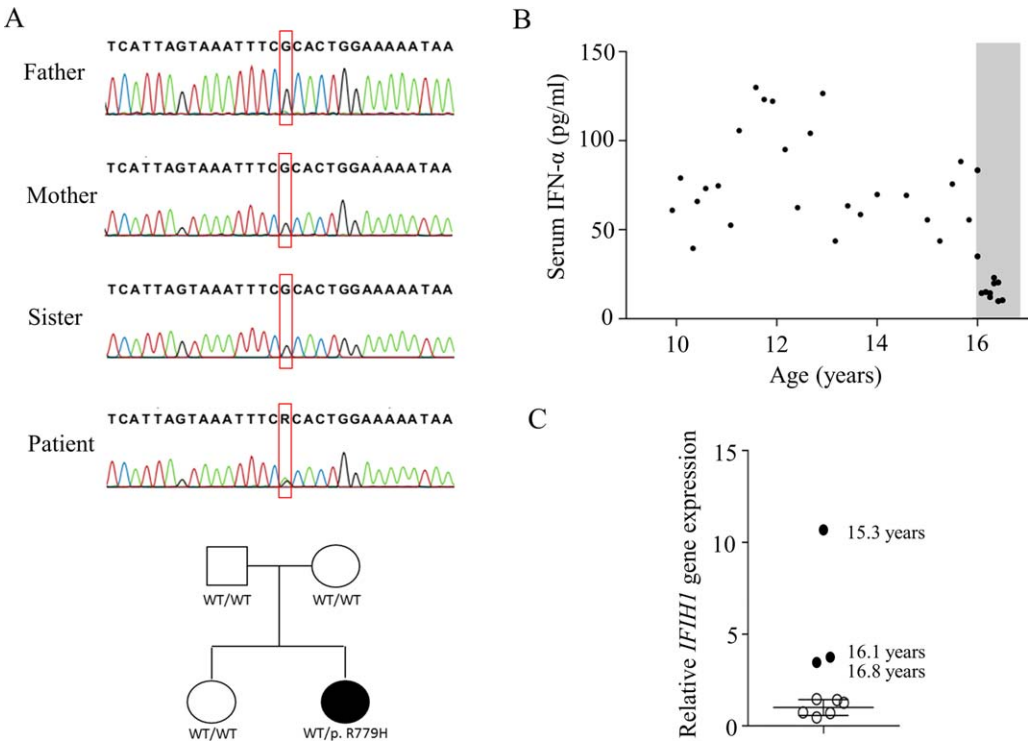

Figure 2. Confirmation of a de novo mutation in *IFIH1*, serum interferon-α (IFNα) levels, and *IFIH1* expression in peripheral blood mononuclear cells (PBMCs) from a systemic lupus erythematosus patient with a gain-of-function mutation in *IFIH1*. **A,** Sanger sequencing of the region of interest in exon 12 of *IFIH1* in samples from the patient and her unaffected family members (top). The pedigree of the affected patient (solid symbol) and her parents and sibling shows the *IFIH1* genotype (wild-type [WT; nonmutated] or p.R779H mutation) (bottom). **B,** Serum IFNα levels in the patient at various ages. Shaded bar indicates the period during which the patient was treated with corticosteroids. **C,** Relative *IFIH1* gene expression in PBMCs from the patient at 3 time points (solid circles) as compared to healthy age-matched controls (n = 6; open circles). Bars show the mean ± SD in the healthy controls.

memory B cells (the majority of which were IgM+ IgD+) was found (Figure 1B), whereas the percentage of switched memory B cells was reduced. Within the CD4+ T lymphocyte population, the percentage of naive cells and antigen-experienced cells in the patient was comparable to that in healthy individuals, and cytokine production after stimulation with PMA/ionomycin appeared normal (Figures 1C and D). CD4+ follicular helper T (Tfh) cell numbers were relatively increased (Figure 1D). The percentage of Treg cells (CD4+ FoxP3+ T cells) was also increased (Figure 1D); however, few of these cells were $CD25^{high}$ (data not shown). Within the CD8+ T lymphocyte population, we found an expansion of antigen-experienced cells (Figure 1C); however, cytokine production after stimulation with PMA/ionomycin was normal (data not shown).

Identification of a de novo mutation in *IFIH1* and *IFIH1* expression in PBMCs. Whole-exome sequencing was performed on genomic DNA from the patient and the patient's unaffected mother. After filtering out common polymorphisms, we identified a heterozygous c.G2336A mutation in *IFIH1*, leading to a p.R779H missense mutation. The mutation in the patient and the de novo status were confirmed by Sanger sequencing of DNA from the patient, both parents, and the patient's sister (Figure 2A).

The same p.R779H missense mutation in *IFIH1* was recently described by Rice et al in 2 patients with AGS (2). They demonstrated gain-of-function properties leading to a type I IFN signature. We also tested serum IFNα and found persistently elevated levels of IFNα in the patient (Figure 2B), whereas serum IFNα was undetectable in healthy individuals. Only after extended treatment with high-dose corticosteroids (which was started because of a severely debilitating disease flare) did we see a reduction in serum levels of IFNα (Figure 2B). Subsequently, autoantibody titers were also reduced and complement activation was no longer present (Table 1), consistent with an IFNα-mediated mechanism of disease. As IFIH-1 is up-regulated by type I IFN (8), *IFIH1* expression in the patient's PBMCs was tested and found to be 10-fold up-regulated (Figure 2C). When

serum levels of IFNα were reduced, the overexpression of *IFIH1* was reduced as well. These findings suggest that excessive activation of IFIH-1 is generated both from the gain-of-function mutation and also from the presence of a positive feedback loop around IFIH-1, leading to persistent production of IFNα and subsequent immune dysregulation.

DISCUSSION

We identified a de novo *IFIH1* mutation in a patient with elevated serum levels of IFNα, severe early-onset SLE, selective IgA deficiency, and mild lower limb spasticity without neuroradiologic manifestations. Strikingly, despite the common molecular etiology, the clinical presentation of this patient varied widely from that of other *IFIH1* gain-of-function patients (2,4). Although several cases in the cohort described by Rice et al manifested some immunologic abnormalities, the primary clinical manifestation was AGS. Conversely, the index patient described herein had no clinical features of AGS, with a clinical presentation dominated by early-onset SLE, IgA deficiency, and spastic paraplegia. In the study by Rice et al, 1 of the 2 AGS patients with the same p.R779H missense mutation developed chilblains at age 6 years and the other presented with generalized urticaria and mildly positive antinuclear antibodies at age 12 years (2). Strikingly, the father and paternal grandmother of the first patient carried the mutation but were asymptomatic. This indicates that the clinical phenotype associated with *IFIH1* mutations is diverse and includes an asymptomatic phenotype, isolated spastic paraplegia, severe inflammatory encephalopathy, and prototypic systemic autoimmune disease. This marked phenotypic heterogeneity demonstrates the importance of genetic, environmental, or stochastic modifying factors in type I interferonopathies caused by mutations in *IFIH1*.

In addition to the SLE manifestation, the patient described herein presented with selective IgA deficiency. We did not formally demonstrate whether selective IgA deficiency in the patient is due to the mutation in *IFIH1*; however, the association between selective IgA deficiency and SLE, as well as the finding of a common *IFIH1* polymorphism conferring risk in both diseases (7,9), indicates a shared genetic predisposition between selective IgA deficiency and SLE through *IFIH1*. An intriguing possibility is that selective IgA deficiency may be the modifying factor that drove the interferonopathy toward the SLE phenotype in this patient. Selective IgA deficiency leads to a defective host defense against viral infections and an abnormal presentation of viral antigens. The possibility of exogenous viral-derived RNA playing a role in the pathogenesis of interferonopathies has been proposed as a possible explanation for the observed clinical nonpenetrance (2). As serum IgA is thought to protect against autoimmunity by helping control inflammation, low levels of IgA or absent IgA may favor the development of autoimmunity and autoantibody production (11). It is therefore possible that selective IgA deficiency directed the interferonopathy in this patient toward the SLE phenotype. Further clinical investigation of patients with *IFIH1* mutations is necessary to determine the relative frequency of SLE and AGS, and whether selective IgA deficiency is disproportionally associated with one of the potential outcomes related to type I interferonopathies.

Immunologic assessment identified disturbances consistent with SLE, with increased levels of CD19+ B cells, CD27+ plasma cells, and a bias toward CD27+ IgM+IgD+ memory B cells. This immunologic signature has previously been described in patients with active SLE (12) and is indicative of a capacity for T cell–independent antibody production (13,14). Potentially contributing to a B cell–driven etiology was a relative increase in Tfh cell numbers and an increase in the CD4+CD25–FoxP3+ T cell population (a proposed dysfunctional Treg cell population) (15). This cell population can suppress T cell proliferation, but not IFNγ production, in vitro (15), so despite their increased proportion, the selective functional defect in these Treg cells might contribute to a failure to control autoimmune dysregulation. As these changes have been identified in other SLE patients (12,15,16), these results suggest that persistently elevated serum IFNα levels distort the immune system into an autoantibody-prone configuration. This mechanistic interpretation is consistent with our clinical observation of persistent inflammation and autoimmune activation despite immunosuppressive treatment. Serum levels of IFNα and, subsequently, autoantibodies were found to be reduced only after several months of treatment with high-dose corticosteroids. While this treatment has been effective, the IFNα-driven nature of the disease process suggests that more targeted inhibition of IFIH-1 or IFNα would produce the same outcome with fewer side effects.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Liston had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Van Eyck, de Zegher, Wouters, Liston.

Acquisition of data. Van Eyck, De Somer, Pombal, Bornschein, Frans, Moens, Bossuyt, Wouters, Liston.

Analysis and interpretation of data. Van Eyck, Bornschein, Humblet-Baron, Wouters, Liston.

REFERENCES

1. Goubau D, Deddouche S, Reis e Sousa C. Cytosolic sensing of viruses. Immunity 2013;38:855–69.
2. Rice GI, del Toro Duany Y, Jenkinson EM, Forte GM, Anderson BH, Ariaudo G, et al. Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling. Nat Genet 2014;46:503–9.
3. La Piana R, Uggetti C, Olivieri I, Tonduti D, Balottin U, Fazzi E, et al. Bilateral striatal necrosis in two subjects with Aicardi-Goutières syndrome due to mutations in ADAR1 (AGS6). Am J Med Genet A 2014;164A:815–9.
4. Crow YJ, Zaki MS, Abdel-Hamid MS, Abdel-Salam G, Boespflug-Tanguy O, Cordeiro NJ, et al. Mutations in ADAR1, IFIH1, and RNASEH2B presenting as spastic paraplegia. Neuropediatrics 2014;45:386–93.
5. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci U S A 2003;100:2610–5.
6. Costa-Reis P, Sullivan KE. Genetics and epigenetics of systemic lupus erythematosus. Curr Rheumatol Rep 2013;15:369.
7. Cunninghame Graham DS, Morris DL, Bhangale TR, Criswell LA, Syvanen AC, Ronnblom L, et al. Association of NCF2, IKZF1, IRF8, IFIH1, and TYK2 with systemic lupus erythematosus. PLoS Genet 2011;7:e1002341.
8. Liu S, Wang H, Jin Y, Podolsky R, Reddy MV, Pedersen J, et al. IFIH1 polymorphisms are significantly associated with type 1 diabetes and IFIH1 gene expression in peripheral blood mononuclear cells. Hum Mol Genet 2009;18:358–65.
9. Ferreira RC, Pan-Hammarstrom Q, Graham RR, Gateva V, Fontan G, Lee AT, et al. Association of IFIH1 and other autoimmunity risk alleles with selective IgA deficiency. Nat Genet 2010;42:777–80.
10. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_t}$ method. Methods 2001;25:402–8.
11. Jacob CM, Pastorino AC, Fahl K, Carneiro-Sampaio M, Monteiro RC. Autoimmunity in IgA deficiency: revisiting the role of IgA as a silent housekeeper. J Clin Immunol 2008;Suppl 1:S56–61.
12. Jacobi AM, Odendahl M, Reiter K, Bruns A, Burmester GR, Radbruch A, et al. Correlation between circulating $CD27^{high}$ plasma cells and disease activity in patients with systemic lupus erythematosus. Arthritis Rheum 2003;48:1332–42.
13. Seifert M, Kuppers R. Molecular footprints of a germinal center derivation of human IgM+IgD+CD27+ B cells and the dynamics of memory B cell generation. J Exp Med 2009;206:2659–69.
14. Weller S, Braun MC, Tan BK, Rosenwald A, Cordier C, Conley ME, et al. Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire. Blood 2004;104:3647–54.
15. Bonelli M, Savitskaya A, Steiner CW, Rath E, Smolen JS, Scheinecker C. Phenotypic and functional analysis of CD4+ CD25–Foxp3+ T cells in patients with systemic lupus erythematosus. J Immunol 2009;182:1689–95.
16. Simpson N, Gatenby PA, Wilson A, Malik S, Fulcher DA, Tangye SG, et al. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. Arthritis Rheum 2010;62:234–44.