

# Progressive Multifocal Leukoencephalopathy in Primary Immune Deficiencies: Stat1 Gain of Function and Review of the Literature

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**Background.** Progressive multifocal leukoencephalopathy (PML) is a rare, severe, otherwise fatal viral infection of the white matter of the brain caused by the polyomavirus JC virus, which typically occurs only in immunocompromised patients. One patient with dominant gain-of-function (GOF) mutation in signal transducer and activator of transcription 1 (STAT1) with chronic mucocutaneous candidiasis and PML was reported previously. We aim to identify the molecular defect in 3 patients with PML and to review the literature on PML in primary immune defects (PIDs).

**Methods.** STAT1 was sequenced in 3 patients with PML. U3C cell lines were transfected with STAT1 and assays to search for STAT1 phosphorylation, transcriptional response, and target gene expression were performed.

**Results.** We identified 3 new unrelated cases of PML in patients with GOF STAT1 mutations, including the novel STAT1 mutation, L400Q. These STAT1 mutations caused delayed STAT1 dephosphorylation and enhanced interferon-gamma-driven responses. In our review of the literature regarding PML in primary immune deficiencies we found 26 cases, only 54% of which were molecularly characterized, the remainder being syndromically diagnosed only.

**Conclusions.** The occurrence of PML in 4 cases of STAT1 GOF suggests that STAT1 plays a critical role in the control of JC virus in the central nervous system.

**Keywords.** progressive multifocal leukoencephalopathy; PML; primary immunodeficiency; STAT1 gain of function; PID.

Progressive multifocal leukoencephalopathy (PML) is a rare, severe, demyelinating disease of the brain white matter caused by reactivation of the neurotropic human polyomavirus JC PyV or JC virus (JCV). The virus targets oligodendrocytes, leading to widespread regions of demyelination in the cerebral and cerebellar hemispheres, with insidious progression over weeks to months. Clinically, PML often presents with aphasia, hemianopsia, hemimotor phenomena, or cognitive decline, usually without seizures [1]. Previously it was seen predominantly in the profoundly immunocompromised (eg, advanced human immunodeficiency virus [HIV] disease), as well as those under intense immune suppression (eg, chemotherapy, graft-vs-host therapy, organ transplantation). With the advent of new biologic agents for immune modulation, PML has been a rare but severe

complication, although its underlying mechanisms remain unclear [2]. While it has been suggested that higher antibody levels increase the likelihood of reactivation of JCV in certain settings [2, 3], no distinct monogenic predisposition to JCV reactivation has been described, and the molecular events that underlie the pathogenesis and reactivation of infection are not well understood. PML remains a largely untreatable and incurable disease.

The recognition of 3 new cases of PML in signal transducer and activator of transcription 1 (STAT1) gain-of-function (GOF) mutation, in addition to 1 reported previously [4], led us to review the literature on PML in primary immune defects. Dominant hypermorphic GOF STAT1 mutations are associated with chronic mucocutaneous candidiasis (CMC), other fungal infections, autoimmunity, and Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX)-like syndrome [4–7]. These mutations are characterized by enhanced STAT1 phosphorylation (pSTAT1), diminished interleukin (IL)-17-producing T cells, and increased in vitro interferon-gamma (IFN- $\gamma$ ) target response. While single cases of PML have been reported in a variety of conditions, STAT1 GOF appears to confer a considerable predisposition to PML, apparently more than do other primary immune defects.

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## METHODS

### Patients

All patients provided informed consent on approved National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH) protocols.

### Viral Detection

Evaluation of JCV viral load was assessed using real-time polymerase chain reaction (PCR; performed at the Microbiology Service, Department of Laboratory Medicine, Clinical Center, and at the Laboratory of Molecular Medicine and Neuroscience, National Institute of Neurological Disorders and Stroke, NIH), which targets a 78 base-pair region of the JCV large tumor (T-antigen) gene. This PCR system uses a single probe specific for the JCV sequence between the forward and reverse PCR primer sites [8]. PCR detection of JCV was performed in cerebrospinal fluid.

### Experimental Procedures

The STAT1-deficient U3C fibrosarcoma cell line (a generous gift of Dr JL Casanova) was maintained in complete Roswell Park Memorial Institute medium supplemented with 10% fetal calf serum and antibiotics. Mutated STAT1 expression constructs were created (BioInnovatise Inc., Rockville, Maryland) and all mutations verified by sequencing. All experimental procedures were as previously described [4, 6]. For evaluation of STAT1 activation, tyrosine pSTAT1 was assayed in U3C transfected cells stimulated with IFN- $\gamma$  (400 IU/mL) or IFN- $\alpha$  (1000 IU/mL). For dephosphorylation experiments, the kinetics of pSTAT1 increase and decline were assayed in cells stimulated with IFN- $\gamma$  for 30–120 minutes. Cell lysates were recovered and analyzed using immunoblotting and flow cytometry as previously described [4, 6]. For reporter gene assays, U3C cells were cotransfected with wild type (WT) or mutant STAT1 constructs along with plasmid-containing tandem gamma activated sequence (GAS) elements driving a luciferase reporter gene (Panomics, Fremont, California). A renilla expression vector was cotransfected to measure transfection efficiency. Cells were stimulated with human IFN- $\gamma$  or IFN- $\alpha$  (1000 IU/mL) for 6 hours. Luciferase activity was evaluated using a dual luciferase assay (Promega, Madison, Wisconsin). For analysis of gene expression, cells were stimulated or not with IFNs, then total RNA was extracted from transfected cells using the RNeasy mini kit (QIAGEN, Valencia, California). RNA was reverse transcribed (Invitrogen), and the resulting cDNA amplified by PCR using the ABI 7500 Sequencer and TaqMan expression assays (Applied Biosystems, Carlsbad, California). Glyceraldehyde-3-phosphate dehydrogenase was used as the normalization control.

### Literature Review

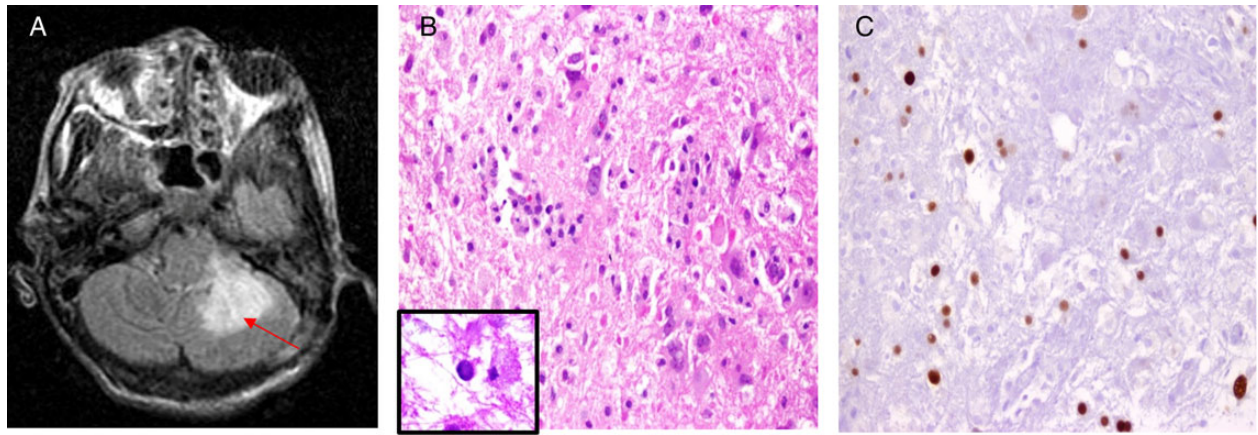
Since our goal was to identify genetic pathways that control JCV in the brain, we conducted a comprehensive literature search of PML in primary immunodeficiencies in MEDLINE through June 2015. The search strategy included the following keywords:

progressive multifocal leukoencephalopathy, JC virus (AND) primary immunodeficiency, immunodeficiency (NOT) HIV. In addition, a manual search for additional references was done to ensure complete coverage of the available linked literature. Scanning for possible suitable reports (conferences and other published materials) was made in Scopus. Literature outside of the above sources was sought through Google using the same terms. More recently, Hatchwell [9] summarized some of the genes and syndromes known or thought likely to be related to PML. We reviewed all published cases of PML outside of the recognized risk factors such as HIV and cancer chemotherapy. We excluded cases in which the immunodeficiency was likely acquired or secondary to infections and cases in which CD4 lymphocytopenia was detected simultaneous with the encephalopathy and without a previous history compatible with primary immunodeficiency (PID). In the early 1970s, few PML cases classified as either spontaneous or without known risk factor were reported [10]. Later, 38 cases of idiopathic CD4+ T-cell lymphocytopenia (ICL) with minimal or occult immunosuppression and PML were reviewed by Gheunes et al [11]. However, a potentially causal association between ICL and JCV was difficult to identify, since most patients presented with both features simultaneously. In addition, in most of those cases, neither complete laboratories nor HIV infection could be definitively excluded. Those who presented with ICL were relatively older than those with recognized PIDs. Subsequently, Delgado-Alvarado et al [12] reviewed the association of PML with previously confirmed ICL and added 9 new cases. The reported cases had PML as a sole manifestation, with onset later in life and better overall survival. Nevertheless, the low CD4 cell count was, in general, found at the time of PML presentation.

## RESULTS

### Patients' Clinical Description

Patient 1 was a 20-year-old African American woman diagnosed at age 2 years with CD4+ T-lymphocytopenia. Her family history was unremarkable; no immunogenetic data were available. At age 6 years she presented with *Candida* esophagitis, onychomycosis, and diffuse cutaneous *Trichophyton tonsurans* infections. From age 10 to age 15 years she had multiple hospitalizations for pneumonias. At age 17 years she had persistent pericardial and chylous pleural and peritoneal effusions. *Mycobacterium avium* complex (MAC) grew from the pleural and peritoneal effusions, which required weekly drainage of 6–10 liters. Mycobacterial and *Candida* infections were aggressively but unsuccessfully treated. Experimental IL-12 (1.4  $\mu$ g/day) did not confer benefit and was stopped after several months. At age 19 years she was severely malnourished and had an unsteady gait. Neurologic symptoms including a left VI nerve palsy, facial numbness, diplopia, nystagmus, hyperreflexia, and cognitive impairment rapidly progressed. Magnetic resonance imaging (MRI) suggested PML, which was confirmed by brain biopsy



**Figure 1.** Patient 1: (A) Brain magnetic resonance imaging shows a demyelinating lesion in the left cerebellum (arrow) consistent with progressive multifocal leukoencephalopathy. (B) Brain biopsy shows foamy macrophages, bizarre astrocytes, minimal lymphocytes, and oligodendrocytes with viral inclusions (highlighted in insert). Hematoxylin and eosin, 200× original magnification; insert, 600× original magnification. (C) SV-40 viral antigen immunostaining shows numerous infected oligodendroglia (200× original magnification).

(Figure 1A–C). Despite the lymphopenia, immunoglobulins were normal. Her mental status continued to decline and she died 3 months later; an autopsy was not performed. Subsequent sequencing of STAT1 identified the novel heterozygous mutation c.1199T>A, p.L400Q in the DNA binding domain, which was predicted to be deleterious by Polyphen and not found in dbSNP 132, the 1000 Genomes Project database, or ExAC. Family members were not screened.

Patient 2 was a 20-year-old white man delivered at term to a mother who subsequently died with recurrent CMC and presumed *Aspergillus* infection in her 30s. At the age of 2 years he had mouth sores, which healed slowly. A chorioretinal scar in the left eye was thought to be due to congenital toxoplasmosis. At around age 12 years he had recurrent pneumonias and oral candidiasis. At age 19 years a staphylococcal empyema required drainage. Subsequently, a cold agglutinin hemolytic anemia was treated with prednisone, followed by rituximab with resolution. Approximately 2 weeks later he noticed right arm and right leg weakness, mild aphasia, and dysarthria. MRI showed demyelination in bilateral thalami, midbrain, cerebellar peduncle, and left cerebellar hemisphere. Lumbar puncture was positive for JCV by PCR. He had low CD19, CD3, CD4, and CD8 cells. Immunoglobulin levels were low despite replacement. Sequencing of STAT1 identified the previously recognized GOF heterozygous mutation c.1154C>T, p.T385M in the DNA binding domain [4, 7]. Cerebral spinal fluid (CSF) JC viral load after 8 weeks of therapy was 27 049 copies/mL and increased to 87 562 copies/mL. MRI showed extensive white matter destruction with neuronal death. Neither mirtazapine nor IFN- $\alpha$  led to improvement, and he died of aspiration pneumonia. No autopsy was performed. Family members were not screened.

Patient 3 was a 32-year-old Hispanic man whose father had CMC. He had had recurrent thrush, onychomycosis, warts,

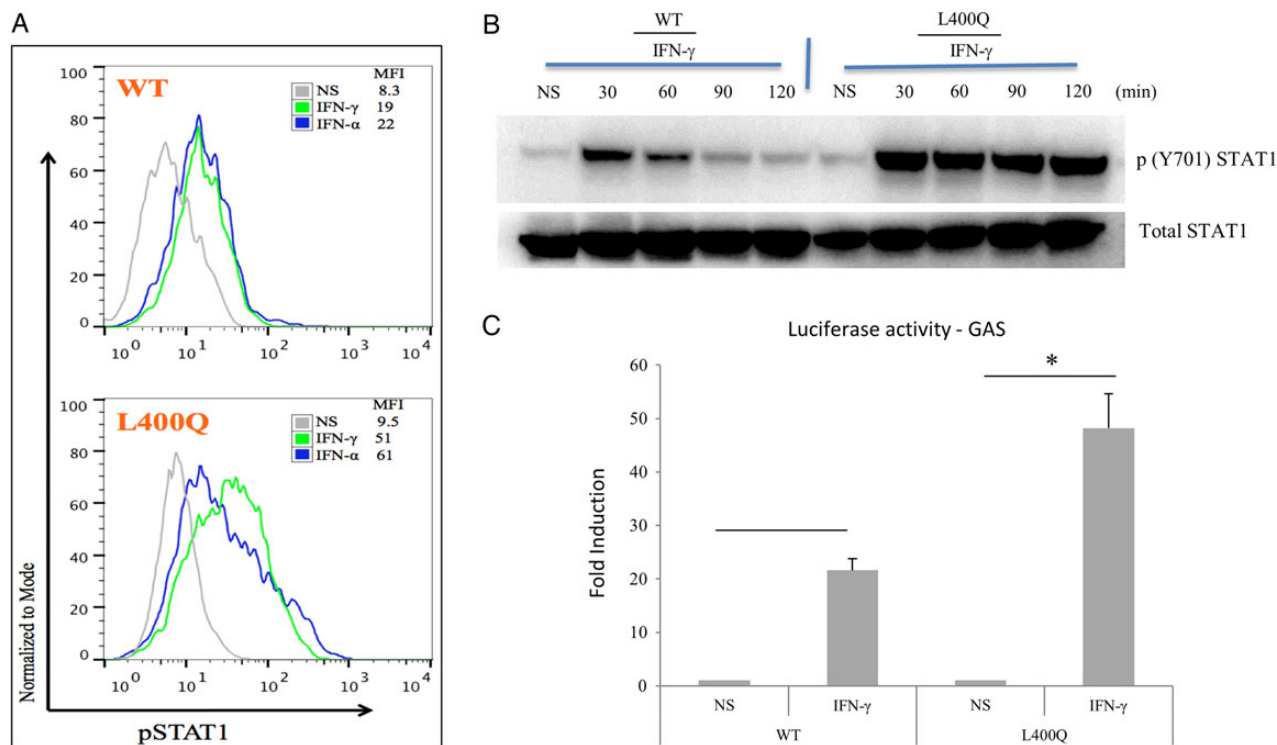
and skin ulcers since infancy and developed his first pneumonia at age 11 months. At age 14 years he had a *Nocardia* pneumonia that resolved with surgery and antibiotics. He had persistent lymphopenia and at age 24 years developed progressive ataxia with cerebellar atrophy, hepatosplenomegaly, and transient pancytopenia. CD19-, CD3-, and CD4-positive cells were persistently low, but CD8+ cells and immunoglobulins were normal. Bone marrow biopsy showed normal cellularity with mild erythrocytosis. STAT1 sequencing identified the recognized GOF heterozygous mutation c.821G>A, p.R274Q in the coiled-coil domain [5]. MRI showed a large enhancing lesion in the left frontal and parietooccipital lobes; in situ hybridization in brain biopsy confirmed JCV. He died within 3 months of diagnosis. No autopsy was performed. Family members were screened and unaffected.

#### L400Q Leads to Enhanced IFN- $\gamma$ -Driven Response

To assess the functional impact of the new mutation described for patient 1, we examined mutant pSTAT1 in comparison with WT STAT1 response. Similar to the previously described STAT1 GOF mutants, transfection of STAT1 L400Q into the STAT1-deficient U3C cell line resulted in enhanced pSTAT1 upon stimulation with IFN- $\gamma$  and IFN- $\alpha$  for 30 minutes (Figure 2A) as well as delayed dephosphorylation (Figure 2B). GAS-driven transcriptional response (Figure 2C) and gene expression (*CXCL10*, *CXCL9*; Figure 2D) in stimulated STAT1 L400Q transfected cells were also elevated following stimulation with IFN- $\gamma$  and IFN- $\alpha$  compared with WT (Figure 2D), which is similar to what has been reported for T385M and R274G [4]. Expression of *MX1* and *OAS2*, induced mainly by IFN- $\alpha$ , was not differentially modulated between GOF and WT STAT1 (Figure 2E).

#### Review of the Literature

Our literature review identified 26 articles in which cases of syndromic or genetically defined PIDs were associated with PML.



**Figure 2.** (A) Signal transducer and activator of transcription 1 phosphorylation (pSTAT1) assayed in transfected U3C cells using flow cytometry after stimulation with interferon-gamma (IFN- $\gamma$ ) or IFN- $\alpha$  for 30 minutes. (B) Immunoblotting for total and tyrosine 701 pSTAT1 in transfected U3C cells stimulated or not with IFN- $\gamma$  (1000 IU/mL). Note prolonged pSTAT1 (30–180 minutes) in cells transfected with the STAT1 mutant L400Q compared with wild type (WT) STAT1. (C) Luciferase gamma activated sequence–induced activity. (D) Gene expression in U3C cells transfected with WT or STAT1 mutant L400Q, T385M, and R274G following stimulation with IFN- $\gamma$  or IFN- $\alpha$  (1000 IU/mL). Data are expressed as fold increase in response to IFN over the nonstimulated cells, performed in triplicate. Results are mean ( $\pm$ standard deviation) of 3 individual experiments. Differences between STAT1 mutant cells and WT were assessed using the Student *t* test. \**P* < .05. Abbreviations: GAS, gamma activated sequence; MFI, mean fluorescence intensity; NS, not stimulated.

All cases, including the patients reported here, are summarized in Table 1. Many of the described cases (12 of 26) had clear clinical features of PID but no molecular diagnosis, with combined and severe immunodeficiencies being more prominent.

Among the molecularly defined PIDs associated with PML (14 cases, 53.8%), only STAT1 GOF deficiency (4 patients, 3 described here), Wiskott-Aldrich syndrome (3 cases) [17, 18, 22], and DOCK8 (dedicator of cytokinesis 8 protein) deficiency (2 patients) [32, 33] were found in more than 1 case. The only previously reported patient with STAT1 GOF was a 31-year-old man with oral candidiasis who developed disseminated histoplasmosis and PML. His mutation was c.820C>G, p.R274G in the coiled-coil domain [4]. X-linked agammaglobulinemia (XLA) [24] and CD40 ligand (CD40L) deficiency [28] were found in 1 case each. Similarly, purine nucleoside phosphorylase deficiency [29] and adenosine deaminase deficiency [19], which have abnormal thymocyte development and peripheral T-cell activation, and the combined defect immunodeficiency, centromeric instability, and facial dysmorphism (ICF syndrome) [25] were also identified in 1 case each.

The clinically defined combined immunodeficiencies associated with PML were severe combined immune deficiency (SCID),

combined immune deficiency (CID), and common variable immune deficiency (CVID) (7 patients) [13–15, 21, 27, 30, 34], hypogammaglobulinemia (1 patient) [31], hyper-IgE (1 patient) [23], and hyper-IgM (3 patients) [16, 20, 26] (Table 1). Because the molecularly defined PIDs identified in PML can also be characterized as cases of CID or hyper-IgM syndrome, the specific etiologies in those syndromic cases remain unclear.

## DISCUSSION

The prevalence of PML in the general population has been estimated at 4.4 cases per 100 000 individuals, according to data from medical services and outpatient prescription claims databases. It is believed to occur exclusively in the setting of significant immune dysfunction, either inborn or acquired. Antibodies to JCV are found in approximately 85% of adults, presumably following inapparent acquisition of infection in childhood [35].

The specific immune factors that hold JCV in check are unknown. The acquired conditions that allow the clinical manifestations of the virus are characterized by profound immune compromise, such as advanced HIV infection and certain immune modulating biologics. The association of PML and rituximab therapy has been reported as 2.3/100 000 patient-years [36],

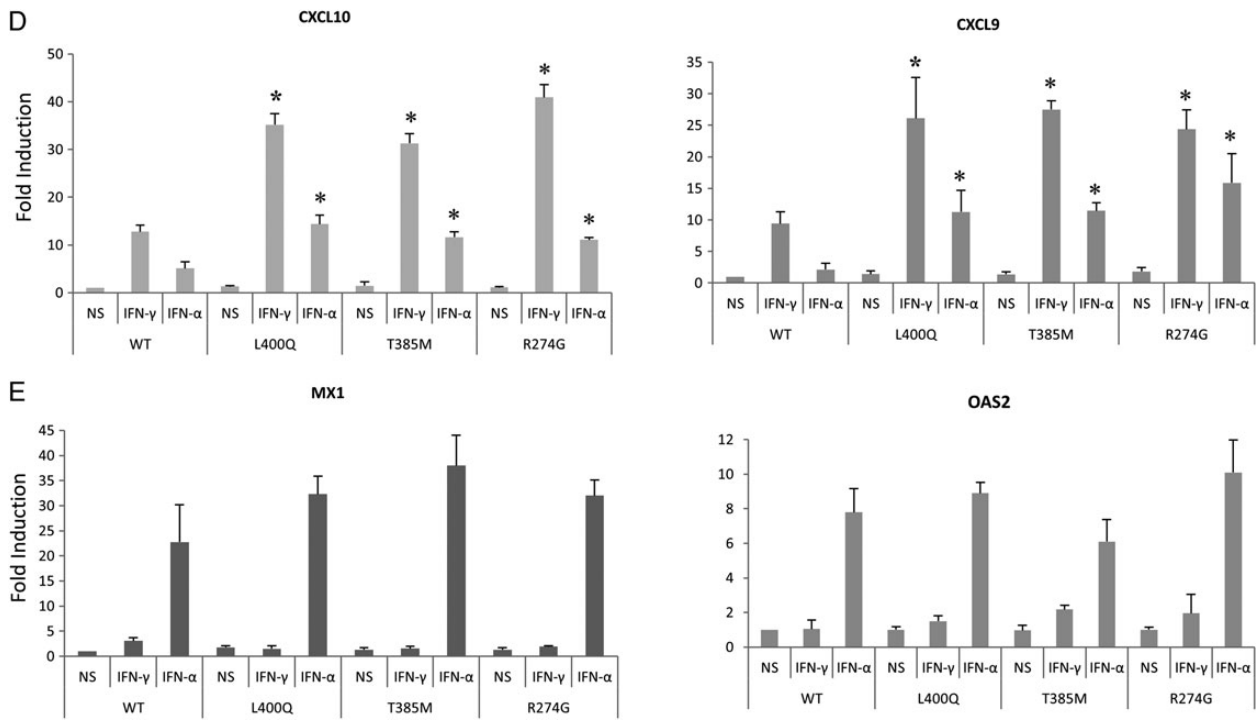


Figure 2 continued.

whereas for PML and natalizumab, it varies from 10/100 000 in JCV-seronegative patients to 1120/100 000 in seropositive patients who have taken natalizumab for more than 24 months [37]. Beyond the necessity of JCV infection, it remains unclear what factors are necessary or sufficient for the development of PML. The specific relevant mechanisms targeted by either the biologic therapeutics or HIV are unknown. Cell-mediated immunity is clearly crucial for controlling JCV, as reflected by high rates of the disease in advanced HIV infection, especially when the CD4+ lymphocyte count is <100 cells/mm<sup>3</sup>. However, B cells and CD34+ progenitors also play roles in the pathophysiology of PML, acting as viral reservoirs. These observations have been reinforced by the observation of high JC viral DNA loads in CD34+ and CD19+ cells in patients treated with natalizumab [38].

The difficulty of determining causal mechanisms in the setting of global defects such as HIV, iatrogenic immune suppression, and syndromic CD4 lymphocytopenia might be addressed through the study of monogenic immune deficiencies associated with PML, since these presumably are due to single gene defects that might show the effects of single pathways on PML development. Consistent with its association with HIV infection and iatrogenic immune suppression, many of the PML cases associated with PIDs are characterized by combined B- and T-cell impairment (eg, DOCK8 deficiency, SCID, CID, CVID). The report of Teramoto et al [24] of PML in a patient with XLA, a relatively pure B-cell disorder, may reflect the previously recognized susceptibility of these patients to central nervous

system (CNS) viral infections. While this case suggests that antibody may in fact be important in PML, it may also reflect a critical role for B-cell-T-cell interaction independent of antibody production, such as antigen presentation.

STAT1 is a transcription factor critical for the mediation of types I, II, and III IFN responses. Loss-of-function (LOF) mutations are associated with viral, mycobacterial (disseminated bacille Calmette-Guérin and nontuberculous mycobacterial), and bacterial infections, while GOF mutations are associated with mucocutaneous and invasive fungal infections, viral infections, arterial aneurysms, and squamous cell carcinomas. Patient 1 had a novel STAT1 GOF mutation (L400Q) that presented with severe CMC, consistent with the GOF phenotype. However, she also developed disseminated MAC infection, which is more commonly seen with LOF mutations in STAT1.

Viral susceptibility in STAT1 GOF includes herpes simplex virus (recurrent oral disease) [39], varicella zoster virus (encephalitis and CNS vasculitis), and 1 report of death ultimately due to Jamestown Canyon encephalitis, cytomegalovirus, and Epstein-Barr virus infection [40]. To date, other common viral infections such as influenza and adenovirus have not been unusually severe in STAT1 GOF patients. Despite the acquisition of JCV early in life, the development of PML during childhood or adolescence is extremely uncommon. We presume that our GOF STAT1 patients had reactivation of JCV infection, but this cannot be proven.

The identification of 4 unrelated patients with PML in the setting of STAT1 GOF disease suggests that STAT1 plays an

**Table 1. Published Case Reports of Progressive Multifocal Leukoencephalopathy Associated With Primary Immunodeficiencies**

Reference/Year of Publication	Primary Immunodeficiency/ Genetic Diagnosis	Gender/Age Onset PML Symptoms (Years)	Survival (Months) Since Onset	Immunodeficiency-related Clinical Syndromes Other Than PML	Medication Prior to PML	PML Diagnosis	Treatment for PML
Knight et al 1972 [13]	Combined immunodeficiency; no molecular diagnosis	M/22	3	Recurrent skin and upper respiratory infections; <i>Salmonella</i> sepsis; pneumococcal pneumonia; malabsorption syndrome; schizophrenia	IVIG steroids	Autopsy	None
Zu Rhein et al 1978 [14]	Severe combined immunodeficiency; no molecular diagnosis	M/11	2	n/a	None	Autopsy	None
Misbah et al 1992 [15]	Combined immunodeficiency; no molecular diagnosis	M/24	4	Warts; bronchiectasis; recurrent otitis	None	Cerebellar biopsy	None
Redfearn et al 1993 [16]	Hyper IgM syndrome; no molecular diagnosis	M/6	9	Recurrent pneumonia; pneumococcal sepsis	IVIG since 3 y	CSF; brain biopsy	Cytosine arabinoside
Katz et al 1994 [17]	Wiskott-Aldrich syndrome; no molecular diagnosis	M/15	10	Candida sepsis; recurrent herpes; pneumonia; liver abscess	None	Brain biopsy	IFN- $\alpha$ intrathecal
Matsushima et al 1997 [18]	Wiskott-Aldrich syndrome; <sup>a</sup> no molecular diagnosis	M/36	n/a	Idiopathic thrombocytopenic purpura; atopic dermatitis; tuberculosis	None	Brain biopsy	Steroids, $\gamma$ -globulin
Jochum et al 1997 [19]	Adenosine deaminase deficiency; no molecular diagnosis	F/39	n/a	n/a	n/a	Brain biopsy	n/a
Bezrodnik et al 1998 [20]	Hyper IgM syndrome; no molecular diagnosis	M/5	36 (alive)	PML was first manifestation	None	Stereotactic brain biopsy	Cytosine arabinoside (IV and intrathecal) IFN- $\alpha$
Scotton et al 1998 [21]	CVID; no molecular diagnosis	M/38	n/a	Cerebral toxoplasmosis	n/a	Brain biopsy and CSF	n/a
Downes et al 2001 [22]	Wiskott-Aldrich syndrome (WAS, c.155C>T)	M/20	2	n/a	IVIG since 12 y	Autopsy	Methylprednisolone IV
Angelini et al 2001 [23]	hyper IgE recurrent infection syndrome; no molecular diagnosis	M/8	5	Skin infections; cold; abscesses; chronic dermatitis; pneumonias due to <i>Candida albicans</i> , <i>Staphylococcus aureus</i> , <i>Haemophilus influenzae</i>	None	Brain biopsy; CSF	Cidofovir; methylprednisolone IV
Teramoto et al 2003 [24]	X-linked agammaglobulinemia (BTK, c.1053A>G)	M/37	9	None	IVIG	Autopsy	Immunoglobulin replacement; cytosine arabinoside intrathecal; IFN- $\alpha$
Colucci et al 2004 [25]	Immunodeficiency, centromeric instability, and facial dysmorphism syndrome; no molecular diagnosis	F/35	2.5	Gastrointestinal infections; facial paralysis; seizures	None	CSF	None
Han et al 2005 [26]	Hyper IgM syndrome; no molecular diagnosis	M/37	10	Pneumonia; chronic otitis and sinusitis	None	Brain biopsy	IVIG
Snyder et al 2005 [27]	CVID; no molecular diagnosis	M/21	2	Recurrent sinusitis and bronchitis; pneumonias; herpes simplex; esophagitis; squamous cell carcinoma	None	CSF	pegylated IFN- $\alpha$ 1.5 mcg/kg/ week
Suzuki et al 2006 [28]	Hyper IgM (CD40L missense mutation)	M/37	n/a	None	n/a	Brain biopsy	IVIG
Parvaneh et al 2007 [29]	Purine nucleoside phosphorylase deficiency; no molecular diagnosis <sup>b</sup>	M/9	3 wks	Sinopulmonary infections; knee arthritis	None	CSF	None

Table 1 continued.

Reference/Year of Publication	Primary Immunodeficiency/ Genetic Diagnosis	Gender/Age Onset PML Symptoms (Years)	Survival (Months) Since Onset	Immunodeficiency-related Clinical Syndromes Other Than PML	Medication Prior to PML	PML Diagnosis	Treatment for PML
Narula et al 2007 [30]	CVID <sup>b</sup>	M/40	n/a	Recurrent sinopulmonary infections; <i>Haemophilus influenzae</i> , <i>Pneumocystis jiroveci</i> ; autoimmune lung disease	IVIg steroids	Brain biopsy	IL-2 IFN- $\gamma$ Cidofovir
Tasca et al 2009 [31]	Hypogammaglobulinemia (gamma- heavy chain disease); no molecular diagnosis	M/72	4	Adenocarcinoma	5-fluorouracil radiotherapy	Brain biopsy	IVIg; cidofovir
Dellepiane et al 2010 [32]	DOCK8 mutation not available	n/a	n/a	n/a	n/a	n/a	n/a
Sampaio et al 2013 [4]	STAT1 GOF (c.820C>G)	M/31	n/a (died from <i>Pseudomonas aeruginosa</i> sepsis)	Recurrent ear infections; <i>Candida</i> esophagitis; disseminated histoplasmosis	None	Brain biopsy in situ hybridization	IL-2
G Day-Williams et al 2015 [33]	DOCK8 (large deletions in chromosome 9p)	M/30	24 (lost to follow- up)	<i>Klebsiella</i> and $\beta$ -hemolytic <i>Streptococcus</i> pneumonia; recurrent herpes skin infection	Steroids (topical)	CSF JCV DNA	n/a
Kurmann et al 2015 [34]	CVID; CD4+T lymphocytopenia; no molecular diagnosis	M/56	23 (alive)	Familial severe hypogammaglobulinemia; immune thrombocytopenia; autoimmune hemolytic anemia; pseudo T lymphoma; recurrent sinopulmonary infections	IVIg	CSF JCV DNA	Mirtazapine mefloquine
Patient 1	STAT1 GOF (c.1199T>A)	F/19	3	<i>Candida</i> esophagitis; <i>Mycobacterium avium</i> complex; recurrent pneumonias	IL-12	Brain biopsy	None
Patient 2	STAT1 GOF (c.1154C>T)	M/20	6	Congenital toxoplasmosis; recurrent pneumonias; candidiasis; <i>Staphylococcus empyema</i> ; hemolytic anemia	Rituximab	CSF JCV	Mirtazapine IFN- $\alpha$
Patient 3	STAT1 GOF (c.821G>A)	M/32	3	Pneumonias; candidiasis; lymphocytopenia	None	Brain biopsy	None

Abbreviations: BTK, Bruton tyrosine kinase; CSF, cerebrospinal fluid; CVID, common variable immunodeficiency; DOCK8, dedicator of cytokinesis; GOF, gain of function; IFN, interferon; IgE, immunoglobulin E; IgM, immunoglobulin M; IL, interleukin; IV, intravenous; IVIG, intravenous immune globulin; JCV, JC virus; n/a, not available; PML, progressive multifocal leukoencephalopathy; STAT1, signal transducer and activator of transcription 1; WAS, Wiskott-Aldrich Syndrome.

<sup>a</sup> CD43 defect detected.

<sup>b</sup> Enzyme activity was not performed.

<sup>c</sup> B-cell deficiency; X-linked agammaglobulinemia excluded (no BTK mutation detected).

important role in the control of JCV in the CNS. Three of these patients had unprovoked PML, while 1 developed PML following the use of rituximab. Precisely how STAT1 is involved in the JCV regulatory pathway is unknown, but we presume that this reflects reactivation of JCV, as it does in the general population. De-Simone et al [41] identified IFN- $\gamma$  as an important regulator that controls JCV replication and expression of large T-antigen. Interestingly, increased programmed cell death 1 (PD-1) expression, which has been detected in T cells from PML patients, is suppressible by pembrolizumab [42]. Binding of PD-1 to its ligands, programmed cell death protein ligands (PD-L) 1 and 2, renders T cells anergic and may lead to T-cell exhaustion and dysfunction. Along these lines, enhanced expression of PD-L1 has been detected in T cells from STAT1 GOF patients [43]. These phenomena may partially explain the pathogenesis of PML in STAT1 GOF. While hyperresponsiveness to IFN- $\gamma$  in vitro is a hallmark of GOF STAT1, it is clear that some of the STAT1 GOF manifestations are more consistent with attenuated IFN- $\gamma$  response. Even though pSTAT1 induced by IFNs is high in STAT1 GOF, the specific IFN-driven dysregulated genes are not clear. Definition of the affected relevant genes in this pathway will help to elucidate the pathogenesis and control of PML in the human brain. It is noteworthy that both DOCK8 deficiency and the syndromic hyper-IgE also predispose to PML, suggesting that their likely overlap in effects on the IL-17 pathway may also be relevant.

Most of the cases of PML in PID identified in this review were reported before the era of genetic identification, preventing molecular confirmation of the underlying immunodeficiency. This may be important as there is considerable overlap between genotypes such as DOCK8 and CID. Over the last decade it has become abundantly clear that the diagnoses of CVID and hyper-IgM include a variety of distinct primary immune defects. Therefore, some cases of the syndromically defined hyper-IgM and other immunodeficiencies without molecular definition could possibly be unrecognized STAT1 GOF or other defects.

It is apparent from this review that defined primary immunodeficiencies underlie a significant number of cases of PML. As molecularly and functionally directed therapies for immune deficiencies become more available, the importance of identifying underlying defects will increase. Although identified only 4 years ago, STAT1 is already a major defined genetic risk for PML. Mutations in STAT1 and other relevant genes should be sought in patients with PML without other clear predisposing factors

## Notes

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## References

- Krupp LB, Lipton RB, Swerdlow ML, Leeds NE, Llena J. Progressive multifocal leukoencephalopathy: clinical and radiographic features. *Ann Neurol* **1985**; 17:344–9.
- Ferenczy MW, Marshall LJ, Nelson CD, et al. Molecular biology, epidemiology, and pathogenesis of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. *Clin Microbiol Rev* **2012**; 25:471–506.
- Gorelik L, Lerner M, Bixler S, et al. Anti-JC virus antibodies: implications for PML risk stratification. *Ann Neurol* **2010**; 68:295–303.
- Sampaio EP, Hsu AP, Pechacek J, et al. Signal transducer and activator of transcription 1 (STAT1) gain of function mutations and disseminated coccidioidomycosis and histoplasmosis. *J Allergy Clin Immunol* **2013**; 131:1624–34.
- Liu L, Okada S, Kong XF, et al. Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. *J Exp Med* **2011**; 208:1635–48.
- Uzel G, Sampaio EP, Lawrence MG, et al. Dominant gain-of-function STAT1 mutations in FOXP3 wild-type immune dysregulation-polyendocrinopathy-enteropathy-X-linked-like syndrome. *J Allergy Clin Immunol* **2013**; 131:1611–23.
- Takezaki S, Yamada M, Kato M, et al. Chronic mucocutaneous candidiasis caused by a gain-of-function mutation in the STAT1 DNA-binding domain. *J Immunol* **2012**; 189:1521–6.
- MacKenzie J, Wilson KS, Perry J, Gallagher A, Jarrett RF. Association between simian virus 40 DNA and lymphoma in the United Kingdom. *J Natl Cancer Inst* **2003**; 95:1001–3.
- Hatchwell E. Is there a (host) genetic predisposition to progressive multifocal leukoencephalopathy? *Front Immunol* **2015**; 6:216.
- Fermaglich J, Hardman JM, Earle KM. Spontaneous progressive multifocal leukoencephalopathy. *Neurology* **1970**; 20:479–84.
- Gheuens S, Pierone G, Peeters P, Koralnik JJ. Progressive multifocal leukoencephalopathy in individuals with minimal or occult immunosuppression. *J Neurol Neurosurg Psychiatry* **2010**; 81:247–54.
- Delgado-Alvarado M, Sedano MJ, Gonzalez-Quintanilla V, de Lucas EM, Polo JM, Berciano J. Progressive multifocal leukoencephalopathy and idiopathic CD4 lymphocytopenia. *J Neurol Sci* **2013**; 327:75–9.
- Knight A, O'Brien P, Osoba D, et al. "Spontaneous" progressive multifocal leukoencephalopathy. Immunologic aspects. *Ann Intern Med* **1972**; 77:229–33.
- Zu Rhein G, Padgett BL, Walker DL, Chun RW, Horowitz SD, Hong R. Progressive multifocal leukoencephalopathy in a child with severe combined immunodeficiency. *N Engl J Med* **1978**; 299:256–7.
- Misbah SA, Spickett GP, Zeman A, et al. Progressive multifocal leukoencephalopathy, sclerosing cholangitis, bronchiectasis and disseminated warts in a patient with primary combined immune deficiency. *J Clin Pathol* **1992**; 45:624–7.
- Redfearn A, Pennie RA, Mahony JB, Dent PB. Progressive multifocal leukoencephalopathy in a child with immunodeficiency and hyperimmunoglobulinemia. *Pediatr Infect Dis J* **1993**; 12:399–401.
- Katz DA, Berger JR, Hamilton B, Major EO, Post MJ. Progressive multifocal leukoencephalopathy complicating Wiskott-Aldrich syndrome. Report of a case and review of the literature of progressive multifocal leukoencephalopathy with other inherited immunodeficiency states. *Arch Neurol* **1994**; 51:422–6.
- Matsushima T, Nakamura K, Oka T, et al. Unusual MRI and pathologic findings of progressive multifocal leukoencephalopathy complicating adult Wiskott-Aldrich syndrome. *Neurology* **1997**; 48:279–82.
- Jochum W, Weber T, Frye S, Hunsmann G, Luke W, Aguzzi A. Detection of JC virus by anti-VP1 immunohistochemistry in brains with progressive multifocal leukoencephalopathy. *Acta Neuropathol* **1997**; 94:226–31.
- Bezrodnik L, Samara R, Krasovec S, Garcia Erro M, Sevlever G. Progressive multifocal leukoencephalopathy in a patient with hypogammaglobulinemia. *Clin Infect Dis* **1998**; 27:181–4.
- Scotton PG, Vaglia A, Carniato A, Marchiori GC. Progressive multifocal leukoencephalopathy in a patient with common variable immunodeficiency. *Clin Infect Dis* **1998**; 26:215–6.
- Downes SM, Black GC, Hyman N, Simmonds M, Morris J, Barton C. Visual loss due to progressive multifocal leukoencephalopathy in a congenital immunodeficiency disorder. *Arch Ophthalmol* **2001**; 119:1376–8.



23. Angelini L, Pietrogrande MC, Delle Piane MR, et al. Progressive multifocal leukoencephalopathy in a child with hyperimmunoglobulin E recurrent infection syndrome and review of the literature. *Neuropediatrics* **2001**; 32:250–5.
24. Teramoto T, Kaneko H, Funato M, et al. Progressive multifocal leukoencephalopathy in a patient with X-linked agammaglobulinemia. *Scand J Infect Dis* **2003**; 35:909–10.
25. Colucci M, Cocito L, Capello E, et al. Progressive multifocal leukoencephalopathy in an adult patient with ICF syndrome. *J Neurol Sci* **2004**; 217:107–10.
26. Han GP, Miura K, Ide Y, Tsutsui Y. Genetic analysis of JC virus and BK virus from a patient with progressive multifocal leukoencephalopathy with hyper IgM syndrome. *J Med Virol* **2005**; 76:398–405.
27. Snyder MD, Storch GA, Clifford DB. Atypical PML leading to a diagnosis of common variable immunodeficiency. *Neurology* **2005**; 64:1661.
28. Suzuki H, Takahashi Y, Miyajima H. Progressive multifocal leukoencephalopathy complicating X-linked hyper-IgM syndrome in an adult. *Intern Med* **2006**; 45:1187–8.
29. Parvaneh N, Ashrafi MR, Yeganeh M, Pouladi N, Sayarifar F, Parvaneh L. Progressive multifocal leukoencephalopathy in purine nucleoside phosphorylase deficiency. *Brain Dev* **2007**; 29:124–6.
30. Narula S, LaRosa DF, Kamoun M, Dalmau J, Levinson AI. Progressive multifocal leukoencephalopathy in a patient with common variable immunodeficiency and abnormal CD8+T-cell subset distribution. *Ann Allergy Asthma Immunol* **2007**; 98:483–9.
31. Tasca G, Iorio R, Basile U, et al. Progressive multifocal leukoencephalopathy in a patient with Franklin disease and hypogammaglobulinemia. *J Neurol Sci* **2009**; 284:203–4.
32. Dellepiane RM, Engelhardt K, Pfeifer D, et al. Hyper IgE syndrome: fatal progressive multifocal leukoencephalopathy in an Italian child with DOCK8 mutation. In: Meeting of the European Society for Immunodeficiencies (ESID, conference 14). Istanbul, 2010.
33. G Day-Williams A, Sun C, Jelcic I, et al. Whole genome sequencing reveals a chromosome 9p deletion causing DOCK8 deficiency in an adult diagnosed with hyper IgE syndrome who developed progressive multifocal leukoencephalopathy. *J Clin Immunol* **2015**; 35:92–6.
34. Kurmann R, Weisstanner C, Kardas P, et al. Progressive multifocal leukoencephalopathy in common variable immunodeficiency: mitigated course under mirtazapine and mefloquine. *J Neurovirol* **2015**; 21:694–701.
35. Beltrami S, Gordon J. Immune surveillance and response to JC virus infection and PML. *J Neurovirol* **2014**; 20:137–49.
36. Mok CC. Rituximab for the treatment of rheumatoid arthritis: an update. *Drug Des Devel Ther* **2013**; 8:87–100.
37. Chalkias S, Dang X, Bord E, et al. JC virus reactivation during prolonged natalizumab monotherapy for multiple sclerosis. *Ann Neurol* **2014**; 75:925–34.
38. Frohman EM, Monaco MC, Remington G, et al. JC virus in CD34+ and CD19+ cells in patients with multiple sclerosis treated with natalizumab. *JAMA Neurol* **2014**; 71:596–602.
39. Tóth B, Méhes L, Taskó S, et al. Herpes in STAT1 gain-of-function mutation [corrected]. *Lancet* **2012**; 379:2500.
40. Sharfe N, Nahum A, Newell A, et al. Fatal combined immunodeficiency associated with heterozygous mutation in STAT1. *J Allergy Clin Immunol* **2014**; 133:807–17.
41. De-Simone FI, Sariyer R, Otolara YL, et al. IFN-gamma inhibits JC virus replication in glial cells by suppressing T-antigen expression. *PLoS One* **2015**; 10:e0129694.
42. Tan CS, Bord E, Broge TA Jr, et al. Increased program cell death-1 (PD-1) expression on T lymphocytes of patients with progressive multifocal leukoencephalopathy (PML). *J Acquir Immune Defic Syndr* **2012**; 60:244–8.
43. Romberg N, Morbach H, Lawrence MG, et al. Gain-of-function STAT1 mutations are associated with PD-L1 overexpression and a defect in B cell survival. *J Allergy Clin Immunol* **2013**; 131:1691–3.