Brief report

Successful treatment of a novel type I interferonopathy due to a *de novo PSMB9* gene mutation with a Janus kinase inhibitor

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Background: Type I interferonopathies are a recently established subgroup of autoinflammatory diseases caused by mutations in genes associated with proteasome degradation or cytoplasmic RNA- and DNA-sensing pathways.
Objective: This study aimed to unveil the molecular pathogenesis of a patient with novel type I interferonopathy, for which no known genetic mutations have been identified.
Methods: We performed the whole-exome sequencing of a 1-month-old boy with novel type I interferonopathy. We also investigated proteasome activities using patient-derived B lymphoblastoid cell lines (LCLs) and normal LCLs transduced with the mutant gene.
Results: Whole-exome sequencing identified a *de novo* proteasome 20S subunit beta 9 (*PSMB9*) p.G156D mutation in the patient who developed fever, a chilblain-like skin rash, myositis, and severe pulmonary hypertension due to the hyperactivation of IFN-α. Patient-derived LCLs revealed reduced proteasome activities, and exogenous transduction of mutant *PSMB9* p.G156D into normal LCLs significantly suppressed proteasome activities, and the endogenous PSMB9 protein was lost along with the reduction of other immunoproteasome subunits, PSMB8 and PSMB10 proteins. He responded to the administration of a Janus kinase inhibitor, tofacitinib, and he was successfully withdrawn from venoarterial extracorporeal membranous oxygenation. At age 7 months, he received an unrelated cord blood transplantation. At 2 years posttransplantation, he no longer required tofacitinib and experienced no disease recurrence.
Conclusions: We present the case of a patient with a novel type I interferonopathy caused by a *de novo PSMB9* p.G156D mutation that suppressed the wild-type PSMB9 protein expression. Janus kinase inhibitor and stem cell transplantation could be curative therapies in patients with severe interferonopathies. (J Allergy Clin Immunol 2021;148:639-44.)

Key words: *JAK inhibitor, interferonopathy, pulmonary hypertension, proteasome 20S subunit beta 9 (*PSMB9*)*

INTRODUCTION

Autoinflammatory diseases are characterized by dysregulation of innate immunity and systemic inflammation in the absence of antigen-specific T cells or high-titer autoantibodies.[1]

Type I interferonopathies are a recently established subgroup of autoinflammatory disease that includes Aicardi-Goutières syndrome, STING-associated vasculopathy with onset in infancy, and chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature syndrome. Patients with type I interferonopathy shared several clinical characteristics, including bilateral calcifications of the basal ganglia, chilblain-like rashes, and liver dysfunction.[2] Each subtype includes disease-specific severe complications, such as early-onset encephalopathy associated with Aicardi-Goutières syndrome[3] and pulmonary hypertension observed in patients diagnosed with STING-associated vasculopathy with onset in infancy.[4]

The common pathogenic mechanism shared by all of these diseases is overactivation of the type I interferon pathway caused

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Abbreviations used
CPK: Creatine phosphokinase
IB: Immunoblot
JAK: Janus kinase
LCL: Lymphoblastoid cell line
PSMB9: Proteasome 20S subunit beta 9
STAT1: Signal transducer and activator of transcription 1
VA-ECMO: Venoarterial extracorporeal membranous oxygenation
WT: Wild-type

by mutations in genes associated with proteasome degradation or cytoplasmic RNA- and DNA-sensing pathways.[2] *In vitro* experiments using patient-derived primary cells[4] and anecdotal case reports have suggested that Janus kinase (JAK) inhibitors, including tofacitinib[5] and ruxolutinib,[6] may be effective for the treatment of these disorders. Here, we report the case of a novel type I interferonopathy in a patient with a *de novo* proteasome 20S subunit beta 9 (*PSMB9*) mutation who was successfully treated with tofacitinib as a bridging therapy prior to allogeneic stem cell transplantation.

METHODS

For detailed methods, please see the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS AND DISCUSSION

A 1-month-old boy visited a hospital for evaluation and treatment of skin rashes, fever, pale face due to respiratory failure, and cluster seizures. Initial blood tests revealed pancytopenia (white blood cell count at 3.83×10^9/L, hemoglobin at 7.6 g/dL, and platelets at 52×10^9/L), elevated levels of liver enzymes (aspartate aminotransferase at 923 IU/L, alanine aminotransferase at 426 IU/L), marked elevation of creatine phosphokinase (CPK at 26,839 IU/L), and abnormal coagulation test results, including prothrombin time-international normalized ratio at 1.40 seconds, fibrinogen at 105 mg/dL, fibrin degradation products at 29.8 mg/L, D-dimers at 21.0 mg/L, and antithrombin at 33 %. Cerebrospinal fluid tests were notable for increased levels of total protein (148.2 mg/dL), normal glucose (45 mg/dL), and a normal cell count (11×10^6/L). Acute encephalopathy was suspected; the patient was initially treated with acyclovir, cefotaxime, intravenous immunoglobulin, and methylprednisolone pulse therapy. His symptoms improved temporarily, but fever, elevation of CPK, and seizures reappeared rapidly.

Serologic screening results for infectious pathogens and various encephalopathy-associated autoantibodies were uniformly negative. Although serum PCR test results for BK virus and JC virus were transiently positive after methylprednisolone pulse therapy, we confirmed that viremia test had negative outcomes without any specific antiviral treatment before hematopoietic stem cell transplantation. The lymphocyte subset analysis (CD3, CD4, CD8, and CD19 cell counts) revealed mild lymphocytopenia, whereas the lymphocyte stimulation tests with PHA and concanavalin A were within the normal limits (see Table E1 in this article's Online Repository at www.jacionline.org). Head computed tomography revealed bilateral calcification of the basal ganglia (Fig 1, *A*), which led to the suspicion of a type I interferonopathy. Although we could not eliminate the possibility of the effect of viral infection, elevated levels of IFN-α were detected in both serum (36.5 pg/mL) and spinal fluid (6.9 pg/mL) (Fig 1, *E*). A biopsy of the left quadriceps revealed primarily immature muscle fibers that were round and small, measuring 7 to 16 microns in diameter. Immunohistochemical staining confirmed the expression of HLA class I molecule and deposition of the membrane attack complex on myofibrotic membranes; these results suggested immune-mediated damage to the muscle tissue (Fig 1, *B*).

Chest computed tomography scan revealed mild pulmonary infiltrative shadows bilaterally in the dorsal lung fields and mild cardiomegaly, and an echocardiogram revealed severe pulmonary hypertension with tricuspid regurgitation at a peak velocity of 5.18 m/s; the estimated pressure gradient between the right ventricle and the right atrium at peak systole was 107 mmHg (Fig 1, *C*; see Videos E1 and E2 in this article's Online Repository at www.jacionline.org). The pulmonary hypertension was unresponsive to multiple lines of treatment, including epoprostenol sodium, sildenafil citrate, milrinone, macitentan, and inhalation of nitric oxide (NO). Venoarterial extracorporeal membranous oxygenation (VA-ECMO) was introduced due to progression of right heart failure.

Under the approval from the institutional review board, we initiated therapy with the JAK inhibitor, tofacitinib, at a dose of 0.2 mg/kg/d twice daily on day 1 after the VA-ECMO induction; the dose was increased to 0.3 mg/kg/d after 2 weeks. Shortly after starting on tofacitinib, the serum level of CPK normalized and chilblain-like rashes improved. VA-ECMO and NO inhalation were successfully withdrawn after 16 days and 39 days, respectively, without further complications. His pulmonary hypertension was well controlled on sildenafil citrate and macitentan.

At age 7 months, he underwent an HLA 7/8 allele-matched unrelated cord blood transplantation after a reduced-intensity conditioning regimen that included fludarabine (120 mg/m^2) and melphalan (140 mg/m^2) with total body irradiation at 3 Gy. Tacrolimus and short-term methotrexate were used as prophylaxis against graft versus host disease. He was engrafted at day 14 after transplantation; he developed mild skin graft versus host disease, which improved without additional treatment. He also developed posttransplant nephrotic syndrome at day 104, which responded well to steroids and cyclosporin A. Hematopoiesis was evaluated with short tandem repeat analysis, which revealed stable mixed donor chimerism undergoing gradual increase, determined at 38.1% on day 28, 47.1% on day 116, 61.7% on day 179, 67.5% on day 350, and 63.1% on day 645.

At 2 years posttransplantation, the patient no longer requires tofacitinib or vasodilator drugs and is in good health without fever, skin rash, pulmonary hypertension, or other symptoms associated with type I interferonopathy.

Whole-exome sequencing analysis identified a *de novo* heterozygous missense variant of *PSMB9* (NM_002800.5:c. 467G>A, p.G156D) in our patient/proband, which has not been previously reported as a causative gene for human disease (Fig 1, *D*), and we confirmed that his parents had only wild-type (WT) alleles in *PSMB9*. Three-dimensional structure analysis revealed that the PSMB9 substitution sites were adjacent to one another in the 2 beta-rings of the immunoproteasome (Fig 1, *F* and *G* and *H* and *I*). No other pathogenic variants associated with any known inherited diseases including autoinflammatory diseases were detected.

A proteasome activity assay revealed that proband-derived lymphoblastoid cell lines (LCLs) (Y375 cells) that included

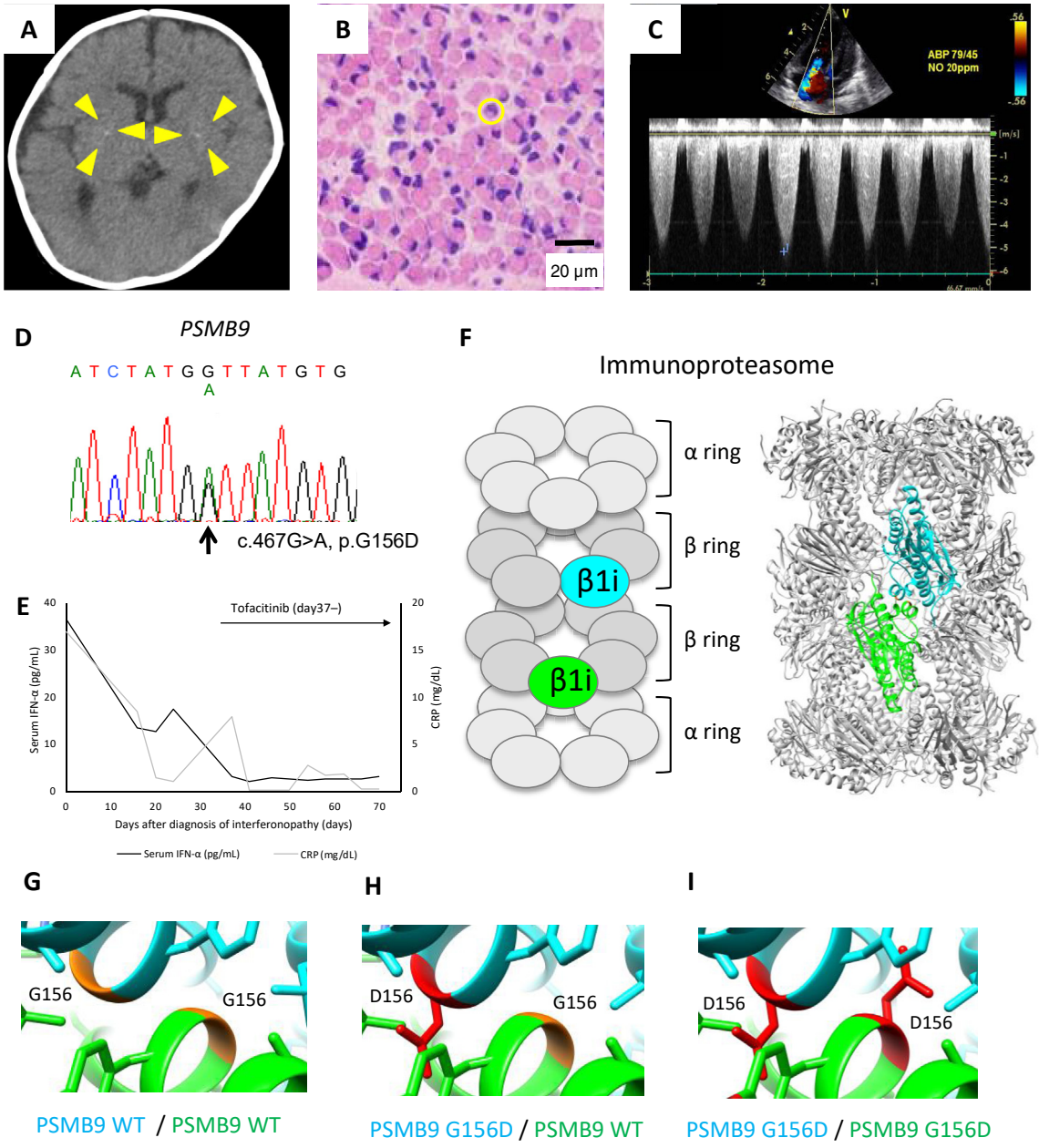

FIG 1. Clinical presentations and *in silico* modeling of mutated PSMB9 protein. **A,** Head computed tomography; *yellow arrowheads* indicate calcifications of basal ganglia. **B,** Biopsy specimen from the quadriceps femoris muscle; *yellow circle* indicates a muscle fiber with the aberrant small and round shape. **C,** Echocardiogram documenting pulmonary hypertension; 4-chamber view with color Doppler prior to the initiation of tofacitinib and VA-ECMO. **D,** Sanger sequencing; a *de novo* heterozygous c.467G>A (p.G156D) mutation was identified in proband. **E,** Transition of IFN-α and C-reactive protein (CRP) value; the black line and the gray line indicate the transitions of the IFN-α value and the CRP value, respectively. Tofacitinib was initiated on day 37 after interferonopathy diagnosis at a dose of 0.2 mg/kg/d twice daily; the dose was increased to 0.3 mg/kg/d after 2 weeks. **F,** Three-dimensional structure of PSMB9; the impact of the *PSMB9* mutation was modeled on the basis of Cryo-electron microscopy structure of the human immunoproteasome (PDB entry code: 6AVO). Blue and green ribbons show PSMB9 protein on 2 separate β-rings. **G- I,** Close-up views show 3 patterns where the 2 PSMB9 proteins are WT or mutant type. The positions of the mutant amino acid are shown in orange for the WT (G156) and red for the mutant type (D156), respectively.

the heterozygous *PSMB9* mutation had lost most of the chymotrypsin-like, trypsin-like, and caspase-like protease activities; in contrast, the LCLs derived from the father (A151) and mother (A139) that both maintained WT *PSMB9* alleles have activity that was comparable to that detected in LCLs derived from 3 unrelated healthy volunteers (Fig 2, *A* and *B*). The proteasome inhibitor, epoxomicin, completely suppressed protease activities detected in all LCLs. We also confirmed that IFN-α in

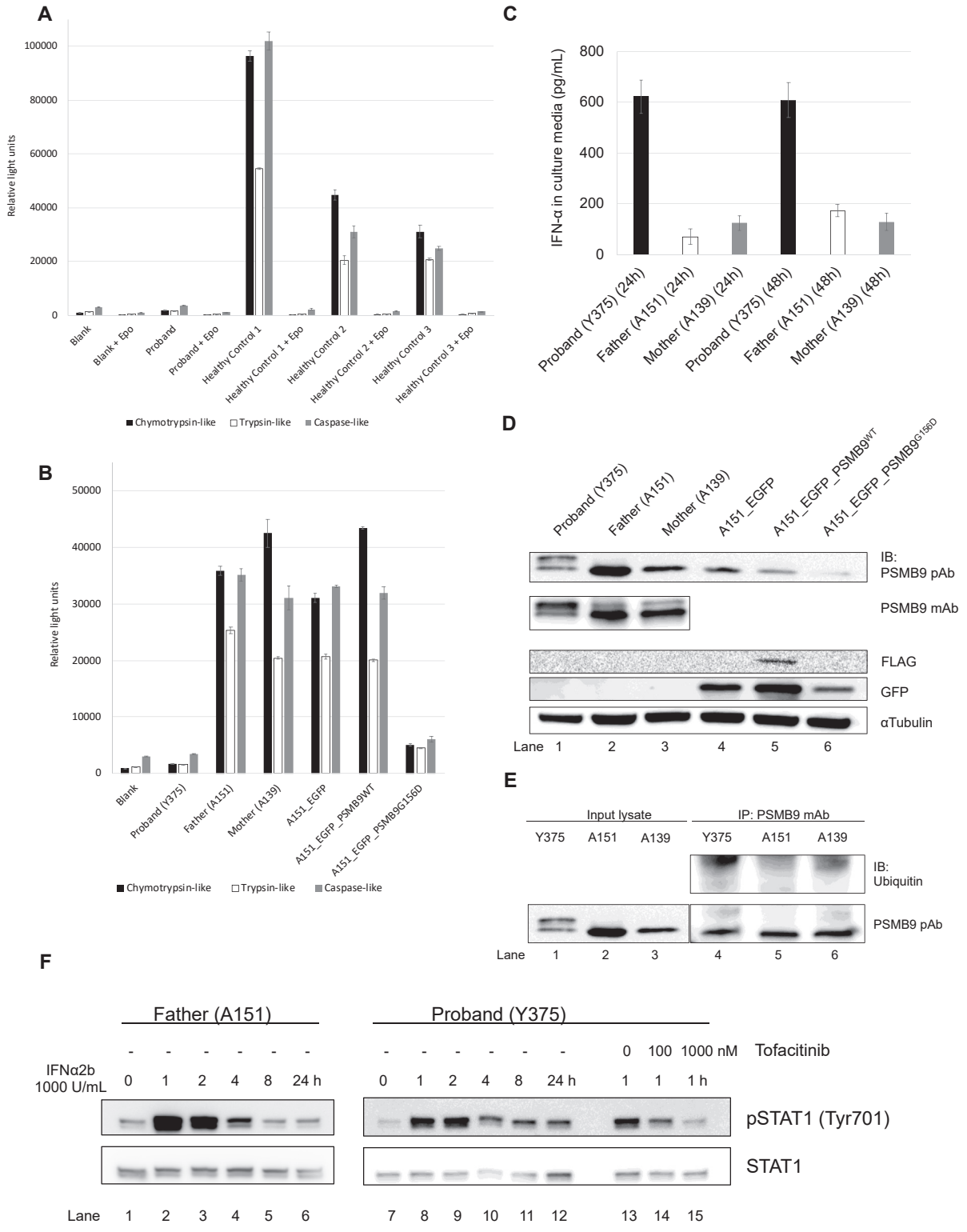

FIG 2. Functional analysis of *PSMB9* G156D mutation. **A,** Proteasome activity in proband-derived LCLs (Y375). Black bar, white bar, and gray bar indicate chymotrypsin-like, trypsin-like, and caspase-like activities, respectively. The addition of epoxomicin results in the suppression of the proteasome activities in control LCLs established from 3 healthy volunteers. **B,** The proteasome activity in LCLs from the proband (Y375), mother (A139), father (A151), A151 transduced with mutant *PSMB9* (A151_EGFP_PSMB9^{G156D}), with WT *PSMB9* (A151_EGFP_PSMB9^{WT}), and with the empty vector (A151_EGFP). **C,** The IFN-α levels in LCLs cultured media after 24-hour and 48-hour culture. The black, white, and gray bars indicate proband-derived LCLs, father-derived LCLs, and mother-derived LCLs, respectively. **D,** Immunoblot probed with polyclonal and monoclonal anti-PSMB9, anti-FLAG, and anti-GFP antibodies. **E,** Immunoprecipitation with monoclonal anti-PSMB9; antiubiquitin and monoclonal anti-PSMB9 antibodies were used for IB. **F,** Immunoblot probed with antiphosphorylated STAT1 (Tyr 701) and anti-STAT1 antibodies in proband-derived and father-derived LCLs after exogenous IFN-α stimulation without and with tofacitinib. *GFP*, Green fluorescent protein.

cultured media was significantly elevated in proband-derived LCLs (Y375) when compared with healthy control cells (A151 and A139) (Fig 2, *C*).

We established A151 cells with lentivirus-mediated overexpression of FLAG-tagged mutant PSMB9 (A151_EGFP_PSMB9^{G156D}) and FLAG-tagged WT PSMB9 (A151_EGFP_PSMB9^{WT}); A151 cells transduced with empty vector (A151_EGFP) served as a control. Exogenous expression was confirmed via bicistronic expression of EGFP (data not shown). Similar levels of proteasome activity were detected in the primary A151 cells, the A151_EGFP_PSMB9^{WT} cells, and A151_EGFP cells. In contrast, the A151_EGFP_PSMB9^{G156D} cells had significantly lower proteasome activity; these results suggested that this mutation suppressed protease activity via a dominant negative mechanism (Fig 2, *B*).

Immunoblot (IB) with polyclonal and monoclonal anti-PSMB9 antibodies uncovered a 10-kDa larger protein band in lysates from the proband-derived LCLs (Y375) compared with bands identified in LCLs from the father (A151) and mother (A139) (Fig 2, *D*), which led us to hypothesize that the larger protein band is a ubiquitinated PSMB9 protein. The level of total ubiquitin-coupled proteins remained constant when comparing Y375 to those of A151 and A139. IB using an anti-ubiquitin antibody to coprecipitate proteins detected with the anti-PSMB9 antibody revealed enhanced ubiquitination in Y375 compared with A151 and A139 (Fig 2, *E*). These results suggested enhanced ubiquitination of the PSMB9 protein in the proband cells, which served to promote its degradation.

IB with the anti-FLAG antibody confirmed exogenous expression of PSMB9^{WT} protein in A151_EGFP_PSMB9^{WT} cells; in contrast, no exogenous PSMB9^{G156D} expression was detected in A151_EGFP_PSMB9^{G156D} cells (Fig 2, *D*). Furthermore, IB using anti-PSMB9 antibody revealed decreased levels of endogenous PSMB9^{WT} protein expression in A151_EGFP_PSMB9^{G156D} cells compared with A151_EGFP_PSMB9^{WT} cells and A151_EGFP cells. IB for other subunits of immunoproteasome with anti-PSMB8 and anti-PSMB10 antibodies revealed that both PSMB8 and PSMB10 were downregulated in proband-derived LCLs (Y375) and father-derived LCLs with mutant PSMB9 G156D (A151_EGFP_PSMB9^{G156D}) (see Fig E1 in this article's Online Repository at www.jacionline.org). These results suggest that the mechanism by which mutant PSMB9 p.G156D protein impairs immunoproteasome function is due to the isolated role of PSMB9 and its impact on other elements of the immunoproteasome, such as PSMB8 and PSMB10. However, whether other PSMB9 amino acid substitution mutations could have similar or different physiological effects needs to be clarified in the future studies.

To investigate the relationship between IFN-α elevation and the JAK-signal transducer and activator of transcription 1 (STAT1) pathway in his disease, we evaluated the phosphorylation of STAT1 in LCLs. We found that endogenous STAT1 was constitutively phosphorylated in both proband-derived LCLs (Y375) and healthy control LCLs (A151 and A139), although the phosphorylation was weak (see Fig E2 in this article's Online Repository at www.jacionline.org). Therefore, we next measured the phosphorylation levels of STAT1 in the presence of exogenous IFN-α. The phosphorylation levels of STAT1 in healthy control (A151) decreased to the basal levels after 8 hours of IFN-α stimulation, whereas Y375 maintained high phosphorylation levels even 24 hours after the IFN-α stimulation. Furthermore, we confirmed that this exogenous IFN-α–induced STAT1 phosphorylation in Y375 was inhibited by the addition of the JAK inhibitor tofacitinib (Fig 2, *F*).

Taken together, these results suggested that mutant PSMB9 p.G156D protein had a dominant negative effect via its capacity to promote degradation and to inhibit the function of normal PSMB9 protein. Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature syndrome, a typical type I interferonopathy, is caused by a biallelic loss-of-function mutation in the *PSMB8* gene.[7] *PSMB9* and *PSMB8* encode the catalytic subunits of the immunoproteasomes beta-1i and beta-5i, respectively. The *PSMB9* p.G156D mutation is a new proteasome-associated autoinflammatory syndrome with symptoms and molecular mechanisms that are similar to those described previously.

In vitro experiments using patient-derived primary cells[4] together with anecdotal case reports suggested that JAK inhibitors, such as tofacitinib[5] and ruxolutinib,[6] may be promising drugs to be used to treat type I interferonopathies. In our patient case, administration of tofacitinib resulted in complete resolution of clinical symptoms, including normalization of life-threatening pulmonary hypertension and elevated serum levels of CPK. Following bridging therapy with the JAK inhibitor, our patient underwent cord blood transplantation and achieved long-term survival; we were able to discontinue tofacitinib without flare-ups of symptoms associated with interferonopathy.

In conclusion, we present here the case of a patient with a novel type I interferonopathy caused by a *de novo PSMB9* p.G156D mutation with dominant negative effect. He was successfully treated with tofacitinib followed by allogeneic cord blood stem cell transplantation. These results suggest the use of JAK inhibitor and stem cell transplantation as curative therapies in patients with severe interferonopathies.

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Clinical implications: A *de novo PSMB9* mutation causes novel type I interferonopathy. The use of a JAK inhibitor along with stem cell transplantation may treat the severe form of type I interferonopathies.

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