

Letter to the Editor

Gain-of-function signal transducer and activator of transcription 1 (STAT1) mutation-related primary immunodeficiency is associated with disseminated mucormycosis*To the Editor:*

Signal transducer and activator of transcription 1 (STAT1) belongs to a family of transcription factors that mediate growth factor and cytokine signaling. Complete recessive mutations in *STAT1* cause susceptibility to viral, mycobacterial, and bacterial infections and are fatal early in life. In contrast, heterozygous dominant negative mutations in *STAT1* cause mild disseminated mycobacterial infections.¹ Recently, heterozygous dominant gain-of-function (GOF) mutations in *STAT1* were described as causing impaired STAT1 dephosphorylation, diminished IL-17-producing T-cell numbers, and chronic mucocutaneous candidiasis (CMC).² These GOF mutations have also been associated with intracellular dimorphic fungal infections, disseminated coccidioidomycosis, histoplasmosis,³ and cases of wild-type forkhead box protein 3 (*FOXP3*) immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX)-like syndrome.⁴ Progressive multifocal leukoencephalopathy, autoimmunity,

arterial aneurysms, and squamous cell cancers have also been reported.²⁻⁵ We identified a novel GOF *STAT1* mutation in a patient with disseminated mucormycosis caused by *Apophysomyces trapeziformis*. Previous reports of *A. trapeziformis* infection were associated with penetrating trauma in immunocompetent hosts.⁶ This is the first description of a primary immunodeficiency linked to disseminated mucormycosis.

A 24-year-old man of mixed African and Asian descent presented with 3 weeks of fever (104°F), myalgia, and night sweats, 2 weeks of headache associated with right-sided facial numbness and swelling, pleuritic chest pain, and shortness of breath. Tender, enlarged, mobile bilateral inguinal and axillary lymphadenopathy was present along with discrete subcutaneous nodules in the right deltoid, right scapula, and right paraspinal muscles. Facial swelling and numbness were associated with weakness of the right muscles of mastication. He had bibasilar crackles and a triphasic pericardial friction rub without murmurs. The WBC count was $27 \times 10^9/L$ with 28% eosinophils, and the troponin I level was 2.4 ng/mL. Chest computed tomography showed a 2-cm nodule in the right lower lobe (Fig 1, A, upper panel) and lesions at the right shoulder, supraspinatus (Fig 1, A, middle panel), and deltoid (Fig 1, A, lower panel). Magnetic resonance imaging of the brain detected a 1.7×1.1 -cm

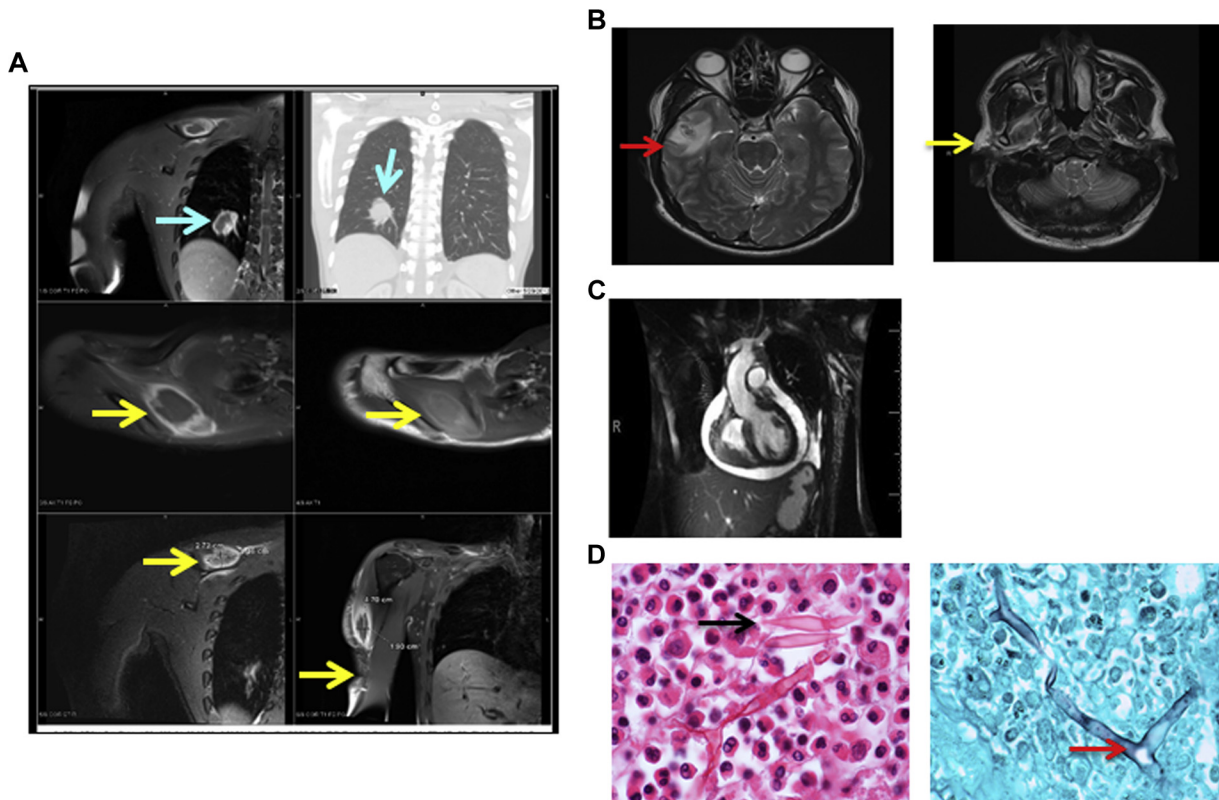


FIG 1. **A,** Chest magnetic resonance imaging. Lesions are visible in the right lung (top panels), right supraspinatus (arrows, middle panels), and deltoid (lower panels). **B,** Brain magnetic resonance imaging T2W images: thick-walled right temporal lobe lesion (left) and enhancement of the right muscles of mastication (right). **C,** Cardiac magnetic resonance imaging: pericardial effusion with mild right ventricular collapse. **D,** Hematoxylin and eosin inguinal lymph node biopsy (left) with intense eosinophilic infiltrate and Charcot-Leyden crystals (arrow). Silver stain of the deltoid muscle is shown at right. Aseptate fungal elements with 90° branching hyphae were observed (arrow).

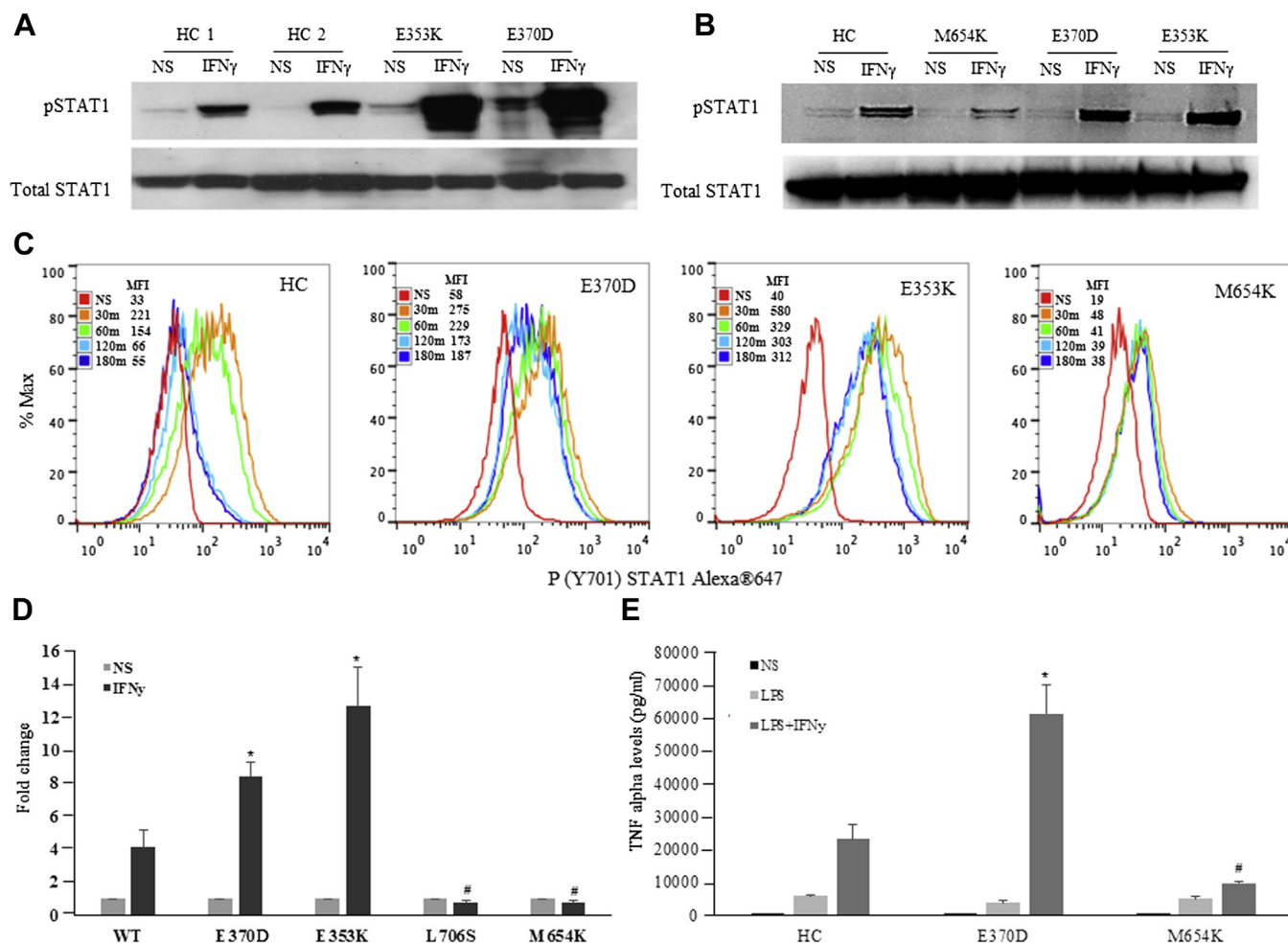


FIG 2. A and B, Immunoblotting (STAT1 phosphorylation [pSTAT1]) of PBMCs (Fig 2, A) and EBV-B cells (Fig 2, B) from patients (E370, current patient; E353K, control subject with GOF mutation; M654K, control subject with LOF mutation) and healthy control subjects (HC) were stimulated or not (NS) with IFN- γ . C, pSTAT1 dephosphorylation (30-180 minutes) in EBV-B cells determined by means of flow cytometry. MFI, Mean fluorescence intensity. D, Luciferase activity on U3A cells transfected with wild-type or mutant STAT1 (E370D, E353K, L706S, and M654K-LOF controls). Data are means $\pm$ SEMs (n = 3). E, Evaluation of TNF- α production (in picograms per milliliter) using the bead cytokine assay from PBMCs of patients and healthy control subjects (n = 4) in response to LPS and IFN- γ . *, #P < .05 versus wild-type.

thick-walled, rim-enhancing lesion in the anterior temporal lobe and enhancement of the right masticatory muscles (Fig 1, B). Cardiac magnetic resonance imaging showed myopericarditis with pericardial effusion and tamponade (Fig 1, C). Biopsy of the deltoid muscle and lymph node showed an eosinophilic infiltrate surrounding aseptate fungal hyphae with right-angle branching (Fig 1, D), which is consistent with mucormycosis.⁷ Cultures from the right deltoid muscle biopsy specimen and blood grew *A. trapeziformis*, which was identified by using colony and microscopic morphology and confirmed by sequencing. The isolate was susceptible to amphotericin B, itraconazole, and posaconazole. Results of serum galactomannan, urine histoplasma antigen, serum cryptococcal antigen, serum *Coccidioides* species antigen, *Trichinella* species and neurocysticercosis serologies, mycobacterial staining, and culture from multiple sites of infection were all negative.

The patient was from northern Florida, had not travelled outside the southern United States, and had no history of previous infections, sick contacts, penetrating trauma, or CMC. Other

conditions predisposing to mucormycosis, such as diabetes and hemochromatosis, were excluded. The patient was formerly healthy, with the exception of episodes of "prostatitis"; no further records were available. The family history was not significant.

The patient was treated with liposomal amphotericin B (7.5 mg/kg/d) and posaconazole. Blood cultures cleared in 36 hours after initiation of treatment. However, his old lesions continued to worsen, and he had new ones. Liposomal amphotericin was increased to 10 mg/kg, and he was started on micafungin (150 mg/d).⁶ After 2 weeks, subcutaneous IFN- γ (50 μ g/m²) administered 3 times weekly was added to augment the cellular immune response. He received this combination for 8 weeks, followed by oral posaconazole. Follow-up imaging studies showed a progressive decrease and disappearance of lesions, along with improvement in symptoms. The patient was discharged on long-term therapy with posaconazole. He has been well at 3, 6, and 12 months of follow-up.

The identification of a disseminated fungal infection in an otherwise healthy patient, let alone an unusual mold, initiated a

search for an immune predisposition. No identified genetic predispositions to disseminated *Mucorales* infections are known. The recent description of several cases of fatal *A trapeziformis* associated with trauma and diabetes did not apply in this case.^{6,8} Mutations in caspase-associated recruitment domain 9 were not found, and reduced nicotinamide adenine dinucleotide phosphate oxidase activity was normal, excluding chronic granulomatous disease. The association of cases of disseminated fungal infections in patients with GOF mutations in *STAT1*, including some with adult onset, prompted us to sequence *STAT1*. Full-length sequencing of *STAT1* genomic DNA and cDNA identified the novel heterozygous mutation c.1110G>C; p.E370D, which resides in the DNA-binding domain and is predicted to be deleterious. The mutation was not found in dbSNP 138 or the 1000 Genomes Project. The patient's parents were not available for mutation screening.

To investigate its function, PBMCs and transformed EBV-B cells from normal donors, the patient with a E370D mutation, a patient with a GOF *STAT1* mutation (E353K),³ and a patient with a loss-of-function (LOF) mutation (M654K) were stimulated with IFN- γ (400 IU/mL for 30 minutes). *STAT1* phosphorylation was examined by using immunoblotting and flow cytometry. *STAT1* phosphorylation was increased in cells from the patient with a GOF mutation (Fig 2, A and B) with characteristic delay in *STAT1* dephosphorylation (Fig 2, C) and was reduced in cells from the patient with an LOF mutation (Fig 2, A-C).

Transcriptional activity of the mutants was evaluated in the *STAT1*-deficient U3A cell line based on luciferase activity of a reporter gene under the control of the gamma-activating sequences promoter. Transfection of the *STAT1* E370D construct into the U3A cells led to enhanced IFN- γ stimulation compared with the wild-type gene (Fig 2, D), which is similar to the E353K GOF mutant and strikingly different from the dominant negative *STAT1* mutants L706S and M654K. Consistent with these observations, expression of IFN- γ -induced chemokine genes (*CXCL9* and *CXCL10*) was enhanced in transfected U3A cells, PBMCs, and EBV-B cells (not shown). IFN- γ upregulation of LPS (200 ng/mL)-induced TNF- α (18-hour cultures) was greater in patients' PBMCs (16-fold induction) versus that seen in normal (4-fold) or LOF cells (Fig 2, E). When PBMCs were stimulated with phorbol 12-myristate 13-acetate/ionomycin, IL-17-producing T cells were mildly reduced: E370D mutant cells, 1.07%; E353K mutant cells, 1.27%; and control cells, $2.0 \pm 0.87\%$. Overall, these laboratory findings are characteristic of a dominant GOF *STAT1* mutation leading to hyperresponsiveness to IFN- γ .

A trapeziformis is a recently described cause of mucormycosis, which rarely infects human subjects. It causes environmentally acquired infections predominantly in the immunocompromised host.⁸ Infections with *Apophysomyces* species have been identified in traumatically inoculated immunocompetent hosts and might also result from inhalation of spores into the sinuses.^{8,9} In 2011, 15 cases of *A trapeziformis* infection were identified in patients injured in an Enhanced Fujita Scale 5 tornado in Joplin, Missouri.⁶ Treatment descriptions are limited to case reports, including extensive tissue damage and debridement and use of liposomal amphotericin B in combination with posaconazole or micafungin.⁶

This novel GOF mutation in *STAT1* in a patient with disseminated *A trapeziformis* infection who had never had CMC or

autoimmunity represents the first genetic predisposition to *A trapeziformis*. *STAT1* GOF mutations have not previously been associated with filamentous molds, let alone members of the *Mucorales* order.

The finding of disseminated *A trapeziformis* occurring in a patient with a *STAT1* GOF mutation without associated trauma or any other predisposing clinical conditions suggests that GOF *STAT1* mutations can also underlie disseminated mucormycosis.

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