A novel NEMO/*IKBKG* mutation identified in a primary immunodeficiency disorder with recurrent atypical mycobacterial infections

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INTRODUCTION

Hypomorphic mutations in the *IKBKG* gene, which encodes nuclear factor-κB (NF-κB) essential modulator (NEMO), on the X chromosome, can lead to the ectodermal dysplasia and immunodeficiency (EDA-ID) syndrome.[1] The clinical presentation of EDA-ID is protean depending on factors, including penetrance and the impact of specific NEMO mutations.[2] In some cases, patients may present with primary immunodeficiency disorders (PID) without evidence of ectodermal dysplasia.[1] Here, we report a novel mutation of *IKBKG* (NEMO) at Y308 in a middle-aged man, identified due to recurrent mycobacterial infections without evidence of ectodermal dysplasia.

Abbreviations used:

EDA-ID:	ectodermal dysplasia and immunodeficiency
NEMO:	nuclear factor-κB (NF-κB) essential modulator
NF-κB:	nuclear factor-κB
PID:	primary immunodeficiency disorder

CASE REPORT

A 38-year-old Caucasian man reported a history of recurrent sinus and otitis media infections, multiple staphylococcal skin infections, and pneumonia since childhood. He never had problems with hair development, perspiration, or problems with his skin. The patient first noted a rash in approximately 2002, which appeared as papules and plaques on his trunk, limbs, and face. Physical examination demonstrated the absence of thickened skin, abnormal nails, or sparse hair. He was treated empirically for psoriasis, atopic dermatitis, and tinea infection, without complete resolution of the rash. In 2008, the patient was referred to a dermatology clinic, where biopsies of the arm and back revealed sarcoidal granulomatous dermatitis and were negative for infectious organisms by PAS and Fite stains. The patient was tentatively diagnosed with cutaneous sarcoidosis, treated with topical steroids and a prednisone taper, and lost to follow-up. In 2010, the patient presented with widespread red-brown papules and plaques involving most of his body and worsening numbness of his hands and feet. Biopsies of the lesions on the thigh and forearms revealed granulomas and lymphohistiocytic infiltrates with prominent perineural involvement. The Fite stain revealed numerous acid-fast bacilli, consistent with the clinical diagnosis of lepromatous leprosy. *Mycobacterium leprae* was confirmed in the biopsy by molecular testing. The patient denied exposure to infected individuals or animals. Treatment with dapsone, rifampin, clofazimine, and prednisone improved most lesions, but a distinctive 8 × 5 cm red-brown plaque with regions

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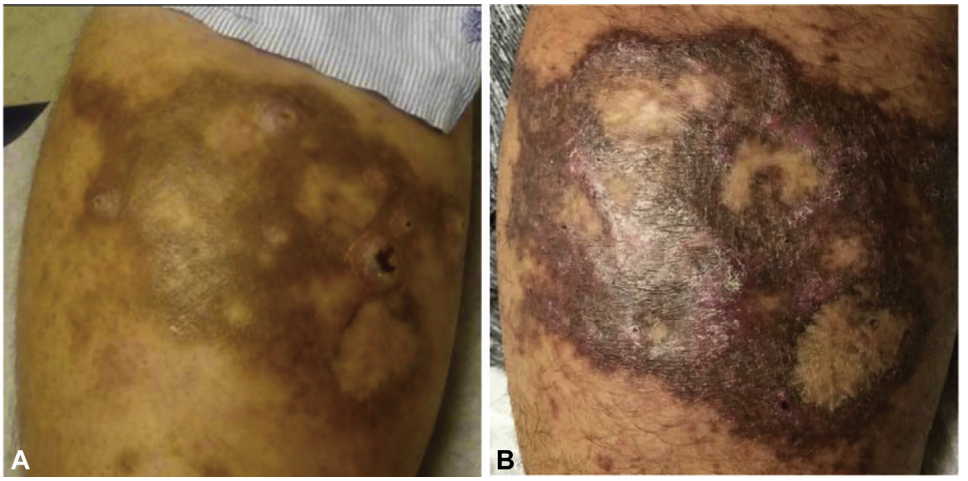

Fig 1. A photograph of a lesion on the posterior aspect of the left calf, which grew *M fortuitum* in 2013 prior to treatment (**A**) and after recurrence in 2016 (**B**).

of hypopigmentation, hyperpigmentation, and scales on the posterior aspect of his left calf persisted (Fig 1, *A*). A biopsy and Fite stain of this lesion revealed abundant acid-fast bacilli, with a culture showing *Mycobacterium haemophilum*. Azithromycin was added to his existing lepromatous leprosy regimen, which was continued for 24 months. After completing therapy, the rash on the posterior aspect of his left calf began to recur (Fig 1, *B*), and many new skin lesions developed, including a red-brown papule with a keratotic plug on his left forearm. Biopsy with Fite stain of the lesion on the left calf revealed yet another mycobacterial infection (Fig 2, *A* and *B*), with cultures now revealing *Mycobacterium fortuitum* on both upper and lower extremities. Immunology completed a workup for primary immunodeficiency, which showed normal immunoglobulin levels, no abnormalities in CD8/CD4 cell counts, normal lymphocyte responses to mitogen and *Candida*, normal IFN-γ level, and an HIV-negative status. After reporting respiratory symptoms in 2016, a chest CT revealed granulomatous changes in the lungs, suggesting an infectious etiology. Sputum studies revealed both *Mycobacterium avium* complex and *M fortuitum*. He was admitted to the NIH clinical center, where he underwent CT scans (abdomen/pelvis/femur/tibia/fibula) and comprehensive dental, skin, and CNS evaluations, which revealed no abnormalities. Whole-exome sequencing did not identify any mutations. However, RNA sequencing identified a hemizygous mis-sense change in NEMO/*IKBKG* exon 8, c.923A>G, causing p.Y308C. While mutations at this site have not been previously reported in patients, the tyrosine residue at this site is evolutionarily conserved, and the presence of a hydrophobic residue at tyrosine 308 has been demonstrated to be essential to polyubiquitin binding and NF-κB activation in independent studies.[3,4]

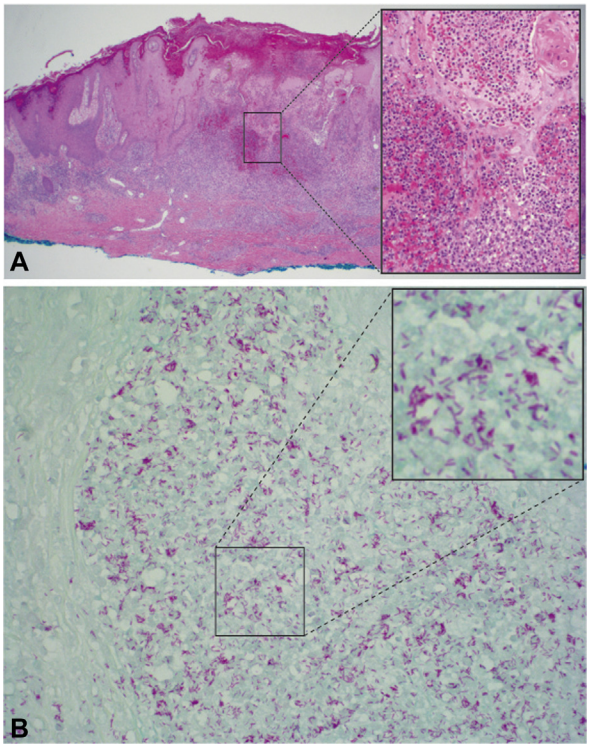

Fig 2. A, A hematoxylin-eosin–stained section of the biopsy from a plaque on the posterior aspect of the left calf. There was pseudoepitheliomatous hyperplasia and an intraepidermal abscess. The inset shows a mixed infiltrate in the dermis, consisting of neutrophils, histiocytes, lymphocytes, and plasma cells. **B**, Fite–stained section of the biopsy from the left calf. The inset highlights numerous rod-shaped and filamentous acid-fast bacilli.

DISCUSSION

This case describes a previously unreported NEMO mutation in exon 8, causing PID without ectodermal dysplasia. Proper NF-κB functioning is necessary for skin development through TNF/TNFr superfamily signaling (ectodysplasin A) and immune function through roles in CD40, TNFR, IL-1R, and Toll-like receptor signaling.[1] NEMO plays a regulatory role in the IκB kinase protein complex, which is required for the activation of NF-κB, a critical transcriptional regulator.[2] Deletions in the NEMO-coding gene lead to fetal death in males and incontinentia pigmenti in females, while hypomorphic mutations of NEMO in males typically lead to EDA-ID.[1] EDA-ID manifests with ectodermal dysplasia in the form of brittle nails, hypotrichosis, hypohidrosis, conical teeth, and hypodontia. Susceptibility to atypical mycobacterial infections is a well-established clinical manifestation of defects in the NEMO protein.[5] Disseminated *M avium* complex infections, without any stigmata of ectodermal dysplasia, have been reported in patients with reduced levels of NEMO protein caused by splice-site mutations in *IKBKG*.[5-7] Infections by multiple distinct atypical mycobacteria have been reported in patients without evidence of ectodermal dysplasia[5] but are more commonly seen in patients with evidence of ectodermal dysplasia.[8] The absence of ectodermal dysplasia in this patient could be due to the "hemizygous" presence of the mutation or because the Y308C mutation results in selective, rather than global, defects in NF-κB signaling, as has been reported for other mutations in this domain of the NEMO protein.[8] Notably, *M leprae* infection has yet to be reported in patients with *IKBKG* gene variants.[5] This case emphasizes the need to consider primary immunodeficiencies, including *IKBKG* mutations, in the differential diagnosis of adults with recurrent atypical mycobacterial infections.

Identifying NEMO mutations can be challenging because exons 3-10 of the *IKBKG* gene encoding NEMO are duplicated in the *IKBKG1* pseudogene.[1,9] The *IKBKG1* pseudogene can not only mask genuine mutations in PID patients but also be inaccurately reported as NEMO mutations in unaffected individuals.[9] Long-range or site-specific PCR, combined with either Sanger or whole-exome sequencing, have been reported as solutions for this diagnostic challenge.[1,9] RNA sequencing is an alternative approach to identify mutations in patients with PID. RNA sequencing has an additional advantage over currently described methods as it does not require the additional customized amplification steps required by DNA sequencing techniques.

This patient is overwhelmed by the mycobacterial infections, and it will be important to continue his prophylactic antibiotic therapy. Hematopoietic stem cell transplantation has been performed for hypomorphic NEMO mutations, with variable outcomes. It has been shown to be an effective modality for the correction of PIDs but is usually reserved for severe cases.[10] Other recommendations have included abstaining from tattoos, good hygiene, and regular follow-up, which would help with the early identification of any infectious diseases until better treatments for NEMO-mediated primary immunodeficiencies are identified.

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