
Congenital alterations of NEMO glutamic acid 223 result in hypohidrotic ectodermal dysplasia and immunodeficiency with normal serum IgG levels

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Background: Hypomorphic mutations in the nuclear factor- κ B (NF- κ B) essential modulator (*NEMO*) gene result in a variable syndrome of somatic and immunologic abnormalities. Clinically relevant genotype-phenotype associations are essential to understanding this complex disease.

Objective: To study 2 unrelated boys with novel *NEMO* mutations altering codon 223 for similarity in phenotype in consideration of potential genotype-phenotype associations.

Methods: Clinical and laboratory features, including cell counts, immunoglobulin quantity and quality, natural killer cell cytotoxicity, and Toll-like and tumor necrosis factor receptor signaling, were evaluated. Because both mutations affected *NEMO* codon 223 and were novel, consideration was given to new potential genotype-phenotype associations.

Results: Both patients were diagnosed as having hypohidrotic ectodermal dysplasia and had severe or recurrent infections. One had recurrent sinopulmonary infections and the other necrotizing soft tissue methicillin-resistant *Staphylococcus aureus* infection and *Streptococcus anginosus* subdural empyema with bacteremia. *NEMO* gene sequence demonstrated a 3-nucleotide deletion (c.667_669delGAG) in one patient and a substitution (667G>A) in the other. These findings predict either the deletion of *NEMO* glutamic acid 223 or it being replaced with lysine, respectively. Both patients had normal serum IgG levels but poor specific antibodies. Natural killer cell cytotoxicity and Toll-like and tumor necrosis factor receptor signaling were also impaired. Serious bacterial infection did not occur in both patients after immunoglobulin replacement therapy.

Conclusions: Two different novel mutations affecting *NEMO* glutamic acid 223 resulted in clinically relevant similar phenotypes, providing further evidence to support genotype-phenotype correlations in this disease. They suggest *NEMO* residue 223 is required for ectodermal development and immunity and is apparently dispensable for quantitative IgG production but may be required for specific antibody production.

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INTRODUCTION

Ectodermal dysplasia with anhidrosis (EDA) is a group of related syndromes resulting from aberrant development of ectodermal tissues. It is characterized by fine sparse hair,

conical or missing teeth, dysplastic nails, periorbital skin wrinkling, and a lack of eccrine sweat glands.¹ A variety of forms of EDA exist with known genetic causes. Isolated EDA is due to genetic mutations affecting the tumor necrosis factor (TNF) superfamily member ectodysplasin A, its receptor, or the EDA receptor-associated death domain containing adaptor protein. A related syndrome is associated with impaired immunity resulting in severe or recurrent infections and is known as EDA with immune deficiency (EDA-ID) or more recently nuclear factor- κ B (NF- κ B) essential modulator (*NEMO*) deficiency with immune deficiency (*NEMO*-ID) or *NEMO* syndrome to reflect the occasional absence of an accompanying ectodermal defect.² Genetic lesions in this form affect the *IKBKG* gene, which encodes *NEMO* and is also referred to as the *NEMO* gene. The EDA-ID syndrome can also and much more infrequently result from mutation of *NFKBIA*, which encodes $I\kappa B\alpha$, the inhibitor of NF- κ B.¹

NEMO is a 419-amino acid regulatory protein encoded by 10 exons on the X chromosome.³ *NEMO* is the catalytically inactive subunit of the inhibitor of the NF- κ B kinase (IKK) complex, which is required for canonical pathway activation

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of the NF- κ B family of transcription factors. NF- κ B is found in most cells and functions to regulate the transcription of genes involved in development, immunity, inflammation, cell growth, and programmed death.⁴ NF- κ B is typically retained in the cytoplasm of cells bound to I κ B but can translocate to the nucleus to promote transcription after I κ B is phosphorylated. In the absence of NEMO, the IKK complex is inactive and hence unable to phosphorylate I κ B α , thus blocking NF- κ B activation because it is unable to become free of its inhibitor to translocate into the nucleus. Mutations that partially disrupt NEMO activity (hypomorphic mutations) result in impaired NF- κ B activation, leading to both humoral and cellular immune abnormalities in an X-linked recessive pattern, because under normal circumstances a single functional copy in mothers is sufficient to enable IKK activity. Some NEMO function, however, is a prerequisite for in utero development, and thereby males inheriting a *NEMO* gene that completely interrupts NEMO function are not viable. Thus, all known *NEMO* gene mutations resulting in NEMO-ID are hypomorphic.

The clinical and immunologic phenotypes attributed to NEMO hypomorphism were recently reviewed.² Despite clinical variability of disease expression, which is inherent in human genetic disease due to genomic and environmental variability, patterns are emerging in which specific phenotypes are suggestive of specific mutations. For example, among the mutations, which have appeared in more than 1 individual, the classic hyper-IgM syndrome phenotype (low immunoglobulin production, normal or elevated IgM level, and a deficiency in specific IgG production, class switching, and B-cell activation) is seen primarily in individuals with mutations at cysteine 417. In contrast, mutation in the NOA/UBAN/NUB domain (residues 289–320) in 100% (6/6) of cases leads to mycobacterial susceptibility compared with 37% (9/24) with mutations outside this region. Furthermore, 100% (6/6) have normal immunoglobulin levels and production of specific antibodies compared with only 12% (15/17) that have mutations outside this region. Comparison of these 2 genotypic patterns suggests clinically relevant phenotypic associations and raises the importance of further work in this disease with regard to genotypes and phenotypes. Irrespective, we have proposed that detailed phenotypic characterization will lead to improved prognostic information and patient management.²

We describe 2 unrelated boys with novel *NEMO* mutations within exon 5, which result in predicted amino acid changes at glutamic acid 223. Both had ectodermal dysplasia characteristics, similar infectious susceptibilities, and similar immune abnormalities with normal total IgG levels. We studied their clinical and immunologic characteristics, including endogenous antibody production and, ex vivo, TNF and Toll-like receptor (TLR) signaling. Because mutations affecting this specific residue within NEMO have not been previously described, further consideration was given to a potential genotype-phenotype association.

METHODS

Parental Consent

Investigations of the patients were performed under an institution review board–approved protocol of the Children’s Hospital of Philadelphia. Parents of both patients provided written informed consent for this research.

Immunologic Assays

Serum immunoglobulin concentrations were determined by nephelometry and compared with age-related normal values. The number and percentage of lymphocytes in various subsets were compared with age-related normal values. Mitogen-induced proliferation was performed using phytohemagglutinin, pokeweed, and concanavalin A, and antigen-induced proliferation to tetanus, *Candida*, and diphtheria was similarly tested.

NEMO Sequence Analysis

Genomic DNA sequencing of patient 1 was performed by polymerase chain reaction amplification, with sequencing of the entire coding region and long-range polymerase chain reaction of the *NEMO* pseudogene by Correlagen Laboratory (Waltham, Massachusetts). Genomic DNA analysis of patient 2 was performed in the Clinical Immunology Unit, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.

TLR Function Assay

TLR function assay was performed as previously described.⁵ Briefly, mononuclear cells isolated from whole blood were incubated with media or ligands to TLR-1/2, -2/6, -3, -4, -5, and -7/8 with subsequent measurement of TNF- α , interleukin (IL) 1 β , and IL-6 in the supernatant by the Luminex multiplex assay using cytokine detection kits (Fluorokine; R&D Systems Inc, Minneapolis, Minnesota; or Miliplex; Millipore, Billerica, Massachusetts).

Natural Killer Cell Studies

Natural killer (NK) cell cytotoxicity was evaluated on the basis of ⁵¹Cr release from radiolabeled K562 erythroleukemia cells as described previously.⁶ Briefly, K562 cells were labeled by incubation with ⁵¹Cr (Na₂CrO₄) (PerkinElmer Inc, Waltham, Massachusetts) at 37°C. Labeled target cells were mixed with NK cells at specified effector to target cell ratios and incubated for 4 hours at 37°C. ⁵¹Cr released into supernatants was measured, and the percentage of lysis was determined. Cytotoxicity was reported as LU₂₀ NK, which is defined as the number of effector cells (expressed in 10⁷) required to achieve 20% lysis of target cells per 10 million NK cells.

TNF- α -Induced I κ B α Degradation

Peripheral blood mononuclear cells (PBMCs) from the patient and a healthy control were isolated using Ficoll-Hypaque lymphocyte separation media. A total of 5 $\times$ 10⁵ cells per condition were stimulated with TNF- α and lysed after 0, 10, and 60 minutes. Cells were then heated in sodium

dodecyl sulfate lysis buffer after the addition of protease inhibitor cocktail as previously described.² After denaturing protein electrophoresis of lysates, protein was detected with rabbit polyclonal anti-I κ B α . To demonstrate equal loading of samples, the membrane was stripped and reprobed for total actin using anti-myosin-IIB antibody. Quantitation of I κ B α and actin for each lane was performed by densitometric analysis using ImageJ software (freeware; National Institutes of Health, Bethesda, Maryland). Ratios of I κ B α band intensity were calculated and depicted graphically in adjacent histograms to facilitate interpretation of the results.

RESULTS

Patient 1

Patient 1 is a 5-year-old boy born to unrelated white parents. He had recurrent sinopulmonary infections beginning before 1 year of age and hospitalization on 2 occasions due to bacterial pneumonia. He had mildly conical shaped teeth and hypohidrosis, consistent with ectodermal dysplasia (Fig 1). An immunologic evaluation at 4 years of age revealed normal IgG (1,035 mg/dL; reference range, 423-1184 mg/dL), low IgM (37 mg/dL; reference range, 48-168 mg/dL), and ele-

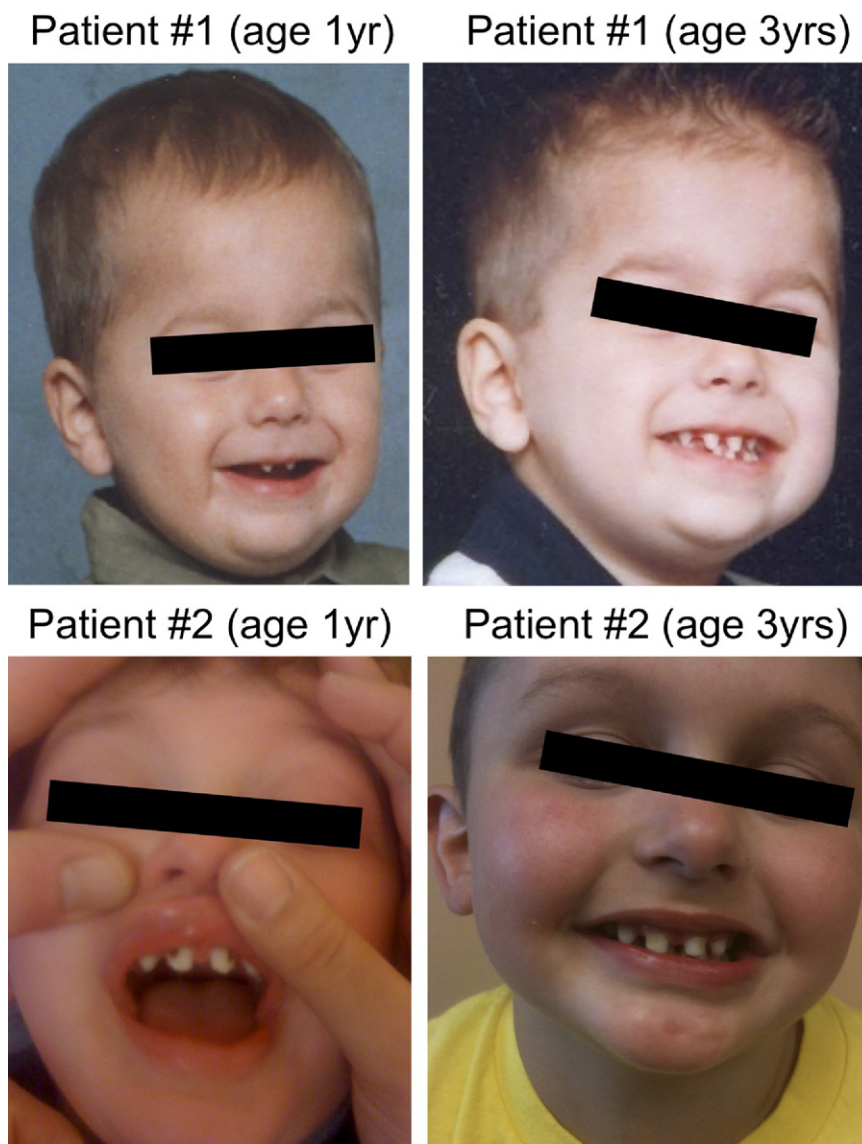


Figure 1. Facial features of nuclear factor- κ B essential modulator (NEMO) syndrome in the 2 patients under consideration. Two images from patient 1 (*top*) and patient 2 (*bottom*) are shown, with the first taken at 1 year of age (*left*) and the second at 3 years of age (*right*). The patients demonstrate mild ectodermal dysplasia with frontal bossing and sparse, hypodontia, and somewhat conical teeth. The caries evident in incisors of patient 2 at 1 year of age are not uncommon in ectodermal dysplasia and may represent a feature of the abnormal dentition.

vated IgA (169 mg/dL; reference range, 24-121 mg/dL) levels. IgG subclass levels were within normal limits. Serum antibody titers to diphtheria toxoid were low normal, and antibodies against *Streptococcus pneumoniae* were protective to only 1 of the 11 serotypes longitudinally tested after 4 doses of the 7-valent pneumococcal conjugate vaccine (Prevnar). He had a normal distribution of lymphocyte subsets, and in vitro lymphocyte proliferation in response to both mitogens and antigens was within normal limits. Subsequent to this testing, he was vaccinated with the pneumococcal polysaccharide vaccine (Pneumovax), and when tested 6 weeks later, titers against 4 of these 11 previously tested serotypes had risen into protective ranges (Table 1). TLR ligand-induced cytokine production was decreased in response to all agonists tested. NK cell cytotoxicity was reduced (Table 1).

Because of the combination of infectious disease history, ectodermal features, and functional studies, we sought molecular genetic analysis of the *NEMO* gene. Sequencing revealed a novel 3 nucleotide deletion, c.667_669delGAG (Fig 2), predicting the deletion of glutamic acid at position 223 (Δ 223). The patient's mother and 1 sister were found to be carriers of this mutation. Because *NEMO* hypomorphic mutations have been shown to have differential effects on NF- κ B activation in response to stimulation with different immune receptors,² we sought to further characterize the immune signaling phenotype in this individual. To determine the integrity of the signaling pathway downstream of the TNF receptor (TNFR), primary mononuclear cells were isolated from the peripheral blood and left in media or stimulated with TNF- α for 10 and 60 minutes. After this treatment, lysates were derived and separated by gel electrophoresis, and Western blot analysis was performed to measure I κ B α degradation at different time points (Fig 3). Stimulation of control donor PBMCs with TNF- α for 10 minutes resulted in reduction of I κ B α levels (lane 5), which was normalized to myosin-IIB levels, quantitated by optical densitometry, and determined to be reduced by 80% (right). The patient's PBMCs were unable to respond to TNF stimulation as evidenced by complete lack of I κ B α degradation (lane 2).

Patient 2

Patient 2 is a 3-year-old boy born to unrelated white parents. He was treated for a necrotizing soft tissue methicillin-resistant *S aureus* infection at 16 months of age, which resolved without sequelae. At 26 months, he was hospitalized with a left temporal lobe brain abscess and temporal convexity empyema, likely secondary to seeding from 3 rapidly progressive cavities in the upper incisor teeth and a lower molar tooth and left maxillary sinusitis. During this course he also experienced *Streptococcus anginosus* bacteremia. Physical examination findings for patient 2 were notable for sparse, conical shaped teeth consistent with mild features of ectodermal dysplasia (Fig 1) and the 4 cavities (Fig 1, left), which progressed rapidly during 2 weeks until topical fluoride was applied as a temporizing measure every 2 weeks until oral

Table 1. Initial Immunologic Laboratory Values in 2 Patients With NEMO Residue 223 Alteration

Laboratory value ^a	Patient 1	Patient 2
IgG (423-1,184 mg/dL)	1,035	735
IgA (25-154 mg/dL)	169 $\uparrow$	151
IgM (48-168 mg/dL)	37 $\downarrow$	34 $\downarrow$
IgE (0-60 IU/mL)	<4	<4
Tetanus titer	<0.10	0.19
Diphtheria titer	0.02	0.16
Pneumococcal titer before vaccination	1-1.1	ND
	3-0.4	
	4-<0.6	
	6b-0.5	
	7f-0.5	
	9-0.1	
	12f-0.4	
	14-<0.5	
	18C-<0.5	
	19f-<0.4	
	23f-<0.3	
Pneumococcal titer 6 weeks after vaccination	1-0.51	ND
	3-6.43	
	4-0.37	
	6b-0.45	
	7f-0.27	
	9-0.17	
	12f-1.73	
	14-2.12	
	18C-0.41	
	19f-0.90	
	23f-1.43	
NK cell cytotoxicity (LU ₂₀ NK >317)	47.6	172.6
Abs CD3 ⁺ (2,100-6,200) ^b	3,820	4,933
Abs CD4 ⁺ (1,300-3,400)	2,490	3,603
Abs CD4/CD45RA	ND	3,486
Abs CD4/CD45RO	ND	338
Abs CD8 ⁺ (620-2,000)	1,220	1,158
Abs CD19 ⁺ (600-2,700)	660	1,998
CD19 ⁺ /CD27 ⁺ /IgD ⁺ c	ND	20.3%
CD19 ⁺ /CD27 ⁺ /IgD ⁻ c	ND	2.1%
CD19 ⁺ /IgD ⁺ /IgM ⁺ d	ND	89.9%
CD16/56 ⁺ (180-920)	720	345

Abbreviations: Abs, antibodies; LU₂₀ NK, number of effector cells (expressed in 10⁷) required to achieve 20% lysis of target cells per 10 million NK cells; ND, not done; NEMO, nuclear factor- κ B essential modulator; NK, natural killer.

^a Age-specific normal values are given in parentheses.

^b Subset counts (number of cells per microliter).

^d Expressed as percentage of total CD19⁺ cells.

health care was instituted 3.5 months later. An immunologic evaluation when the patient was 2 years of age revealed normal concentrations of IgG (735 mg/dL; reference range, 423-1,184 mg/dL) and IgA (151 mg/dL; reference range, 25-254 mg/dL) and a low IgM level (34 mg/dL; reference range, 48-168 mg/dL). Serum antibodies to tetanus and diph-

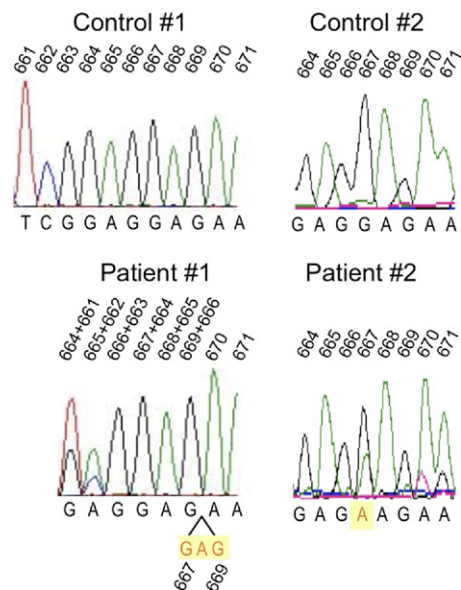


Figure 2. Genomic sequencing tracings of the individuals with the *IKBKG* mutation. The genomic DNA sequence tracings of 2 healthy controls (*top tracings*) and 2 patients with mutations predicting Δ E223 (patient 1, *bottom left*) and E223K (patient 2, *bottom right*) nuclear factor- κ B essential modulator (NEMO) protein. Genomic sequences were obtained from genomic DNA generated from peripheral blood mononuclear cells via long-range polymerase chain reaction. In patient 1 the tracing demonstrates a shift to the right so that with the nucleotides are superimposed as listed, indicative of a trinucleotide deletion c.667_669delGAG (*depicted in yellow, below*) and due to coincidence with the *NEMO* pseudogene. In patient 2, c.667G>A is indicated by the red A, which denotes the purine substitution (*highlighted with the yellow rectangle, bottom right*). The 2 peaks in the patient tracing demonstrate coincident signal from the actual and pseudogene in these original data. The numbers above the sequences denote the complementary DNA reference sequence used throughout, which corresponds to 19416-19423 in the genomic reference.

theria antigens were low normal (Table 1). Pneumococcal antibody titer levels were not evaluated before intravenous immunoglobulin therapy was begun, and so determination of specific antibody production to polysaccharide antigen was not made. The distribution of lymphocyte subsets was normal, but there were a paucity of CD27⁺/IgD⁺ switched memory B cells and an abundance of IgD⁺/IgM⁺ naive B cells (Table 1). Because of infection with *S. anginosus* and subsequent concern about the inability to generate superoxide, dihydrorhodamine 123 conversion in granulocytes was tested and the results were normal. NK cytotoxicity was abnormal (Table 1). Results of TLR testing demonstrated impaired response to all ligands evaluated. *NEMO* sequence analysis revealed that patient 2 has a missense mutation resulting in a nucleotide 667G>A substitution (Fig 2), which predicts the substitution of glutamic acid at position 223 with a lysine (E223K). TNFR signaling was evaluated as for patient 1. Control donor cells demonstrated a 55% reduction in I κ B α 10 minutes after stimulation (lane 5), as determined by densi-

tometry, normalized to the loading control (Fig 3). By contrast, E223K PBMCs demonstrated slightly less degradation of I κ B α but more importantly levels that were near baseline by 60 minutes unlike in control cells where sustained degradation was identified. This finding suggests a lack of robustness in the TNF signal in the patient but not control cells.

Taken together, hypomorphic *NEMO* mutation at amino acid 223 resulting in deletion or substitution leads to a similar phenotype. Specifically, it is associated with ectodermal dysplasia and susceptibility to infections. TLR signaling was impaired with reduced TNF-induced I κ B α degradation. E223 mutation did not result in hypogammaglobulinemia but was associated with weak specific antibody titers and impaired the ability to produce specific antibodies to polysaccharide antigen (in the 1 patient evaluated) without affecting mitogen- and antigen-induced lymphocyte proliferation.

After diagnosis of a *NEMO* mutation with functional abnormality, both patients underwent replacement immunoglobulin therapy and did not have recurrence of severe bacterial infection. Although both boys did not have preexisting evidence of mycobacterial infection, they were additionally maintained with mycobacteria prophylaxis with azithromycin and neither has had suggestions of disease caused by mycobacteria.

DISCUSSION

We describe 2 previously unreported mutations of human *NEMO* associated with immunodeficiency and ectodermal dysplasia. These mutations affect glutamic acid 223, resulting in deletion of the residue or lysine substitution. Patient 1 had recurrent sinopulmonary infections and 2 hospitalizations for suspected bacterial pneumonia. Patient 2 had *S. aureus* soft tissue infection in addition to *S. anginosus* subdural empyema and bacteremia. The organism has not previously been reported in individuals with NEMO syndrome but has been reported in individuals with AIDS and secondary immunodeficiency states.⁷ Because of the unusual features in both patients, NEMO-ID was suspected and diagnosed. Defective NK cell function was identified in our patients and is one of the most consistently observed immunologic defects in individuals with hypomorphic *NEMO* mutation, having been found in 10 of 10 different individuals with *NEMO* mutation as previously reported.² Although not specific to NEMO-ID,⁸ a deficiency of NK cell function can be a useful tool, which can be used to refine consideration of *NEMO* mutation. The infectious susceptibilities found in our patients, however, were likely the result of other immunologic impairments. We hypothesize that the infections occurred due to a combination of specific antibody deficiency and impairments in innate immunity, including TLR and TNFR signaling. We believe, therefore, that the susceptibility is likely to represent a combination of immune deficits because our patients' severity of infections was disproportionate to what might be expected in routine specific antibody deficiency.

Although mutations affecting position 223 are unprecedented for NEMO-ID, an L227P mutation, shared by 3 broth-

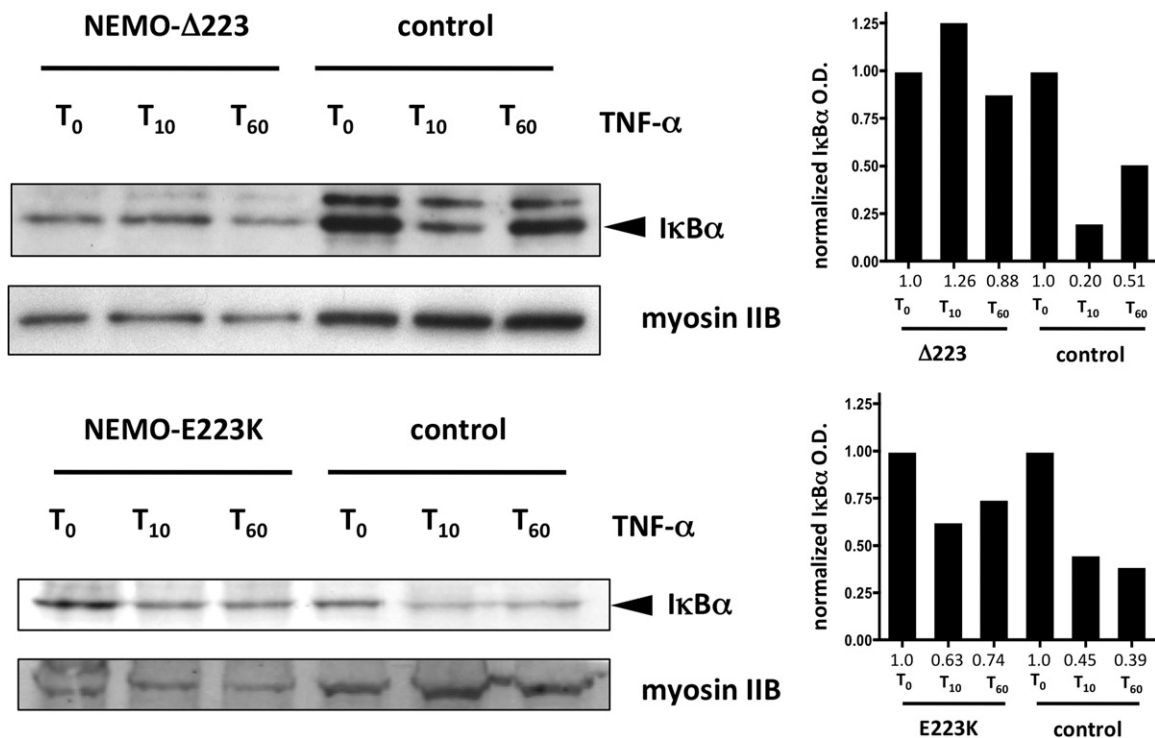


Figure 3. Tumor necrosis factor α (TNF- α)-induced I κ B α degradation time course in patient and control peripheral blood mononuclear cells (PBMCs). The PBMCs from the patients containing the mutant Δ E223 (top left) and E223K (bottom left) nuclear factor- κ B essential modulator (NEMO) or from healthy control donors (top and bottom right) were stimulated with 10 μ g/mL of human recombinant TNF for the specified times. Cell lysates were separated using polyacrylamide gel electrophoresis and then evaluated via Western blot analysis for the presence of I κ B α . To evaluate protein loading, membranes were stripped and reprobed for myosin-IIB. In the Δ E223 NEMO analysis, control I κ B bands are darker at least in part due to protein loading as demonstrated by increased myosin-IIB signal. The lower band in the I κ B Western blot analyses represents the I κ B α protein and is denoted with an arrowhead. An upper band can be noted, which is a feature of sample handling, protein loading, and length of electrophoresis, but is likely to contain phosphorylated I κ B α . Densitometric analyses of the I κ B α band (using the lower, major band) were performed, and the measured values were normalized to those obtained in the unstimulated cells (to compensate for differences in protein loading between patient and control). Normalized densitometric measurements are shown in graphic format to the right of each Western blot analysis, with the values provided below each time point (red). These experiments were performed twice with the Δ E223 NEMO and once for the E223K NEMO patient cells. The data indicate that the patients with E223 mutations fail to effectively inactivate I κ B α .

ers, was associated with hypogammaglobulinemia, inability to make specific antibody after immunization with the 23-valent vaccine, and *S pneumoniae* sepsis, leading to demise in the first decade.⁹ Furthermore, L227P mutation was associated with chronic inflammatory gastrointestinal disease.⁹ A phenotype heat map of inflammatory disease in patients with NEMO-ID has indicated a hot spot of mutations affecting the second half of the first coiled coil domain and including the second α -helix associated with the phenotype.² However, our patients with NEMO residue 223 alterations did not exhibit an overt inflammatory disease as part of the clinical phenotype. This could suggest a nonessential role for NEMO residue 223 in the development of inflammatory disease or a milder impact of this particular residue within this region, which could result in delayed onset of inflammatory disease phenotype. Regardless, these patients and the specific mutations are worthy of further study. In particular, it will be important to determine whether there are differential associations with upstream or downstream signaling intermediates

with NEMO 223 vs 227 variants. Such work is likely, however, to underscore the complex potential genotype-phenotype associations in this disease at a mechanistic level, and additional investigations in consideration of biological and even clinical insights will be necessary.

TNF-induced I κ B α degradation was additionally evaluated in our patients to determine the extent to which the alteration at position 223 affects the diverse immune pathways known to be implicated in NEMO syndrome. TNFR activation engages a distinct signaling cascade from TLR and Toll-IL-1 receptor (TIR) pathways. TLR signaling was impaired because of both mutations; however, a difference in TNFR signaling was observed. The deletion of residue 223 was associated with an inability to induce I κ B α degradation, whereas the mutation resulting in E223K resulted in slightly reduced TNF- α -induced I κ B α degradation. That some I κ B α degradation occurred in E223K cells does not exclude the possibility of downstream defects in this pathway because TNF- and TLR-induced gene expression and cytokine pro-

duction have been reported in cells in which I κ B α degradation is normal.^{2,10} TLR vs TNFR signaling impairment differences suggest upstream (and possibly downstream) components of these 2 signaling pathways interact differentially with this region of NEMO or with another domain functionally related to E223. An important task remains to discover how signaling by the TLR/TIR domain containing receptor signaling through NEMO differs from TNFR or T-cell receptor signaling; differences such as the one described here provide a functional clue into the mechanism. That this clue provides no real insight into the mechanism highlights the need to develop more comprehensive readouts to interrogate these signaling pathways and network perturbations due to *NEMO* mutation.

Diagnosis of *NEMO* mutations is challenging in patients with subtle or even without signs of hypohidrotic ectodermal dysplasia. A total of 23% of patients with NEMO-ID had no diagnosis of EDA² in a recent review of 72 individuals. A higher percentage of patients had mild features of the syndrome, which can be overlooked until a serious immunodeficiency is suspected. The patients in this report might have been considered to have had more “mild” features of hypohidrotic ectodermal dysplasia, which further emphasizes the importance of following physical examination–derived clues in determining whether a patient has NEMO. At this point, however, there is no single clear indication suggesting the absolute certainty of NEMO mutations in patients. It should be considered worthwhile to seek *NEMO* mutations in male patients with the hyper-IgM syndrome who do not have any of the other known mutations associated with this phenotype or in boys with a mycobacterial infection who have a humoral abnormality of some type. It is not as clear when to pursue a *NEMO* mutation in a patient with isolated specific antibody deficiency or other mild immunoglobulin abnormalities. In the aforementioned review, defects in specific antibody production occurred in 64%, but only 4 individuals also had normal serum IgG levels. Mutations in these “atypical” individuals span an area that includes first and second coiled coils and C-terminal to the leucine zipper. On the basis of the clinical presentation of our 2 patients and those atypical patients previously described, consideration of *NEMO* mutation in patients with symptoms of immunodeficiency and mild ectodermal abnormalities is reasonable. Recurrent or

severe invasive infections, often including infection with polysaccharide encapsulated organisms and specific antibody deficiency with or without mycobacterial infection, however, should still be considered the sine qua non of the syndrome.

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