NEMO Mutations in 2 Unrelated Boys With Severe Infections and Conical Teeth

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ABSTRACT. X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency is a developmental and immunologic disorder caused by mutations in nuclear factor-κB essential modulator (NEMO), which is essential for nuclear factor-κB activation. Early in life, affected boys present a typical appearance, with hypotrichosis or atrichosis, hypohidrosis or anhidrosis, and hypodontia or anodontia with conical incisors. They are also susceptible to various microorganisms, mostly pyogenic bacteria and mycobacteria. Here we report 2 unrelated boys, aged 6 and 11 years, who have novel mutations in *NEMO* and present conical incisors and hypodontia as their sole and long-unrecognized developmental anomaly. One child had isolated recurrent pneumococcal disease, whereas the other had multiple infections. Our observations indicate that conical incisors should prompt the search for *NEMO* mutations in boys with unusual infectious diseases. *Pediatrics* 2005; 115:e615–e619. URL: www.pediatrics.org/cgi/doi/10.1542/peds.2004-1754; *NEMO, ectodermal dysplasia, conical teeth, immunodeficiency, NF-κB.*

ABBREVIATIONS. XL-EDA-ID, X-linked anhidrotic ectodermal dysplasia with immunodeficiency; NF-κB, nuclear factor-κB; NEMO, nuclear factor-κB essential modulator; TNF, tumor necrosis factor; IL, interleukin; Ig, immunoglobulin; Hib, *Haemophilus influenzae* type b.

X-linked anhidrotic ectodermal dysplasia with immunodeficiency (XL-EDA-ID) is a rare congenital disease, characterized by abnormal development of ectoderm-derived skin appendages and susceptibility to infectious diseases.[1–12] Most affected individuals are male. In early childhood, they generally present multiple overt developmental anomalies, such as hypohidrosis or anhidrosis resulting in intolerance to heat, hypotrichosis or atrichosis, and hypodontia or anodontia with conical incisors, resulting in a typical facial appearance. A small number of patients also present with osteopetrosis and lymphedema. More than half of the known patients died from severe infections, generally caused by pyogenic bacteria and poorly pathogenic mycobacteria. Paradoxically, the only anomaly that is found consistently during routine immunologic tests is an impaired antibody response to polysaccharide antigens. Various hypomorphic mutations have been found in nuclear factor-κB (NF-κB) essential modulator (*NEMO*), which encodes a critical component of the NF-κB signaling pathway. The EDA phenotype results from impaired NF-κB activation by the single ectodysplasin receptor. In contrast, immunodeficiency results from impaired NF-κB activation by multiple immune receptors, such as members of the Toll-like receptor, interleukin (IL)-1 receptor, and tumor necrosis factor (TNF) receptor superfamilies and T/B-cell receptors. We report 2 unrelated boys who bear mutations in *NEMO*. Both experienced severe infectious diseases. Abnormal teeth were their sole developmental anomaly.

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Accepted for publication Nov 18, 2004.
doi:10.1542/peds.2004-1754
No conflict of interest declared.

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CASE REPORTS

Patient 1 is a 6.5-year-old white boy who was born to unrelated French parents. He had been hospitalized previously for 3 episodes of *Streptococcus pneumoniae* arthritis, affecting a knee at the age of 2 years, an ankle at 5 years, and a hip at 5.5 years. At 3 years, the patient was hospitalized for *Haemophilus influenzae* lobar pneumonia. No other unusually severe infections were documented, and the patient has been well on prophylactic antibiotherapy (oracillin) since the age of 6 years.

Patient 1 had a functional spleen and normal complement levels. The lymphocyte subsets were normally distributed, and in vitro T-lymphocyte proliferation in response to both mitogens and antigens was normal (Table 1). At 5 years, patient 1 had normal immunoglobulin (Ig) M and IgA and high IgG serum levels. IgG subclasses were normal. At this age, serum antibodies to the tetanus toxoid recall antigen were within the normal range, but antibodies against *S pneumoniae* were undetectable despite 3 episodes of pneumococcal arthritis (titer of antibodies to a pool of 23 serotypes <0.15 mg/L and no detectable specific antibodies to serotypes 3, 4, and 9). He then was vaccinated with a pneumococcal polysaccharidic vaccine (Pneumovax 23), and 1 month later, no adequate antibody response was detected against serotypes 3, 4, 9N, 18C, and 23F despite an antibody titer of 0.8 μg/mL against a mixture of the 23 serotypes. Antibodies against *H influenzae* type b (Hib) were also detectable after vaccination with a conjugated Hib capsule (46% inhibition, normal value >20%). Anti-B allohemag-

TABLE 1. Serum Immunoglobin Levels, Specific Antibody Titers, and T-Lymphocyte Proliferation

	Patient 1	Patient 2
Serum immunoglobin, g/L		
IgM	0.69 (0.66–1.18)	0.2 (0.68–1.28)
IgG	11.4 (7–12.6)	12.19 (8.3–14.3)
IgA	4.03 (0.78–1.62)	1.25 (1.02–1.94)
Specific antibodies to		
Tetanus	ND	0.32 (>0.17 IU/mL)
Hib*	46% inhibition	0.89 (>0.15 μg/mL)
Pneumococcus	0.8 (>0.3 μg/mL)†	<4.0 (>15.4 IU/mL)
Serotype 3	0.12 μg/mL (>0.5 μg/mL)	ND
Serotype 4	0.07 μg/mL (>0.7 μg/mL)	ND
Serotype 9N	0.7 μg/mL (>1.4 μg/mL)	ND
Serotype 18C	0.08 μg/mL (>0.8 μg/mL)	ND
Serotype 23F	0 μg/mL (>0.15 μg/mL)	ND
T-lymphocyte proliferation, cpm		
Phytohemagglutinin	18 230	19160 (>50 000)
Candidin	1257	250 (>1000)
Tuberculin	4485	640 (>1000)
Tetanus toxoid	6703	3170 (>1000)

Patients 1 and 2 were tested at 5 and 9 years of age, respectively. ND indicates not determined.
* After Hib-conjugated vaccination.
† Vaccinated by Pneumovax 23.

glutinins were barely detectable in serum at the age of 5 years (titer: 1/4; blood group A).

Patient 2 is an 11-year-old white boy who was born to unrelated German parents. From 1 year of age, he had multiple infections: pneumococcal septicemia at the ages of 1.5 and 6 years, 6 episodes of pneumonia between 1.5 and 9 years (1 caused by *Pseudomonas aeruginosa* and 1 by *H influenzae*), and 3 episodes of orbital cellulitis between 2 and 5 years (caused by *Candida albicans* or *Staphylococcus aureus*). The patient has been well on intravenous immunoglobin substitution since the age of 9 years.

The patient had a functional spleen and normal complement levels. In vitro T-lymphocyte proliferation in response to both mitogens and antigens was low (Table 1). At the age of 9 years, patient 2 had low IgM and normal IgG and IgA serum levels. IgG subclasses were normal at the age of 3 years. He displayed normal levels of serum antibodies against tetanus toxoid after vaccination. He was not vaccinated against *S pneumoniae*, but no serum antibodies against *S pneumoniae* could be detected at 2 or 6 years despite multiple episodes of pneumococcal disease. At 5 years of age, his antibody response to Hib conjugate vaccine was normal (0.89 mg/L; for protective values: >0.15 mg/L). He had neither anti-A nor anti-B serum allohemagglutinins at the ages of 5 and 7.5 years (blood group O).

These 2 children display no developmental signs of EDA syndrome other than hypodontia and conical incisors (Fig 1). Their eye brows, eye lashes, and hair are normal, and there is no facial dysmorphy. Both patients sweat normally, with no heat intolerance. Patient 1 had all of his lacteal teeth but with 4 conical-shaped mandibular incisors (teeth 71, 72, 81, and 82; Fig 1A). Mandibular radiography revealed hypodontia with agenesis of 1 adult premolar (tooth 35). The mother of patient 1 presents no immunodeficiency or incontinentia pigmenti. However, her teeth are also sparse and some incisors are conical (not shown). Patient 2 also has hypodontia of adult teeth, and some of his lacteal incisors were conical (Fig 1B). Deciduous incisor 81 is conical shaped, and there is agenesis of adult teeth 12, 22, 31, 32, 41, and 42. The mother of patient 2 displays no clinical abnormalities.

Molecular genetic analysis revealed 2 novel *NEMO* mutations. Patient 1 carries an 18-nucleotide deletion in exon 7, causing the deletion of amino acids 271 to 276 (designated 811_828del). This in-frame small deletion was not found in 50 healthy white control subjects (72 chromosomes), suggesting that this deletion is a

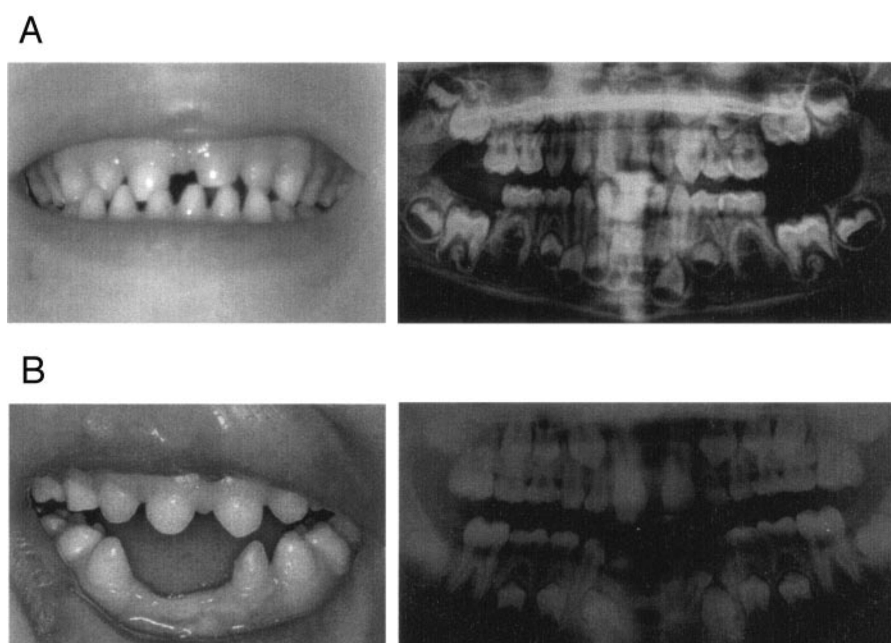

Fig 1. Dysmorphic teeth of the patients. Patients 1 (A) and 2 (B) both present hypodontia and conical-shaped teeth. For patient 1, the photograph and the radiograph were taken at 5 years. For patient 2, the photograph was taken at 8 years and the radiograph was taken at 9 years. Patient 1 has 4 conical-shaped deciduous mandibular incisors (71, 72, 81, and 82) and agenesis of 1 adult premolar (35). Patient 2 has 1 conical-shaped deciduous incisor (81) and agenesis of 6 adult teeth (12, 22, 31, 32, 41, 42).

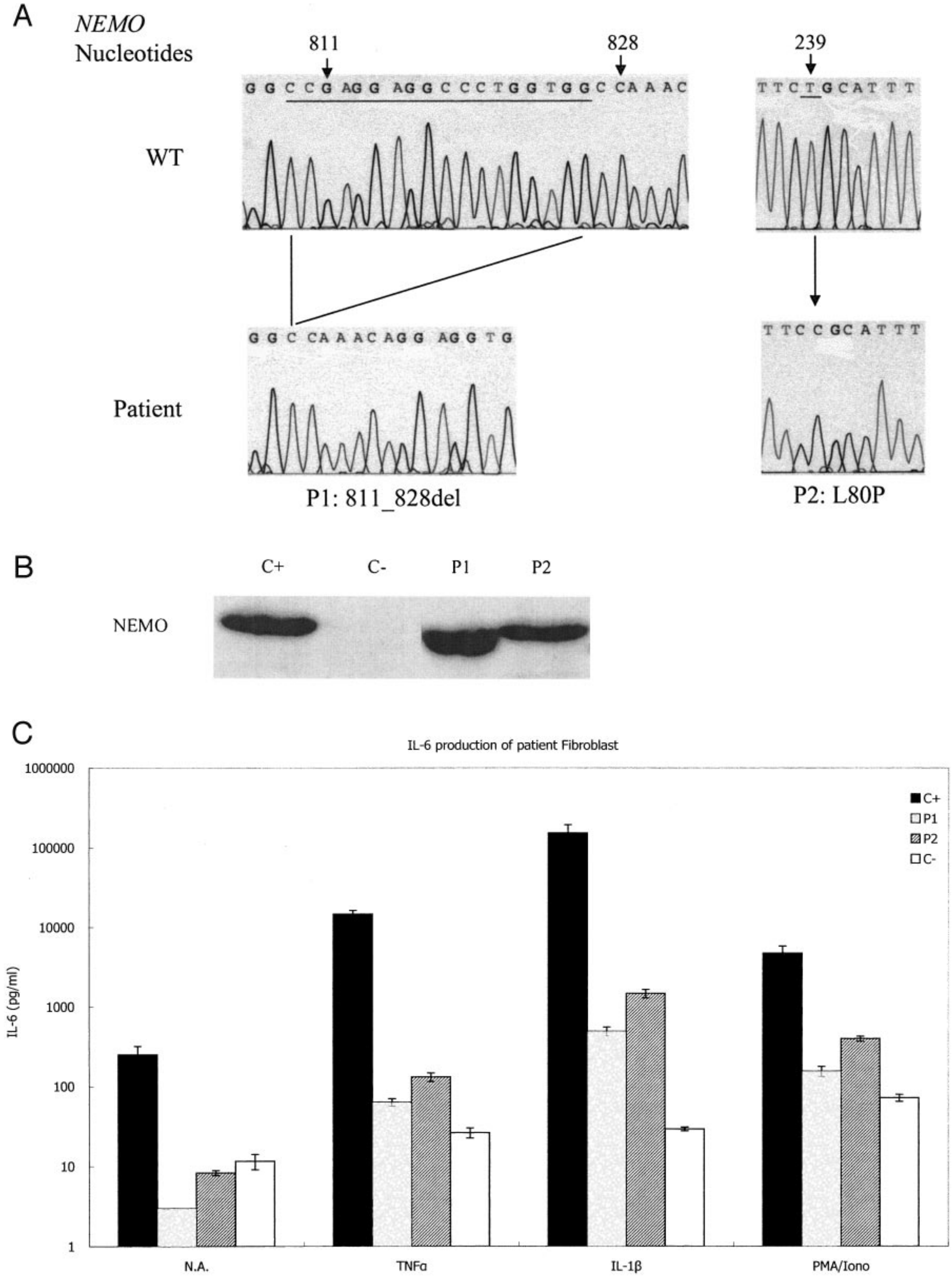

Fig 2. *NEMO* mutations in the patients. A, Mutant (bottom) and wild-type (WT; top) *NEMO* cDNA sequences from patient 1 (811_828del) and patient 2 (L80P). The mutated nucleotides are underlined. B, NEMO protein in fibroblasts from patient 1, patient 2, a healthy individual positive control subject (C+), and a NEMO-deficient fetus negative control subject (C−), as detected by Western blot using the anti-NEMO antibody. C, IL-6 production by fibroblasts from the 2 patients (patient 1 and patient 2), C+, and C−. Fibroblasts were stimulated with TNF-α (20 ng/mL), IL-1β (10 ng/mL), or phorbol 12-myristate 13-acetate/ionomycin ($10^{-7}/10^{-5}$ M). IL-6 production was measured by enzyme-linked immunosorbent assay after 24 hours of activation. D, Known *NEMO* mutations associated with EDA-ID (1–12) and found in our 2 patients (*, patient 1; **, patient 2).

pathogenic mutation rather than an irrelevant polymorphism. This deletion is located in the coiled-coil 2 domain (Fig 2A) and was confirmed to be located in *NEMO* (and not in its nearby paralogous pseudogene) by cDNA sequencing. The patient's mother is heterozygous for this mutation. X-inactivation in her blood, however, is random (data not shown).

In patient 2, a T-to-C mutation (239 T>C) was found in exon 3 of *NEMO*. This mutation results in a leucine to proline substitution

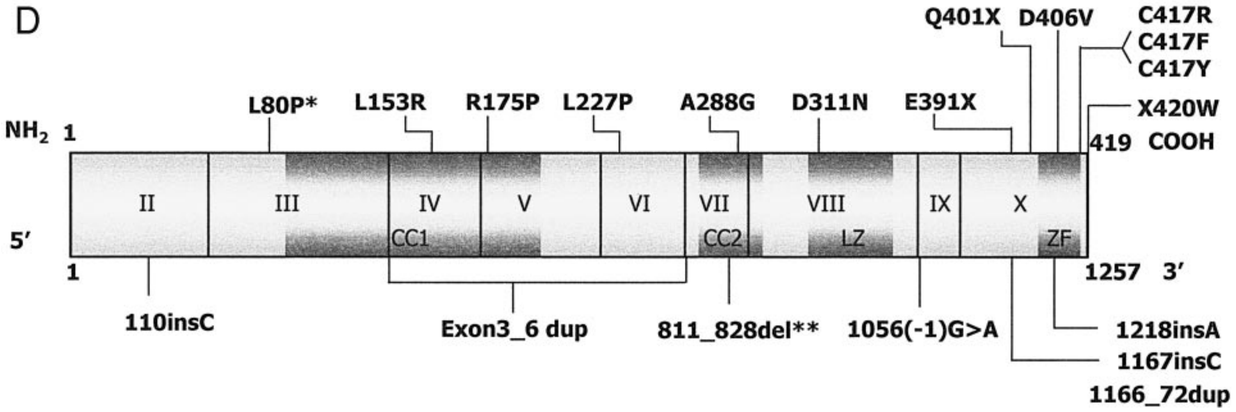

Fig 2. Continued.

(designated L80P) in the N-terminal coiled-coil 1 domain of NEMO. This amino acid substitution was not found in 517 healthy white control subjects (665 chromosomes), strongly suggesting that this missense mutation is a pathogenic mutation and not merely a common polymorphism of the *NEMO* coding region. The mother of patient 2 is also heterozygous for the mutation, and her blood X-inactivation is skewed (data not shown).

By Western blot, NEMO was detected in cellular extract from patient 1 and patient 2 (Fig 2B). However, fibroblasts from patient 1 and patient 2 responded weakly to TNF-α and IL-1β in terms of IL-6 production, when compared with a healthy control subject (Fig 2B). Fibroblasts bearing an amorphic *NEMO* mutation did not respond to these stimulations. These data validated the pathogenic impact of the 2 mutations identified, despite the presence of detectable NEMO protein.

DISCUSSION

Hypomorphic mutations in *NEMO* are associated with XL-EDA-ID.[1–11] We describe 2 novel *NEMO* mutations in patients who have severe pyogenic bacterial infections and in whom dysmorphic conical lacteal incisors were the only overt developmental anomaly and the only clinical clue to the proper diagnosis before hypodontia of adult teeth was documented. The impaired antibody response to polysaccharides is the only immunologic anomaly consistently detected in laboratory tests in patients with XL-EDA-ID. T-cell–independent antibody response to unconjugated polysaccharides was persistently impaired in both patients. However, unlike the previously reported patients with mutations in *NEMO*, both patients mounted an apparently normal T-cell–dependent antibody response to conjugated polysaccharides. The ectodermal dysplasia of our 2 cases is also extremely mild when compared with other patients with XL-EDA-ID. Until a mutation was found in *NEMO*, after the recognition of conical incisors, both patients had received a diagnosis of pure immunodeficiency. The conical teeth were thought to be merely a coincidental curiosity.

Our observations thus indicate that impaired tooth development and defective antipolysaccharide antibody response to unconjugated polysaccharides (allohemagglutinins and antipneumococcal antibodies) are the 2 most consistent clinical indicators of *NEMO* mutations. Our 2 patients extend our description of the clinical phenotype associated with hypomorphic *NEMO* mutations, as not only the immunologic but also the developmental phenotype varies considerably from case to case. The mutations 811_828del (patient 1) and L80P (patient 2) both are associated with a mild developmental impact in vivo in hemizygous male individuals, yet only the heterozygous mother of patient 1 shows abnormal teeth. Paradoxically, the heterozygous mother of patient 2, unlike that of patient 1, shows a skewed X-inactivation in her blood cells. Biochemical studies now are required to understand how the different mutations in *NEMO*[1–12] (this article) result in such different clinical phenotypes. In clinical practice, all boys with unusually severe infections and conical incisors or hypodontia should be investigated for *NEMO* mutations, even if they do not display the canonical EDA phenotype. Moreover, neither an apparently normal antibody response to conjugated polysaccharides nor a random X-inactivation in blood cells from their symptomatic or asymptomatic mothers or a detectable NEMO protein by Western Blot should delay the sequencing of the coding region of the *NEMO* gene.

ACKNOWLEDGMENTS

We thank Laurent Abel, Marion Bonnel, Horst von Bernuth, Huey-Hsuan Chang, Jacqueline Feinberg, and all members of the laboratory of Human Genetics of Infectious Diseases for helpful discussions. We also thank Mario Abinun for collaboration on the investigation of patients with ectodermal dysplasia with immunodeficiency. We are extremely grateful to the 2 families reported for allowing us to share these findings.

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Pediatrics 2005;115;e615
DOI: 10.1542/peds.2004-1754 originally published online April 15, 2005;