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 (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.

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 antibiotics (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 polysaccharide 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-

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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 dysmorphism. 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 811del). This in-frame small deletion was not found in 50 healthy white control subjects (72 chromosomes), suggesting that this deletion is a

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

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum immunoglobin, g/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>0.69 (0.66–1.18)</td>
<td>0.2 (0.68–1.28)</td>
</tr>
<tr>
<td>IgG</td>
<td>11.4 (7–12.6)</td>
<td>12.19 (8.3–14.3)</td>
</tr>
<tr>
<td>IgA</td>
<td>4.03 (0.78–1.62)</td>
<td>1.25 (1.02–1.94)</td>
</tr>
<tr>
<td>Specific antibodies to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetanus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hib*</td>
<td>46% inhibition</td>
<td>0.32 (&gt;0.17 IU/mL)</td>
</tr>
<tr>
<td>Pneumococcus</td>
<td>0.8 (&gt;0.3 μg/mL)†</td>
<td>&lt;4.0 (&gt;15.4 IU/mL)</td>
</tr>
<tr>
<td>Serotype 3</td>
<td>0.12 μg/mL (&gt;0.5 μg/mL)</td>
<td>ND</td>
</tr>
<tr>
<td>Serotype 4</td>
<td>0.07 μg/mL (&gt;0.7 μg/mL)</td>
<td>ND</td>
</tr>
<tr>
<td>Serotype 9N</td>
<td>0.7 μg/mL (&gt;1.4 μg/mL)</td>
<td>ND</td>
</tr>
<tr>
<td>Serotype 18C</td>
<td>0.08 μg/mL (&gt;0.8 μg/mL)</td>
<td>ND</td>
</tr>
<tr>
<td>Serotype 23F</td>
<td>0 μg/mL (&gt;0.15 μg/mL)</td>
<td>ND</td>
</tr>
<tr>
<td>T-lymphocyte proliferation, cpm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytohemagglutinin</td>
<td>18230</td>
<td>19160 (&gt;50 000)</td>
</tr>
<tr>
<td>Candidin</td>
<td>1257</td>
<td>250 (&gt;1000)</td>
</tr>
<tr>
<td>Tuberculin</td>
<td>4885</td>
<td>640 (&gt;1000)</td>
</tr>
<tr>
<td>Tetanus toxoid</td>
<td>6703</td>
<td>3170 (&gt;1000)</td>
</tr>
</tbody>
</table>

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.
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 2B).

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).
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NEMO

the clinical phenotype associated with hypomorphic

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development and defective antipolysaccharide anti-

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31 patients with XL-EDA-ID. Until a mutation was found

in XL-EDA-ID. T-cell–independent antibody response

to unconjugated polysaccharides was persistently

impaired in both patients. However, unlike the pre-

viously reported patients with mutations in

NEMO, both patients mounted an apparently normal T-cell–

dependent antibody response to conjugated polysaccha-

rides. The ectodermal dysplasia of our 2 cases is

also extremely mild when compared with other pa-

tients 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 im-

munodeficiency. The conical teeth were thought to

be merely a coincidental curiosity.

Our observations thus indicate that impaired tooth
development and defective antipolysaccharide antibo-
dy response to unconjugated polysaccharides (al-

lohemagglutinins 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 consider-

ably 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 hemi-
yzogous male individuals, yet only the heterozygous

mother of patient 1 shows abnormal teeth. Parado-
xically, 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

NEMO1–12 (this article) result in such different clini-
cal phenotypes. In clinical practice, all boys with

unusually severe infections and conical incisors or

hypodontia should be investigated for NEMO muta-
tions, 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 detect-
able NEMO protein by Western Blot should delay

the sequencing of the coding region of the NEMO
gene.

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for allowing us to share these findings.

REFERENCES

1. Abinun M. Ectodermal dysplasia and immunodeficiency. Arch Dis

Child. 1995;73:185


immune deficiency and hypohidrotic ectodermal dysplasia is allelic to

incontinentia pigmenti and due to mutations in IKK-gamma (NEMO). Am


dysplasia with immunodeficiency is caused by impaired NF-kB signal-


mutations in NEMO result in hyper-IgM syndrome with hypohydrotic

5. Aradhya S, Courtois G, Rajkovic A, et al. Atypical forms of incontinen-
tia pigmenti in male individuals result from mutations of a cytosine


765–771


surviving male is accompanied by hypohidrotic ectodermal dysplasia


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