Case Report: X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency and an unusual Aspergillus infection [version 1; peer review: 2 approved with reservations]

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Abstract
NEMO (NF-kB essential modulator) is a regulatory factor involved in signaling pathways of the innate and adaptative immune systems. Hypomorphic mutation of the NEMO gene (also called IKBKG gene) on the X chromosome leads to X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency. Affected male children present a developmental phenotype with hypotrichosis, hypohydrosis, and hypodontia with conical incisors and susceptibility to pyogenic bacteria, mycobacteria and viruses. Most also have impaired antibody response to polysaccharide antigens. Here we present the case of a 7-year-old boy with disseminated BCGitis and unusual Aspergillus infection who was later diagnosed with a homozygous mutation of the NEMO gene. Appropriate long term anti-mycobacterial medications, prophylactic anti-fungal therapy and current monthly intravenous immunoglobulin (IVIG) stabilized the patient’s condition and has significantly improved his general health. High incidence of atypical mycobacterial infection in such cases emphasize the need for prophylaxis.

In conclusion, attention to gender, pattern of infections, and precise physical exam helped us to diagnose and appropriately manage this case. We propose prophylactic therapy for mycobacterial and opportunistic infections after the confirmation of homozygous NEMO gene mutation.

Keywords
Anhidrotic Ectodermal Dysplasia, NF-B essential modulator, immunodeficiency, recurrent infections, Aspergillosis
Introduction

X-linked anhidrotic ectodermal dysplasia with immunodeficiency (XL-EDA-ID) is a rare congenital disease, characterized by susceptibility to infectious diseases and abnormal development of ectoderm-derived skin appendages.1-5.

XL-EDA-ID clinical and immunological phenotypes are highly mutation dependent. Previous reports showed the associations of specific mutations with particular phenotypes including susceptibility to poorly virulent mycobacteria, invasive pyogenic bacterial fungal and severe viral infections, due to the essential role of NEMO (nuclear factor-kappa B (NF-xB) essential modulator) in both innate and adaptive immunity.6-14. Both cellular and humoral abnormalities were recorded in XL-EDA-ID cases.15,16.

Most patients bearing NEMO gene mutations (also called IKBKGN, encoder of NEMO protein) have an impaired antibody response, in particular to glycans.17. However, impairments in CD40-mediated B cell activation, isotype class switching, NK cell cytotoxicity, response to LPS stimulation, and production of TNF and IL-12 have been verified by *in vitro* studies for some NEMO-deficient patients.18.

In this report, we present a 7-year-old boy with XL-EDA-ID suffering from disseminated BCGitis and fungal infection with specific antibody deficiency against glycans antigens.

Case presentation

This report describes a male child born to a non-consanguineous parents with no history of immunodeficiency in the family. The mother had a history of Behçet’s disease. Birth growth parameters and mental development were in normal range. Vaccinations were up-to-date without any complication except diffuse lymphadenopathy following Bacillus Calmette-Guérin (BCG) vaccination at the age of 3 days. At 1 month of age, he was admitted to hospital with low fever, dry cough and respiratory distress and was diagnosed with pneumonia. During admission, abnormal signs such as tremor of upper and lower extremities and upward gaze were inspected. Cerebrospinal fluid analysis was normal (sugar: 36mg/dl; protein: 26mg/dl; no cells). He was diagnosed with suspected febrile convulsion due to a viral infection. During the first 9 months, he developed recurrent episodes of respiratory tract infections. Later on, another episode of disseminated BCGitis was detected while he was under continuous phase of isoniazid (INH) and rifampin (RIF) (10 mg/kg daily) for one month. Physical examination found multiple cervical lymphadenopathies, which later revealed caseating granulomatous lymphadenitis on biopsy. Spiral CT-scan of the abdomen illustrated hepatomegaly with inflammatory parenchyma and multiple para-aortic lymphadenopathies. Bone marrow study was normal. Continuation of antimycobacterial therapy at maximum dose of INH (15 mg/kg/day) and RIF (20 mg/kg/day) significantly improved disseminated BCGitis after 18 months.

Two other episodes of pneumococcal pneumonia were reported at the age of 3.5 and 4 years. At the age of 5, the patient experienced severe *Aspergillus nidulans* pneumonia and was started on Voriconazole (8mg/kg) followed by Itraconazole (5 mg/kg) twice a day for 1 year with a favorable outcome.

At the age of 6, the patient was referred to our center for the evaluation of immunodeficiency. Further examination revealed additional features of ectodermal dysplasia including conical lctal teeth without agenesia, ridged nails, sparse hair and skin abnormalities. In terms of problems associated with recurrent opportunistic and unusual infections, laboratory evaluation of the immune system was performed and reported normal immunoglobulin levels, impaired response to pneumococcal vaccine and defective reaction to PPD (<5mm induration) (Table 1). Gene sequencing revealed a homozygous NEMO missense mutation in exon 8, c.932 A>G which led to the substitution of asparagine by glycine at residue 311 (designated D311G). Molecular testing of the patient’s mother, grandmother and second aunt revealed heterozygous NEMO mutations in the corresponding locus. The patient has been administered monthly courses of intravenous immunoglobulin (800 mg/kg) and a prophylactic dose of Itraconazole (5mg/kg/day). Currently, patient is symptom free.

Discussion

Hypomorphic mutations in NEMO are associated with XL-EDA-ID.19-28. Patients with hypomorphic hemizygous *IKBKG* mutation appear to possess some variety of immunodeficiency, regardless of presence or absence of EDA.24-26. This could be due to an abnormal NF-xB which results in defective lymphocytic receptor signaling.24,25. Here we describe the first patient with NEMO deficiency who presents a classical EDA phenotype, disseminated BCGitis and pulmonary Aspergillosis. This patient carries a hemizygous NEMO mutation, D311G, which has also been reported previously in a patient with recurrent mycobacterial infections (*M. avium* and *M. abscessus*) without any history of fungal infections. The mycobacterial susceptibility in both patients can be explained by the impairment of CD40-dependent IL-12 production.27. Defective PPD of our patient is the only document which revealed cellular immune system failure leading to disseminated BCGitis.

The immunological phenotypes of these two cases are comparable, because both patients displayed the same impaired antibody response to glycans as the only detected immunologic abnormality. Almost all patients bearing mutations in NEMO have an impaired antibody response to glycans, including pneumococcal capsules in particular.4,4. Half of them have also hypogammaglobulinemia, probably secondary to CD40 signaling impairment.5 Some mutations in the *IKBKG* gene are associated with T-cell defects, because NEMO is an essential component of the inhibitor of NF-xB (IxB)-kinase (IKK) complex, affecting the phosphorylation of IxB which is necessary for nuclear translocation of NF-xB.24.

Signaling through the IKK complex has been shown to be essential for production of mature/memory T-cells, which may be an explanation for the low memory T-cell phenotype observed in these patients.25,28.

Lastly, regarding the developmental phenotype, the patient reported here displays a more severe EDA phenotype (dysmorphic conical lctal) compared to the patient with the same hemizygous NEMO mutation who has only teeth agenesia of maxillary lateral incisors and premolars.29. Hence, appropriate genetic diagnosis and genetic counseling looks essential and testing for NEMO carriers should be considered (if applicable) as performed in our case for the patient’s mother and maternal aunts. Intravenous immunoglobulins is the treatment of choice in NEMO-deficient patients with...
evidence of impaired antibody production\textsuperscript{5,6}. High incidence of atypical mycobacterial disease infection in these cases emphasize the need of prophylaxis. Prophylaxis against pneumocystis pneumonia should also be considered, specifically in males with low T-cell counts or severely impaired lymphocyte proliferation\textsuperscript{27–29}. Conclusively, attention to gender, pattern of infections, and skin involvements helped us to diagnose and appropriately manage this case.

Consent
Written informed consent for publication of the patient’s details was obtained from the patient’s parents.

Author contributions
TS, ZM conducted the study and prepared the first draft. All authors were involved in data collection and preparation of the written manuscript.

Competing interests
No competing interests were disclosed.

Grant information
The authors declared that no grants were involved in supporting this work.

References

Table 1. Immunologic laboratory results.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
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<tbody>
<tr>
<td><strong>Complete blood count</strong></td>
<td></td>
</tr>
<tr>
<td>WBC (cells/ml)</td>
<td>9850</td>
</tr>
<tr>
<td>Lymphocyte (cells/ml)</td>
<td>5000</td>
</tr>
<tr>
<td>CD3+ T cells (% of lymphocytes)</td>
<td>64.9%</td>
</tr>
<tr>
<td>CD3+CD4+ CD3+ T cells (% of lymphocytes)</td>
<td>44.35%</td>
</tr>
<tr>
<td>CD3+ CD8+ T cells (% of lymphocytes)</td>
<td>17.3%</td>
</tr>
<tr>
<td>CD16+ NK cells (% of lymphocytes)</td>
<td>7.51%</td>
</tr>
<tr>
<td>CD19+ B cells (% of lymphocytes)</td>
<td>21.22%</td>
</tr>
<tr>
<td><strong>Serum immunoglobulins</strong></td>
<td></td>
</tr>
<tr>
<td>IgG (mg/dl)</td>
<td>1131</td>
</tr>
<tr>
<td>IgA (mg/dl)</td>
<td>140</td>
</tr>
<tr>
<td>IgM (mg/dl)</td>
<td>47</td>
</tr>
<tr>
<td>IgE (IU/dl)</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Vaccine-specific serology</strong></td>
<td></td>
</tr>
<tr>
<td>Anti-Tetanus (IU/ml)</td>
<td>0.5</td>
</tr>
<tr>
<td>Anti-Diphtheria (IU/ml)</td>
<td>0.7</td>
</tr>
<tr>
<td>Anti-Pneumonia Ab (IgG) before vaccination (μg/ml)</td>
<td>10.1</td>
</tr>
<tr>
<td>Anti-Pneumonia Ab (IgG) after vaccination (μg/ml)</td>
<td>11.1</td>
</tr>
<tr>
<td>Anti-Pneumonia Ab (IgG2) before vaccination (μg/ml)</td>
<td>2.5</td>
</tr>
<tr>
<td>Anti-Pneumonia Ab (IgG2) after vaccination (μg/ml)</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>T cell proliferations</strong></td>
<td></td>
</tr>
<tr>
<td>Phytahemagglutinin (PHA)</td>
<td>Normal</td>
</tr>
<tr>
<td>Dihydroamine reduction (DHR)</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Tuberculosis skin test (PPD)</strong></td>
<td>&lt; 5mm</td>
</tr>
</tbody>
</table>


The authors reported an interesting clinical and molecular case of a rare entity; this is enough to find the merit to index this case; considering the pathology I would not be surprised by an infection, and not focus in the title on the “unusual Aspergillus infections”; the mutation detected has been already reported, phenotype is typical and it would be of interest knowing something about life quality and expectancy for the incoming years since XL-HED-ID is quite a challenge with all the complications which might arise from the disease.
A reference is attached not mandatory indeed to be cited.

- I recommend to change the title suggesting to be more general (i.e Clinical and molecular study in a case of X linked hypohidrotic ectodermal dysplasia with immunodeficiency). The abstract should begin with an introduction on Ectodermal Dysplasia (EDs) in general and after mentioning the most common form XL-HED, AD-HED etc caused by EDA gene or EDAR, EDARADD, WNT10A could continue and go deep in the analysis of NEMO gene features.

- Design, methods and analysis of the results from the study have not been explained and do not clarify the data exposed and results.

- Conclusions are sensible, balanced and justified on the basis of the results of the study although no novel findings are reported.

- Good information has been provided but indeed more figures such as electropherogram of the mutation, pedigree of the family, OMIM number of the disease, methodology of sequencing plus few more references are required. Furthermore mentioning clinical features (i.e peculiar conical shaped primary –better than “lacteal”- teeth, agenesis better than “agenesia”, sparse hair) a picture of the proband and/or radiographic examination such as orthopantomography should be added.

References

**Competing Interests:** No competing interests were disclosed.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Reviewer Report 02 December 2016

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The authors reported a case with hypomorphic IKBK mutation of a 7-year-old boy with disseminated BCGitis and unusual Aspergillus infection, who was successfully treated and prohibited with INH and RIF, in addition to IVIG administration.

But any evidence about molecular and functional defects were not shown in the table or Figures. They only described. I could not confirm the results. The reviewer needs some figure for them but not precisely.

- The title is appropriate for the content of the article. The abstract represent a suitable and attractive summary of the work.
- The design, methods and analysis of the results from the study been neither explained nor shown any figure or results of functional studies. Although the detail was not required, some figure for them are necessary.
- Conclusions are usual and no novel findings.
- The data shown in Table 1 is not enough to explain patient’s symptom. More critical or positive data should be listed, like a sequence data.

**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
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