Skewed X-inactivation in a Female Carrier with X-linked Chronic Granulomatous Disease

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ABSTRACT

Chronic granulomatous disease (CGD) is a primary immunodeficiency caused by defective phagocytic NADPH oxidase, causing a complete lack or significant decrease in the production of microbicidal reactive oxygen metabolites. It mainly affects male children; however, there are scarce reports of adult females diagnosed with X-linked-CGD attributed to an extremely skewed X-chromosome inactivation. This condition is characterized by severe and recurrent infections that usually develop after childhood. In clinical practice, physicians who usually confront these patients should suspect this entity and differentiate it from a secondary immunodeficiency. Here, we report a 38-year-old Mexican female with juvenile-onset X-linked-CGD, caused by a de novo mutation and extremely skewed X-inactivation, whose clinical features were similar to those in patients with classic X-linked-CGD.

Keywords: Autoimmunity; X-linked chronic granulomatous disease carrier; Discoid lupus; Skewed lyonization; X-inactivation; X-linked chronic granulomatous disease

INTRODUCTION

Chronic granulomatous disease (CGD) is a primary immunodeficiency caused by defective phagocytic NADPH oxidase, causing a complete lack or significant decrease in the production of microbicidal reactive oxygen metabolites.1-4 X-linked (XL)-CGD is caused by a hemizygous mutation of the CYBB gene encoding the gp91phox protein and is the most common form of
CGD, resulting in a greater number of affected males. However, there are scarce reports of adult females diagnosed with XL-CGD attributed to an extremely skewed X-chromosome inactivation.2,5-7

The precise mechanism by which the disease occurs in adults remains unclear, but a positive correlation has been observed between older age and the degree of skewed X-inactivation.3,6,8

XL-CGD in females, due to extremely skewed X-inactivation, is characterized by severe and recurrent infections that generally begin after childhood. An accessible diagnosis of this condition is through the dihydrorhodamine (DHR) assay, which makes evident the percentage of neutrophils with abnormal production of reactive oxygen metabolites.

The study received approval from the Ethical Review Committee of the National Institute of Pediatrics. Written informed consent was obtained from patient. Here, we report on a 38-year-old Mexican female with juvenile-onset XL-CGD caused by a de novo mutation and extremely skewed X-inactivation; she was identified when her son was diagnosed with XL-CGD, and her carrier status was confirmed through DHR assay.

The patient was asymptomatic in childhood and presented at the age of 14 years with a liver abscess, which needed surgical drainage. Four years later, she developed axillar and inguinal hidradenitis suppurativa with a chronic recurrent course. At age 20, she began to gradually lose her teeth secondary to periodontitis. Since the age of 31, she has had recurrent vaginal yeast and lower urinary tract infections. Since the age of 33, she has had recurrent perioral acneiform dermatitis, photosensitivity, gingivitis, and recurrent oral ulcers. Furthermore, she developed a neck abscess that also required surgical drainage.

Upon physical examination, she had melasma; mild perioral acneiform dermatitis with small papules and erythema around the nostrils and chin; and papules, pustules, small cysts and scars consistent with hidradenitis suppurativa on the left axilla. Oral examination showed a severe involvement with inflamed mucosa; periodontal pockets and gingival migration in all teeth; lack of twelve dental pieces; root remains and chronic infection in the apical portion of one dental organ; and a molar presenting total dehiscence of the gum.

Laboratory work-up showed leukopenia of 3,800/µL (4000-12000/µL); with 2,090/µL (55%) neutrophils and 1,216/µL (32%) lymphocytes; a low hemoglobin level of 11.8 g/dl (12.5-16-5 g/dl), and the platelet count of 175 x10^9/l was near the lower limit (150-450 x10^9/l). The immunoglobulins were above normal values: IgG 2,085 mg/dl (610-1,620 mg/dl), IgA 522 mg/dl (85-500 mg/dl), and IgM 275 mg/dl (36-242 mg/dl). Antinuclear antibodies were positive (1:80 titer); anti-Ro/SSA and anti-La/SSB were negative; complement was normal. Urine analysis showed a pH of 6 and the presence of leucocytes and epithelial cells.

Her son suffered from multiple severe episodes of pneumonias since the first year of life and, was diagnosed with XL-CGD at seven years of age (Figure 1). In the familial extended studies, DHR assays showed that only 4.11% of the index case’s total neutrophils produced hydrogen peroxide (H2O2), which is not a characteristic distribution for XL-CGD in female carrier, since the habitual distribution of each population of neutrophils, producers and non-producers of H2O2, is 50 and 50%. The patient’s daughter presented an expected bimodal distribution, and the patient’s mother and three sisters had normal results (Figure 1).

Figure 1. Pedigree of the kindred with Cytochrome b (CYBB) gene mutation, the proband is pointed with an arrow (II.2), her mother (I.2) was not a carrier of the mutation, and all her brothers and sisters are healthy. Her son had chronic granulomatous disease (III.1) and her daughter (III.2) was a carrier.
X-linked Chronic Granulomatous Disease Carriers

Positive NADPH subunit gp91<sup>phox</sup> neutrophil expression was present in only 4% of the patient’s cells. Sanger sequencing of the CYBB gene from peripheral blood DNA showed a previously described heterozygous nonsense variant (www.ncbi.nlm.nih.gov/clinvar/variation/10929/) in exon 7. The patient and her daughter presented p.Arg226*/WT, her son presented a p.Arg226*/Y, and her mother was WT/WT. The pattern of X-chromosome inactivation was determined in the patient and her father by assessing the methylation status of the androgen receptor (AR) gene (HUMARA assay). A heterozygous AR genotype was observed in the undigested DNA sample of the patient; however, only the AR-derived paternal (methylated) allele was seen in her HpaII-digested sample, a finding that is compatible with an extremely skewed X-chromosome inactivation, which also suggests that the active (unmethylated) AR-derived maternal allele carried the CYBB pathogenic variant. These results collectively support that the patient has late-onset XL-CGD due to a <i>de novo</i> CYBB mutation. Based on her extremely skewed X-chromosome inactivation diagnosis, abnormal laboratory results and the knowledge that carriers have a major risk of autoimmunity and infections, the patient was referred to an adult hospital to continue her medical follow-up.

Table 1. Summary of patients with X-linked chronic granulomatous disease secondary to skewed X-inactivation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Author</th>
<th>Present case</th>
<th>Rösen-Wolff et al&lt;sup&gt;2001&lt;/sup&gt;</th>
<th>Lun et al&lt;sup&gt;2002&lt;/sup&gt;</th>
<th>Anderson-Cohen et al&lt;sup&gt;2003&lt;/sup&gt;</th>
<th>Wolach et al&lt;sup&gt;2005&lt;/sup&gt;</th>
<th>Chollet-Martin et al&lt;sup&gt;2007&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of onset of the first manifestation</td>
<td>14 yo</td>
<td>45 yo</td>
<td>17 yo</td>
<td>18 months old</td>
<td>66 yo</td>
<td>P1: 7 yo</td>
<td>P2: newborn</td>
</tr>
<tr>
<td>Percentage of functional neutrophils and techniques used</td>
<td>Dihydrorhodamine (DHR): 4%</td>
<td>DHR: 6–8%</td>
<td>DHR: 2%</td>
<td>DHR: 0.04%</td>
<td>DHR: 0.4%–2%</td>
<td>P1: Dichloro-dihydro-fluorescein diacetate (DCFH-DA)</td>
<td>P2: 7%</td>
</tr>
<tr>
<td>Type of mutation in CYBB&lt;sup&gt;*&lt;/sup&gt;</td>
<td>p.(Arg226*)</td>
<td>p.(His303Thrfs*10)</td>
<td>p.(Tyr137Ilefs*3)</td>
<td>p.(Trp289*)</td>
<td>p.(Tyr30*)</td>
<td>p.(Leu211Profs*16)</td>
<td></td>
</tr>
<tr>
<td>De novo mutation</td>
<td>Yes</td>
<td>ND</td>
<td>ND</td>
<td>Yes</td>
<td>No</td>
<td>ND</td>
<td>P1: maternal</td>
</tr>
<tr>
<td>HUMARA (Maternal or paternal allele)</td>
<td>Maternal</td>
<td>Maternal</td>
<td>Maternal</td>
<td>Maternal</td>
<td>No</td>
<td>ND</td>
<td>P1: maternal</td>
</tr>
</tbody>
</table>

<sup>*</sup> CYBB: Chromosome 17 open reading frame 2, neutrophil cytosolic protein.
Table 1: Characteristics

<table>
<thead>
<tr>
<th>Author</th>
<th>Age of onset of the first manifestation</th>
<th>Percentage of functional neutrophils and techniques used</th>
<th>Clinical manifestations</th>
<th>Infectious agents isolated</th>
<th>Type of mutation in CYBB*</th>
<th>De novo mutation</th>
<th>HUMARA (Maternal or paternal allele)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lewis et al²</td>
<td>16 yo</td>
<td>Chemiluminescence (CL) 4-8%</td>
<td>Community-acquired pneumonia, Recurrent stomatitis, acne, and refractory subcutaneous abscess.</td>
<td>A. fumigatus</td>
<td>p.(Cys85Serfs*24)</td>
<td>No</td>
<td>Maternal</td>
</tr>
<tr>
<td>Gono et al³</td>
<td>21 yo</td>
<td>DHR 9.6%</td>
<td>Cervical herpes zoster, refractory pneumonia, lower respiratory tract infections, and invasive salmonellosis.</td>
<td>P. aeruginosa</td>
<td>p.(Tyr206*)</td>
<td>Yes</td>
<td>Paternal</td>
</tr>
<tr>
<td>Sarwaret al⁴</td>
<td>43 yo</td>
<td>DHR 3%</td>
<td>Recurrent subcutaneous abscesses, pneumonia, lower respiratory tract infections, and invasive salmonellosis.</td>
<td>B. cepacia</td>
<td>c.1152-2A&gt;G</td>
<td>No</td>
<td>ND</td>
</tr>
<tr>
<td>Amaralet al⁵</td>
<td>18 months old</td>
<td>DHR 16%</td>
<td>Recurrent pneumonia, complicated and inflammatory appendageal mass.</td>
<td>S. typhimurium, S. marcescens and S. epidermidis.</td>
<td>p.(Ile325Phefs*22)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Abbreviations. YO: years old, P: patient, ND: Not determined, CL: Chemiluminescence, DHR: Dihydrorhodamine assay, DCFH-DA: Dichloro-dihydro-fluorescein diacetate assay
*Mutations are described in amino acid change nomenclature using the Alamut Visual.

The woman described is an XL-CGD carrier with a sole starting point in adolescence, and a functional study showed that only 4% of her neutrophils were unaffected. It is reported that infections in carriers are present when the percentage of functional neutrophils is less than 10%; recently Marciano et al described a large study involving X-linked CGD carriers and found severe infections in 15% of them, however, the noninfectious manifestations have not been associated with a lower percentage of functional neutrophils. The features presented in late-onset disease are similar to classic XL-CGD in most cases. In clinical practice, physicians who usually confront these patients should suspect this entity and differentiate it from a secondary immunodeficiency. The antimicrobial therapy prescribed for patients with primary immunodeficiencies is different in comparison with the one used for immunocompetent subjects. We recommend AP for XL-CGD carriers with an extremely skewed X-inactivation; furthermore, medical follow-up, including a DHR assay, should be considered in all carriers due to the risk of skewed X-inactivation associated with age. The case reported herein evidence that XL-CGD can be suspected in females who develop clinical features of CGD in adulthood, even in the absence of a family history, since de novo mutations could be the cause.
REFERENCES


