Adult Onset X-Linked Chronic Granulomatous Disease in a Woman Patient Caused by a \textit{de novo} Mutation in Paternal-Origin \textit{CYBB} Gene and Skewed Inactivation of Normal Maternal X Chromosome

Takahisa Gono\textsuperscript{1}, Masahide Yazaki\textsuperscript{1}, Kazunaga Agematsu\textsuperscript{2}, Masayuki Matsuda\textsuperscript{1}, Kozo Yasui\textsuperscript{2}, Maki Yamaura\textsuperscript{3}, Fumio Hidaka\textsuperscript{4}, Tomoyuki Mizukami\textsuperscript{4}, Hiroyuki Nuno\textsuperscript{i}, Takeo Kubota\textsuperscript{5} and Shu-ichi Ikeda\textsuperscript{1}

Abstract

We report a 28-year-old woman patient suffering from refractory subcutaneous abscess. Stimuli-induced microbicidal reactive oxygen metabolites formation test of the patient’s neutrophils revealed that only 9.6% of the neutrophils produced H\textsubscript{2}O\textsubscript{2}. DNA analysis of the \textit{CYBB} that encodes \textit{gp91}\textsubscript{phox} demonstrated that she was heterozygous for a nonsense mutation, \textsuperscript{206}Trp(TGG)/stop(TGA) and therefore, a diagnosis of adult onset X-linked chronic granulomatous disease was made. Our molecular biological study revealed that her disease was caused by a \textit{de novo} mutation in the \textit{CYBB} gene on the paternal-origin X-chromosome and a skewed inactivation of the normal maternal X-chromosome.

Key words: chronic granulomatous disease, \textit{gp91}\textsubscript{phox}, \textit{CYBB}, X-chromosome

(Inter Med 47: 1053-1056, 2008)
(DOI: 10.2169/internalmedicine.47.0919)

Introduction

Chronic granulomatous disease (CGD) is a rare inherited immunodeficiency disorder, caused by a complete lack of or significant decrease in the production of microbicidal reactive oxygen metabolites (ROM) due to a defective phagocytic NADPH-oxidase (1). Approximately 70% of CGD patients have X-linked CGD caused by mutation of the \textit{CYBB} gene that encodes \textit{gp91}\textsubscript{phox}. For many years the onset of X-linked CGD was thought to occur early in infancy in man patients, with a fatal outcome in adolescence due to recurrent severe bacterial or fungal infections. However, late onset cases of X-linked CGD have been recently reported in some adult woman (2-4). While it has been assumed that the late onset of woman X-linked CGD could be associated with age-related skewing of lyonization (2), the detailed mechanism of onset in adult woman patients remains obscure. Here, we report a woman patient with adult onset X-linked CGD caused by a \textit{de novo} mutation in the paternal-origin \textit{CYBB} gene and skewed inactivation of normal maternal X chromosome.

Case Report

A Japanese woman first noticed recurrent stomatitis and acne on her face and trunk at the age of 21. She visited our hospital, and mild hypergammaglobulinemia (IgG: 2,463 mg/dl, normal: 800 to 2,000 mg/dl) was found. Collagen disorders, such as Behçet disease and systemic lupus erythematosus (SLE), were thought to be the differential diagnoses at that time, but a definite diagnosis was not established. At...
the age of 27, she suffered from refractory subcutaneous ulcerative abscess in the left inguinal region and hip (Fig. 1A). Despite therapy including oral antibiotics and draining, the abscess still persisted 10 months after onset.

Skin biopsy was performed at the hip abscess and the histopathology revealed granulomatous formation and severe infiltration of plasma cells and lymphocytes, indicating the presence of chronic inflammation (Fig. 1B). A small amount
of Pseudomonas aeruginosa was cultured in the biopsy specimen but fungus was not detected. Acid-fast bacteria were not detected in Ziel-Neelsen staining and culture of the tissue. She was thought to have impairment of the immune system and was therefore admitted to our hospital for further examination in September 2004 at the age of 28. Physical examination showed no abnormal findings except for the subcutaneous abscess. Laboratory data demonstrated normal white blood cell count (5,850/μl) and neutrophil count (3,040/μl). Serum CRP (1.50 mg/dl, normal: <0.1 mg/dl) and CH50 (74.8 IU/ml, normal: 30 to 53 IU/ml) were elevated. Serum IgG was also high (2,850 mg/dl, normal: 680-1,620 mg/dl) but IgM, IgA, and IgD were within the normal limits. Monoclonal proteins were not detected in the serum or urine. Anti-nuclear antibody was negative and angiotensin-converting enzyme (ACE) was within the normal range. Serum anti-HIV antibody and Treponema pallidum hemagglutination (TPHA) were negative. Stimuli-induced ROM formation test of the patient’s neutrophils revealed that most of the patient’s neutrophils were unable to produce ROMs with dihydrorhodamine 123. Only 9.6% of the neutrophils produced H2O2 (Fig. 1C). Nitroblue-tetrazolium (NBT) slide test demonstrated about 10% positive cells (data not shown). Thus, she was thought to have adult onset X-linked CGD. There were no patients with CGD in her family.

The patient had been treated with intravenous administration of sodium ampicillin/sodium sulbactam (6 g/day) for two weeks from admission and draining, and subsequently oral antibiotics were given for two weeks. The subcutaneous abscess gradually healed and she was discharged in late October 2004. Subsequently, a prophylactic therapy with sulphamethoxazole-trimethoprim (480 mg/day) was begun and thus far, she has not suffered from severe infectious disease.

Materials and Methods

DNA sequencing of CYBB

After informed consent was obtained from the patient, DNA was purified from peripheral leukocytes. Intronic primer pairs flanking each of the 13 exons of CYBB gene were used to amplify each exon (5) and all exons of CYBB gene were analyzed by direct DNA sequencing. In addition, genomic DNA was also extracted from a buccal swab and direct DNA sequencing of exon 6 of CYBB gene was carried out.

Detection of X-chromosome inactivation at the HUMARA locus

After informed consent was obtained from the patient and her mother, DNA was purified from neutrophils. Using the assay of human androgen receptor (HUMARA) locus involving a methylation-specific polymerase chain reaction (M-PCR) technique (6), an X-inactivation pattern based on the ratio of the maternal inactive X to the paternal inactive X was evaluated in the neutrophils of the patient and her mother. Another X-inactivation pattern based on the ratio of the maternal active X to the paternal active X using specific primers for the unmethylated allele was also evaluated.

Results

The direct DNA sequencing of CYBB gene in both the leukocytes and the buccal epithelial cells revealed that our patient was heterozygous for a nonsense mutation, 618 G→A in exon 6, 300Trp(TGG)→stop(TGA) (Fig. 1D), showing that she was a carrier of CYBB gene mutation.

The assay in the HUMARA locus revealed that the maternal allele was inactivated in the patient, but activated in the patient’s mother (Fig. 1E). The paternal allele was activated in the patient (Fig. 1E). The ratio of the paternal active X to the maternal active X was skewed at 93:7 (Fig. 1E). This lyonization ratio corresponded well to the observed phenotype of only 7-9% of normal neutrophils in the patient.

Discussion

Based on the results of the DNA sequence analysis, a diagnosis of adult onset X-linked chronic granulomatous disease was made. The assay of HUMARA locus (6) strongly suggested that the paternal allele harbored the mutant CYBB gene. Unfortunately DNA analysis could not be performed on her father since he had already died in an accident at the age of 60. However, because he had been healthy until he died, he would not have been a CGD patient. Hence, a de novo mutation must have arisen on the paternal allele of the CYBB gene in the paternal germ line. As the maternal normal allele was gradually skewed, the neutrophils with normal function decreased and the patient developed a subcutaneous abscess as a symptom of CGD.

There have been many woman carriers of X-linked CGD to date and most of them have a balanced X inactivation ratio and are commonly healthy. However, some woman carriers with an X inactivation ratio of normal cells between 3 and 30% occasionally suffer from symptoms such as discoid lupus (DLE) photosensitivity of the skin, or aphthous stomatitis (7-9). Only massive exposure to bacteria or fungi can cause severe and life-threatening infection if the percentage of oxidase-positive phagocytes is approximately 5-10% (2). Carriers with less than approximately 3-5% are more at risk for severe infection (3). Skewing of X-inactivation as a cause of the respective diseases in women has been reported in other X-linked recessive disorders (10). To date, the presence of adult onset X-linked CGD has been reported in only 3 woman patients (2-4). The precise mechanism by which the disease occurs in adults remains unclear but a positive correlation was observed between age and degree of skewing in X-inactivation (11). Indeed, age-related skewing was revealed in one adult woman CGD patient (2) and therefore,
the late onset in woman CGD carriers including the present patient could be explained by this age-related skewing of lyonization.

Although a de novo mutation of the CYBB gene was revealed in two patients (4, 12), in the patient described by Anderson-Cohen et al (12) the patient’s parents had no mutation in the CYBB gene. In that patient, the maternal allele of the CYBB gene had a nonsense mutation, leading to a premature stop codon (12). The normal paternal allele was inactivated by skewed lyonization not only in the patient’s leukocytes, but also in the buccal epithelial cells. These results indicated that a de novo mutation had occurred on the maternal allele and skewedly lyonization caused the X-linked CGD (12). In the other patient, a mutation of the CYBB gene, leading to a premature stop codon, was detected in the patient’s leukocytes, but not in the buccal epithelial cells, indicating that somatic mutation arose in the CYBB gene of the hematopoietic stem cell (4).

The present patient initially presented with recurrent stomatitis before the onset of the subcutaneous abscess. Although the clinical pictures of adult onset X-linked CGD in woman patients are not fully understood, skin lesions including subcutaneous abscess, photosensitive dermatitis, and aphthous stomatitis were often seen especially before the onset of life-threatening bacterial and/or fungal infections (2, 3). The combination of DLE-like skin lesions and aphthous stomatitis, which are commonly observed in collagen diseases such as SLE and Behcet disease, were also cardinal clinical symptoms in woman carriers of X-linked CGD (7-9). Because appropriate prophylactic therapy is quite effective for prevention of severe infectious disease (4, 12), we emphasize that a careful differential diagnosis between X-linked CGD and collagen diseases is needed especially in adult woman patients with skin lesions and/or stomatitis. The stimuli-induced ROM formation test of neutrophils is necessary for the differential diagnosis.

Acknowledgement

This work was supported by a grant from Neuroimmunological Disease Research Committee of the Intractable Disease Division, the Ministry of Public Health, Labor and Welfare, Japan.

References