JACC: CASE REPORTS VOL. 2, NO. 10, 2020

MINI-FOCUS ISSUE: HEART FAILURE

ADVANCED

CASE REPORT: CLINICAL CASE SERIES

Endothelial Function and Oxidative Stress in X-Linked, gp91^{phox} Deficiency, Chronic Granulomatous Disease

Yukihito Higashi, MD, PhD, Shinji Kishimoto, MD, PhD, Tatsuya Maruhashi, MD, PhD

ABSTRACT

Two patients with X-linked chronic granulomatous disease without NAPDH oxidase activity and with high responses of flow-mediated vasodilation are reported. Bone marrow transplantation restored oxidative stress to the levels of those in healthy subjects and decreased flow-mediated vasodilation to the levels of those in healthy subjects in both of the patients. (**Level of Difficulty: Advanced.**) (J Am Coll Cardiol Case Rep 2020;2:1480–3) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Chronic granulomatous disease (CGD) is a rare, heterogenous, and inherited phagocyte disorder that affects approximately 1 in 250,000 births (1). X-linked CGD accounts for approximately 70% of cases of CGD and is due to mutation of the *CYBB* gene encoding gp91^{phox}, which is located in the region Xp21.1 of the short arm of the X chromosome (1). NAPDH oxidase activity is diminished in activated polymorphonuclear leukocytes in these patients, leading to a reduction in reactive oxygen species (ROS), which results in severe and recurrent bacterial and fungal infections.

LEARNING OBJECTIVES

- Patients with X-linked CGD, gp91^{phox} deficiency phenotype X91^{0}, and without NAPDH oxidase activity in leukocytes had very low levels of circulating oxidative stress markers and very high responses of flow-mediated vasodilation.
- Superoxide generated by leukocyte NADPH oxidase plays an important role in endothelial function even under a normal condition without cardiovascular risk factors.

A balance between ambient levels of superoxide and nitric oxide release plays a critical role in the maintenance of normal endothelial function (2). We have shown that 1 mechanism of endothelial dysfunction is an increase in oxidative stress in patients with renovascular hypertension, who are ideal models of excess angiotensin II and angiotensin II-related increase in oxidative stress through the activation of NADPH oxidase (3). By contrast, patients with X-linked CGD are ideal models for determining how endothelium-dependent vasodilation is affected by gp91^{phox} deficiency-related decrease in oxidative stress. Indeed, Violi et al. (4) showed an increase in endothelium-dependent vasodilation and a decrease in the oxidative stress marker isoprostan. We hypothesized that the restoration of ROS by bone marrow transplantation (BMT) would decrease

From the Department of Cardiovascular Regeneration and Medicine, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan. The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Case Reports* author instructions page.

Manuscript received March 20, 2020; revised manuscript received April 25, 2020, accepted May 12, 2020.

ISSN 2666-0849 https://doi.org/10.1016/j.jaccas.2020.05.086

endothelium-dependent vasodilation in patients with X-linked CGD by increasing oxidative stress.

We present flow-mediated vasodilation (FMD) and oxidative stress markers before and after BMT in 2 patients with X-linked CGD who had $gp91^{phox}$ deficiency phenotype $X91_o$.

HISTORY OF PRESENTATION

CASE 1. Case 1 was a 26-year-old man who presented with recurrent upper and lower respiratory infections and hepatic abscesses. His first episode of pneumonia was at the age of 11 years, but he had been well until that time. Since then, he had recurrent pneumonia at the ages of 13, 15, 16, 18, 21, and 24 years, and had an episode of liver abscess at the age of 24 years. The number of episodes of infection (e.g., lung, liver, and skin) that required hospitalization was 11 before BMT. Cytochrome b558 protein, gp91phox, NADPH oxidase activity, and superoxide generation in activated leukocytes were totally absent (**Figure 1A**). The mutation in Case 1 was identified as a single-base substitution of adenosine to thymine in base 1309 in exon 10, resulting in the formation of a stop codon 437 Lys (TAA) (**Figure 1B**). This nonsense mutation was located in the putative NADPH oxidase-binding motif (**Figure 1B**). The phenotype of X-linked CGD was $X91_o$.

CASE 2. Case 2 was a 9-year-old boy who presented with recurrent upper and lower respiratory infections and a fistulized infection by a fungus. He had pneumonia and encephalitis at the age of 2 months and had 2 episodes of liver abscess at the ages of 1 and 3 years. Since then, he had some infections every year. In the following 2 years from the age of 7 years, he had fistulized infections by a fungus in the skin. The number of episodes of infection (e.g., lung, liver, brain, skin, and bone) that required hospitalization was 8 before BMT. Cytochrome b558 protein, gp91phox, NADPH oxidase activity, and superoxide generation in activated leukocytes were totally absent (**Figure 1A**). The mutation in Case 2 was identified as a deletion of the nucleotides TTTGGTACACACATCATCT in base 614 in exon 6, resulting in a frameshift and formation of a stop codon (**Figure 1B**). We confirmed that the mutation in Case 2 was de novo in accordance with previous studies (5) and the *CYBB* gene database. This de novo mutation was localized in the transmembrane region (**Figure 1B**). The phenotype of X-linked CGD was $X91_o$.

MANAGEMENT AND INTERVENTIONS

CASES 1 AND 2. The presence of cytochrome b558 on the surface of intact cells was determined by fluorescence-activated cell sorter analysis after binding on monoclonal antibody 7D5 with fluorescein isothiocyanate–conjugated mouse anti-mouse immunoglobulin. Oligonucleotide primers used in this study for the polymerase chain reaction and sequencing for the *CYBB* ($gp91^{phox}$) gene are presented in Supplemental Table 1. BMT was performed in Cases 1 and 2 as previously described (6). FMD was measured before and after BMT as described previously (7).

Mean FMD was 8.6 ± 3.7% (range 1.8% to 18.7%) in 128 healthy young men (22 ± 2 years of age; range, 17 to 28 years of age). Before BMT, FMD was 27.4% in Case 1 and 26.0% in Case 2. FMD in Cases 1 and 2 before BMT was higher than the maximum FMD in healthy young men (**Figure 2**). Serum malondialdehyde-modified low-density lipoprotein (MDA-LDL) concentration, plasma nitrite/nitrate concentration, urinary excretion of 8-OHdG, and urinary excretion of nitrite/nitrate in 51 healthy young men (22 ± 3 years of age; range, 18 to 28 years of age) and 2 patients with CGD are shown in Supplemental Table 2. Oxidative stress marker levels, serum MDA-LDL concentration, and urinary excretion of 8-OHdG in the 2 cases before BMT were smaller than the minimum values in healthy young men. Plasma nitrite/nitrate concentration was in the range of the mean ± SD values in healthy subjects (Supplemental Table 2).

Cases 1 and 2 had no clinical symptoms and were well after BMT. After BMT, levels of 7D5 staining were restored to the levels of those in normal control subjects in both cases (**Figure 1A**). BMT attenuated FMD from 27.4% to 10.2% in Case 1 and from 26.0% to 9.6% in Case 2 (**Figure 2**). After BMT, serum MDA-LDL concentration and urinary excretion of 8-OHdG increased in both cases, and plasma concentration or urinary excretion of nitrite/nitrate was not altered in either case (Supplemental Table 2).

DISCUSSION

Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis. Oxidative stress plays an important role in the pathogenesis and development of cardiovascular diseases (2). Enhanced nitric oxide inactivation caused by excess production of ROS under the condition of NADPH oxidase activation plays an important role in endothelial dysfunction. In the present study, FMD was markedly enhanced in 2 patients with X-linked CGD. In addition, after BMT for treatment of X-linked CGD, FMD decreased to the levels of those in healthy

ABBREVIATIONS AND ACRONYMS

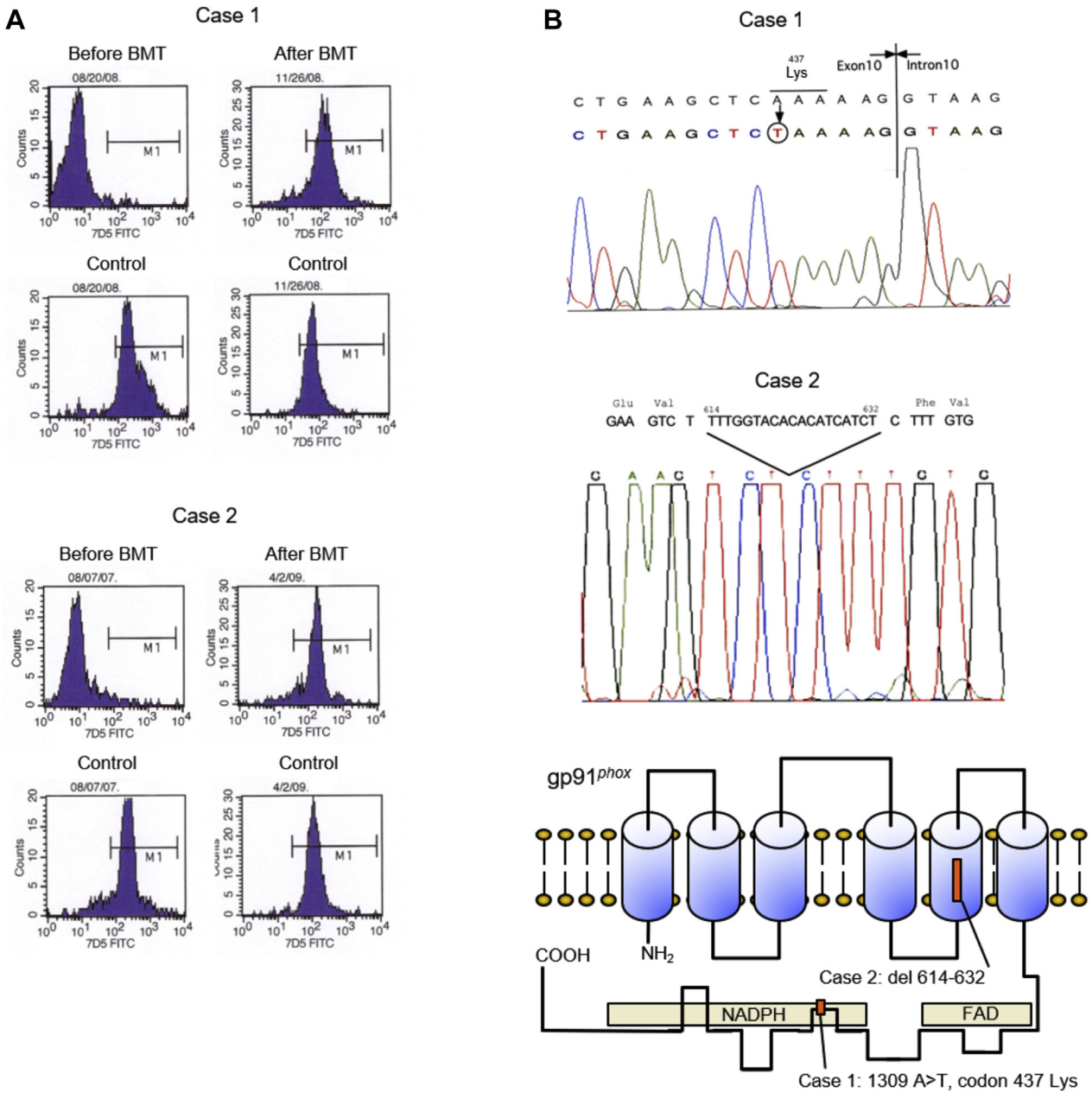
BMT = bone-marrow transplantation

CGD = chronic granulomatous disease

FMD = flow-mediated vasodilation

ROS = reactive oxygen species

FIGURE 1 Superoxide Generation Before and After BMT and gp91phox Mutations in Cases 1 and 2



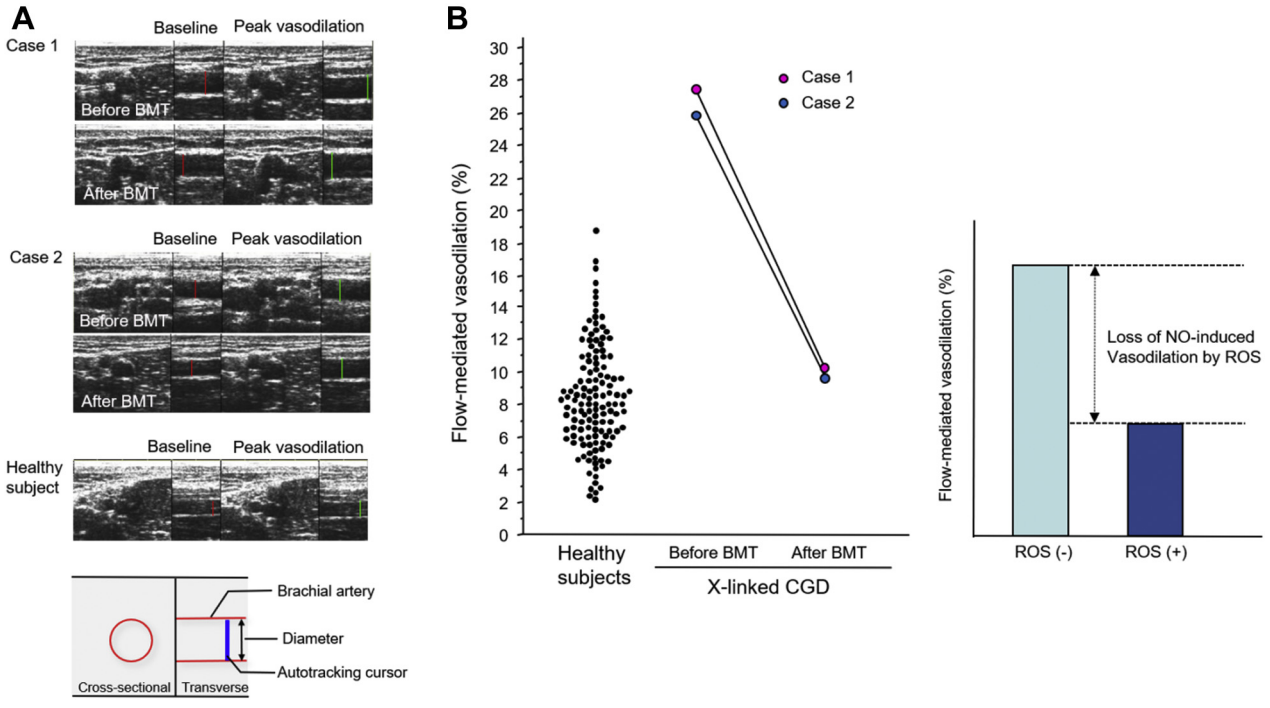
(A) The presence of gp91phox is shown using flow cytometric analysis of monoclonal antibody 7D5 in leukocytes before and after bone marrow transplantation (BMT) in Case 1, Case 2, and normal control subjects. **(B)** Direct nucleotide sequence analysis of all of the exons of gp91^{phox} is shown in Case 1, Case 2, and a normal control subject, and a schematic representation of the gp91^{phox} molecule. Mutation in Case 1: nucleotide 1309 A>T, codon 437 Lys in exon 10. Mutation in Case 2: nucleotide 614-632 deletion (del) TTTGGTACACACATCATCT, frameshift in exon 6. Localization of mutation in gp91phox in Case 1 **(short orange bar)** and that in Case 2 **(tall orange bar)** are presented. FITC = fluorescein isothiocyanate.

subjects by increasing oxidative stress. These findings suggest that even a normal condition of oxidative stress in healthy subjects may induce endothelial dysfunction, leading to atherosclerosis by long-term exposure to a normal range of oxidative stress.

CONCLUSIONS

Oxidative stress in healthy subjects attenuates endothelium-dependent vasodilation by about two-thirds compared with that under the condition in

FIGURE 2 **FMD Before and After BMT in Cases 1 and 2**



(A) Representations of flow-meditated vasodilation (FMD) are shown using an autotracking system before and after bone marrow transplantation (BMT) in Cases 1 and 2 and a healthy young male subject. **(B)** Flow-mediated vasodilation is shown in healthy young male subjects and before and after BMT in Cases 1 and 2 **(left)**. Putative role of oxidative stress, reactive oxygen species (ROS), in nitric oxide (NO)-induced vasodilation in healthy subjects **(right)**.

which oxidative stress is absent. These findings also indicate that oxidative stress is a dual-edged sword for the maintenance of a healthy condition. It is likely that superoxide generated by leukocyte NADPH oxidase plays an important role in endothelial function even under normal conditions without cardiovascular risk factors.

ADDRESS FOR CORRESPONDENCE: Prof. Yukihito Higashi, Department of Cardiovascular Regeneration and Medicine, Research Institute for Radiation Biology and Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. E-mail: yhigashi@hiroshima-u.ac.jp.

REFERENCES

1. Winkelstein JA, Marino MC, Johnston RB Jr., et al. Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine (Baltimore) 2000;79:155–69.

2. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 2000;87:840–4.

3. Higashi Y, Sasaki S, Nakagawa K, et al. Endothelial function and oxidative stress in renovascular hypertension. N Engl J Med 2002;346:1954–62.

4. Violi F, Sanguigni V, Carnevale R, et al. Hereditary deficiency of gp91(phox) is associated with enhanced arterial dilatation: results of a multicenter study. Circulation 2009;120:1616–22.

5. Rae J, Newburger PE, Dinauer MC, et al. X-linked chronic granulomatous disease: mutations in the CYBB gene encoding the gp91-phox component of respiratory-burst oxidase. Am J Hum Genet 1998;62:1320–31.

6. Horwitz ME, Barrett AJ, Brown MR, et al. Treatment of chronic granulomatous disease with nonmyeloablative conditioning and a T-cell-depleted hematopoietic allograft. N Engl J Med 2001;344:881–8.

7. Soga J, Nakamura S, Nishioka K, et al. Relationship between augmentation index and flow-mediated vasodilation in the brachial artery. Hypertens Res 2008;31:1293–8.

KEY WORDS chronic granulomatous disease, endothelial function, gp91phox, NADPH oxidase, nitric oxide, oxidative stress

APPENDIX For supplemental tables, please see the online version of this paper.