—**Report on Experiments and Clinical Cases**—

An Asymptomatic Heterozygous Female with Fabry Disease: Implications for Enzyme Replacement Therapy

Shin-ichiro Inagaki[1], Makoto Migita[1,2], Mari Hayakawa[1], Atushi Fujita[1], Junko Yoshida[1], Masamichi Ishizaki[3], Masaharu Kotani[4], Hitoshi Sakuraba[4], Takashi Shimada[2], Mutsumi Murakami[1] and Yoshitaka Fukunaga[1]

[1]Department of Pediatrics, Nippon Medical School
[2]Department of Molecular and Medical Genetics, Nippon Medical School
[3]Department of Pathology, Nippon Medical School
[4]Department of Clinical Genetics, the Tokyo Metropolitan Institute of Medical Science

Abstract

We report an asymptomatic female with Fabry disease immunohistochemically diagnosed by renal biopsy. She was initially diagnosed as having nephrotic syndrome, and renal biopsy was performed for pathological diagnosis. The renal specimen revealed non-specific findings (minor glomerular abnormalities) for nephrotic syndrome. Numerous laminated bodies in glomerular epithelial cells in electron microscopic findings and accumulations of ceramidetrihexoside immunohistochemically were observed and she was diagnosed with Fabry disease. However, no other laboratory data or clinical findings supported the diagnosis of Fabry disease. Since the efficacy of recombinant human alpha-galactosidase replacement therapy in this disease has been reported, whether enzyme replacement therapy for subclinical Fabry female patients is indicated or not is an important issue.
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Key words: Fabry disease, enzyme replacement therapy, asymptomatic heterozygote, immunohistochemical diagnosis

Introduction

Fabry disease is an X-linked lysosomal storage disease caused by a congenital deficiency of alpha-galactosidase (Gal) that manifests with a wide variety of symptoms, renal failure, heart failure, cerebrovascular attack[1]. To treat this disease, enzyme replacement therapy (ERT) using a recombinant human alpha-Gal (rh-alpha-Gal, Fabrazyme®, Genzyme Corporation) has been established and clinical efficiency has been reported in affected male patients[2,3]. The efficacy of rh-alpha-Gal replacement therapy in subclinical Fabry female patients has not been much discussed.

Case Report

A 15-year-old overweight female (height 152.9 cm, weight 98.3 kg) was admitted to the hospital with marked edema and proteinuria. Hypoproteinemia and hypercholesterolemia were discovered on

Correspondence to Makoto Migita, MD, PhD, Department of Pediatrics, Nippon Medical School, 1–1–5 Sendagi, Bunkyo-ku, Tokyo 113–8603, Japan
E-mail: Migita-Makoto-bmb@nms.ac.jp
Journal Website (http://www.nms.ac.jp/jnms/)

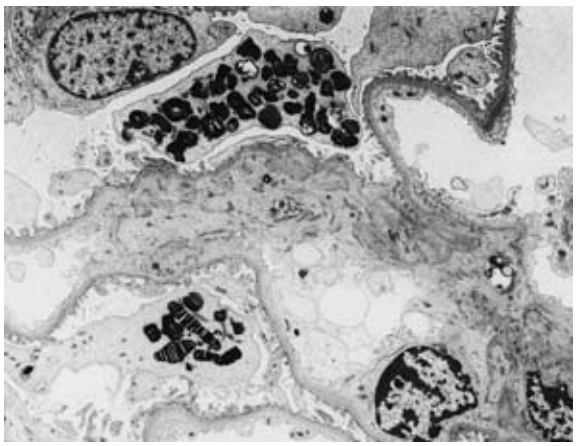

Fig. 1 Electron microscopic image of numerous laminated bodies in glomerular epithelial cells, which are highly characteristic findings in Fabry disease (Magnification, ×3,000)

general examination. The parents were not consanguineous and Fabry disease was not present in either the paternal or maternal families. On admission, she had no angiokeratoma and no corneal opacity on slit-lamp microscopy. Hypoproteinemia (serum total protein 4.0 g/dl; albumin 1.5 g/dl), hypercholesterolemia (total cholesterol 654 mg/dl), and proteinuria (urinary protein excretion ranged from 27.3 to 36.7 g/day) were confirmed. Hematological findings were normal. Serum urea nitrogen was 10.1 mg/dl and serum creatinine was 0.67 mg/dl. Liver function studies were normal. Other laboratory data were as follows: IgG 259 mg/dl, IgA 109 mg/dl, IgM 347 mg/dl, CH50 38.1 IU/ml, C_3 219 mg/dl, C_4 32 mg/dl, antinuclear antibody (−), and anti-DNA antibody ds (−), ss (−). Urine analysis showed no abnormal findings, except proteinuria. She was diagnosed as having nephrotic syndrome based on these data. Proteinuria disappeared in seven days with steroid therapy, and complete remission has been maintained. Renal biopsy was performed for pathological diagnosis. Light microscopy of the renal specimen revealed non-specific findings (minor glomerular abnormalities). Direct immunofluorescent studies showed negative staining for IgG, IgA, IgM, C_{1q}, C_3 and C_4. Electron microscopic findings demonstrated glomerular epithelial cells containing numerous laminated bodies, which are highly characteristic of Fabry disease (**Fig. 1**). However, no other family member had been diagnosed with Fabry disease and no significant laboratory findings specific to Fabry disease were observed on examination: alpha-Gal activity of skin fibroblasts was 68.4 nmol/h/mg protein (normal control: 49.2 nmol/h/mg protein) with 4-methylumbelliferyl-alpha-galactoside used as substrate[4]. Total ceramidetrihexoside (CTH) in urine was 0.13 pg/mg・Cr (normal control 0.12 pg/mg・Cr). No mutations were found in the coding regions of alpha-Gal gene exons 1 to 7. These data did not support the diagnosis of Fabry disease. Indirect immunofluorescent staining was performed using a mouse monoclonal antibody that specifically detects CTH[5]. Accumulation of CTH was observed segmentally in the cytoplasm of the renal tubules (**Fig. 2b**) and glomerulus (**Fig. 2c**). To identify the localization of CTH, skin fibroblasts derived from this patient, a normal control, and an affected male patient with Fabry disease (positive control) were stained with the anti-CTH antibody and anti-Lamp (lysosomal-associated membrane protein)-2 antibody. No accumulation of CTH was detected in the normal control, while CTH was accumulated in the lysosomes and CTH accumulation was seen in the Fabry patient cells (data no shown). In the present case, accumulation of CTH was colocalized in Lamp-2-positive areas, but some Lamp-2 positive cells were negative for CTH (**Fig. 3**), consistent with the diagnosis of heterozygote for Fabry disease.

Discussion

Fabry disease is an X-linked lysosomal storage disorder with accumulation of CTH primarily in the vascular endothelium affecting mainly the kidney, heart and central nervous system. In affected males, the progressive accumulation of CTH leads to symptomatic severity and death usually occurs from renal failure, or from cardiac or cerebrovascular disease[1]. Disease expression is uniform in affected males, but is highly variable in heterozygous females[1,6], because the X chromosome is randomly inactivated[7]. Fabry disease is usually diagnosed by decreased levels of alpha-Gal activity in peripheral leukocytes or cultured skin fibroblasts, but these

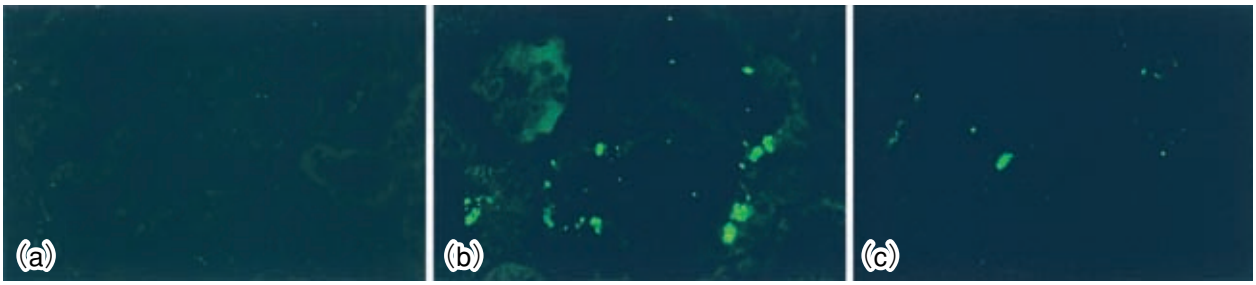

Fig. 2 CTH was visualized using an immunofluorescence-labeled anti-CTH antibody. Negative control: renal tubule in IgA nephropathy (a). Accumulation of CTH was observed segmentally in cytoplasm of the renal tubules (b) and glomerulus (c).

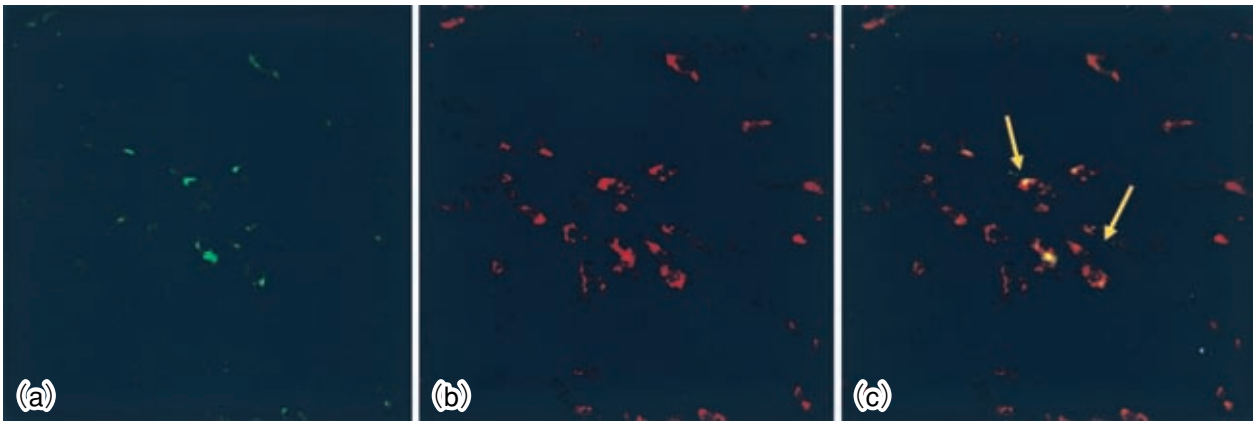

Fig. 3 Localization of CTH in skin fibroblasts derived from this patient.
(a) anti-CTH antibody, (b) anti-Lamp (lysosomal-associated membrane protein)-2 antibody, (c) overlay. Accumulation of CTH was colocalized in Lamp-2-positive areas, but some Lamp-2 positive cells were negative for CTH in this patient.

routine enzyme activity assays do not always distinguish Fabry heterozygotes with high residual enzyme activity from normal individuals with low enzyme activity. Human alpha-Gal cDNA and genomic sequences have been isolated[8], and over 300 mutations have been identified, approximately 90% of which are coding region mutations[9]. They have to find the mutation for the accurate diagnosis of Fabry disease. In this case, no mutations were found in the coding regions of alpha-Gal gene exons 1 to 7, and immunofluorescent staining of skin fibroblasts with anti-CTH antibody is a useful way to diagnose heterozygous Fabry disease, as previously reported[10]. A few Japanese male patients with Fabry disease have been reported, among whom no gene mutations could been detected in the protein-coding region; however, the amounts of the alpha-Gal mRNA were decreased[11]. In these patients, there are presumably some mutations outside the coding region that involve the transcription of alpha-Gal. The present female case might have such a gene mutation or another unknown one on one of the alleles. The underlying pathogenesis should be clarified in the near future.

Until the recent advent of rh-alpha-Gal replacement therapy, little could be done for patients with Fabry disease, but controlled trials of rh-alpha-Gal (Fabrazyme, Genzyme) replacement therapy have demonstrated improvements in clinical symptoms and renal function[2,3]. It has been reported that heterozygotes with Fabry disease may have cerebral infarction, renal failure and/or cardiac involvement in adult life[1,9,12]. The efficacy of rh-alpha-Gal replacement therapy in heterozygotes has been reported[12]. Enzyme replacement therapy has now been approved for Fabry disease all over the world.

This disorder is caused by the gradual accumulation of CTH in multiple organs and because a promising enzyme replacement therapy has now been established, initiation of enzyme replacement early in life may prove to be beneficial. Certainly affected males should begin enzyme replacement early, prior to symptoms. Even though the total alpha-Gal activity in peripheral leukocytes or cultured skin fibroblasts of heterozygous females is within the normal range, the expression of alpha-Gal in the kidneys or cardiovascular system, which are the most significantly affected areas in this disease, may be decreased because of the random inactivation of the X chromosome. When we consider that heterozygotes with Fabry disease may produce symptoms as in Fabry male patients, we do have to consider whether ERT for subclinical Fabry female patients is indicated or not.

References

1. Desnick RJ, Ioannou YA, Eng CM: In The metabolic & molecular bases of inherited disease: a-galactosidase A deficiency: Fabry disease (Scriver CR, Beaudet AL, Sly WS, Valle D, eds), 2001; pp 3733–3774, McGrow-Hill, NewYork.
2. Eng CM, Guffon N, Wilcox WR, Germain DP, Lee P, Waldek S, Caplan L, Linthorst GE, Desnick RJ; International Collaborative Fabry Disease Study Group: Safety and efficacy of recombinant human A replacement therapy in Fabry's disease. N Engl J Med 2001; 345: 9–16.
3. Thurberg BL, Rennke H, Colvin RB, Dikman S, Gordon RE, Collins AB, Desnick RJ, O'Callaghan M: Globotriaosylceramide accumulation in the Fabry kidney is cleared from multiple cell types after enzyme replacement therapy. Kidney Int 2002; 62: 1933–1946.
4. Mayes JS, Scheere JB, Sifers RN, Donaldson ML: Differential assay for lysosomal a-galactosidase in human tissue and its application to Fabry's disease. Clin Chem Acta 1981; 112: 247–251.
5. Kotani M, Kawashima I, Ozawa H, Ogura K, Ariga T, Tai T: Generation of one set of murine monoclonal antibodies specific for globo-series glycolipids: Evidence for differential distribution of the glycolipids in rat small intestine. Arch Biochem Biophys 1994; 310: 89–96.
6. Marguery MC, Giordano F, Parant M, Samalens G, Levade T, Salvayre R, Maret A, Calvas P, Bourrouillou G, Cantala P: Fabry's disease: Heterozygous form of different expression in two monozygous twin sisters. Dermatology 1993; 187: 9–15.
7. Lyon MF: Sex chromatin and gene action in the mammalian X-chromosome. Am J Hum Genet 1962; 14: 135–148.
8. Bishop DF, Calhoun DH, Bernstein HS, Hantzopoulos P, Quinn M, Desnick RJ: Human a-galactosidase A: Nucleotide sequence of a cDNA clone encoding the mature enzyme. Proc Natl Acad Sci USA 1986; 85: 3903–3907.
9. Garman SC, Garboczi DN: The molecular defect leading to Fabry disease: structure of human alpha-galactosidase. J Mol Biol 2004; 337: 319–335.
10. Itoh K, Kotani M, Tai T, Suzuki H, Utsunomiya T, Inoue H, Yamada H, Sakuraba H, Suzuki Y: Immunofluorescence imaging diagnosis of Fabry heterozygotes using confocal laser scanning microscopy. Clin Genet 1993; 44: 302–306.
11. Nakao S, Takenaka T, Maeda M, Kodama C, Tanaka A, Tahara M, Yoshida A, Kuriyama M, Hayashibe H, Sakuraba H: An atypical variant of Fabry's disease in men with left ventricular hypertrophy. N Engl J Med 1995; 333: 288–293.
12. Waldek S: PR interval and the response to enzyme-replacement therapy for Fabry's disease. N Engl J Med 2003; 348: 1187–1188.

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