A novel mutation causing type 1 Gaucher disease found in a Japanese patient with gastric cancer

A case report

Sakura Hosoba, MD, PhD\textsuperscript{a,*}, Katsuyuki Kito, MD, PhD\textsuperscript{a}, Yukako Teramoto, MD\textsuperscript{a}, Kaori Adachi, PhD\textsuperscript{b}, Ryota Nakanishi, BS\textsuperscript{c}, Ai Asai, MD\textsuperscript{a}, Masaki Iwasa, MD, PhD\textsuperscript{a}, Rie Nishimura, MD, PhD\textsuperscript{a}, Suzuko Moritani, MD, PhD\textsuperscript{b}, Masahiro Kawahara, MD, PhD\textsuperscript{a}, Hitoshi Minamiguchi, MD, PhD\textsuperscript{a}, Eiji Nanba, MD, PhD\textsuperscript{b}, Ryoji Kushima, MD, PhD\textsuperscript{a}, Akira Andoh, MD, PhD\textsuperscript{b}

Abstract

Rationale: Gaucher disease (GD) is an autosomal recessive disorder that leads to multigorgan complications caused by \( \beta \)-glucocerebrosidase deficiency due to mutations in the \( \beta \)-glucocerebrosidase-encoding gene (\( \text{GBA} \)). GD morbidity in Japan is quite rare and clinical phenotype and gene mutation patterns of patients with GD in Japan and Western countries differ considerably. Of Japanese patients with GD, 57% develop types 2 or 3 GD with neurologic manifestations and younger onset, whereas only 6% of patients with GD develop those manifestations in Western countries. Thus, it is relatively difficult to find and diagnose GD in Japan.

Patient concerns: A 69-year-old Japanese female with mild anemia and thrombocytopenia but without neurologic symptoms was initially referred for gastric cancer. Preoperative \( ^{18} \text{F}-\text{deoxyglucose positron emission tomography/computed tomography (FDG PET/CT)} \) showed accumulation in the bone marrow and paraabdominal lymph nodes. Following bone marrow aspiration found, abnormal foamy macrophages in the bone marrow and electron microscopy revealed that the macrophages were filled with tubular-form structures. Adding to these signs suggestive of a lysosomal disease, serum \( \beta \)-glucocerebrosidase activity test found decreased. Sequencing of the patient’s \( \text{GBA} \) gene revealed a RecNciI recombinant mutation and the novel mutation K157R (c.587A\( \rightarrow \)G).

Diagnoses: On the basis of these findings and clinical manifestations, the final diagnosis of type 1 GD was made.

Interventions: Enzyme replacement therapy (ERT) with velaglucerase \( \alpha \) was started after the diagnosis of type 1 GD.

Outcomes: The patient’s \( \beta \)-glucocerebrosidase activity as well as hemoglobin and platelet levels were restored by ERT without any side effects. Bone marrow aspirations 10 months after the start of the treatment with velaglucerase \( \alpha \) showed reduction of Gaucher cells in bone marrow to 2% from 4% of total cellularity.

Lessons: This is the first report of \( ^{18} \text{F}-\text{FDG PET/CT} \) application providing a clue for GD diagnosis. A novel mutation in \( \text{GBA} \) is described, which implies a potential pool of patients with GD with this mutation in Japan.

Abbreviations: ERT = enzyme replacement therapy, \( \text{FDG PET/CT} = ^{18} \text{F}-\text{deoxyglucose positron emission tomography/computed tomography, GBA = } \beta \text{-glucocerebrosidase-encoding gene, GD = Gaucher disease.}

Keywords: anemia, fluoro-deoxyglucose positron emission tomography, Gaucher disease, mutation, thrombocytopenia

1. Introduction

Gaucher disease (GD) is an autosomal recessive disease caused by the deficiency of \( \beta \)-glucocerebrosidase, a lysosomal enzyme in monocytes and macrophages that catalyzes hydrolysis of \( \beta \)-glucocerebroside to glucose and ceramide.\textsuperscript{[1]} The deficiency of \( \beta \)-glucocerebrosidase leads to the accumulation of its substrates in lysosomes followed by progressive complications in the liver, spleen, lung, bone, bone marrow, and nervous system.\textsuperscript{[2]} There are 3 subtypes of GD classified by the presence and extent of central nervous system (CNS) involvement and prognosis. Type 1 GD does not include CNS manifestations. Type 2 is associated with acute onset in infancy and severe CNS manifestations. Type 3 develops chronically with mild CNS symptoms.\textsuperscript{[3]}

The definite diagnosis of GD must be based upon experimental proof of the low activity of \( \beta \)-glucocerebrosidase in total leukocytes or mononuclear cells or in fibroblasts cultured from skin biopsies.\textsuperscript{[4]} Genotype identification of the \( \beta \)-glucocerebrosidase gene (\( \text{GBA} \)) supports the definite diagnosis of GD. Genotype identification seems worthy even for the patients who had already been diagnosed with GD for three reasons. First, the patients with type 3 GD maintain relatively normal \( \beta \)-glucocerebrosidase activity and are sometimes difficult to diagnose by the latter parameter only. \( \text{GBA} \) genotype testing should be an essential clue to diagnose GD in such patients. Second, \( \text{GBA} \)
Genotype identification is important to determine if the patient with GD is a candidate for chaperone therapy, which is effective for patients with GD with particular mutations, including N370S (c.1226A>G) and F2131 (c.754T>A). Third, GBA genetic analysis may predict the patient’s prognosis because significant correlations between clinical phenotypes and GBA mutations have been reported. For example, 62% of the patients with type 1 GD have N370S allele, whereas most patients with types 2 and 3 GD have L444P alleles (48% and 69%, respectively). Tajima et al reported that the diagnosis of type 1 GD may change later into type 3 if the patients have L444P allele in GBA. Moreover, because GD is an inherited disease, the patient’s genotype identification may enable the early diagnosis of GD and early start of the treatment of the patient’s children.

There are several therapeutic options for GD, including enzyme replacement therapy (ERT), substrate reduction therapy, chaperone therapy, and allogeneic hematopoietic stem-cell transplantation. ERT has the longest history among them. Since mid-1990’s, imiglucerase, a recombinant form of β-glucocerebrosidase, has been administrated as an ERT drug to treat the patients with GD. Recently, velaglucerase α, which is driven from a gene-activated human cell line, has been approved as a new ERT treatment in Japan.

Here, we report a Japanese patient who presented with slight anemia and thrombocytopenia without major complaints and was initially diagnosed at the preoperative 18F-deoxyglucose positron emission tomography/computed tomography (FDG PET/CT) scan with gastric cancer. Ultimately, the patient was diagnosed with type 1 GD caused by the novel K157R (c.587A>G) mutation in GBA.

2. Case report

A 69-year-old female was referred to our hospital for the treatment of gastric cancer invading the submucosa at the gastric angle, which had been suggested by upper endoscopy during periodic medical checkup. She had no major complaints and was under treatment for hyperlipidemia and type 2 diabetes mellitus. She had a history of left ovarian cancer followed by left ovariectomy. The marriage of her deceased parents was consanguineous.

Her blood test was within normal limits except for slight anemia (hemoglobin 11.8 g/dL), mild thrombocytopenia (platelets 14.8 x 10^4/mL), and high level of ferritin (553.7 ng/mL) (Table 1). Preoperative 18F-FDG PET/CT scan showed strong bilateral accumulations in the bone marrow of humeri and femora and in the paraabdominal aortic lymph nodes in addition to a weaker accumulation in the stomach without accumulation in the gastric lymph nodes.

Table 1

| Laboratory findings at diagnosis. | White blood cell 9000/μL | Total protein 6.1 g/dL | CK 64 IU/L | Leukocyte differential | Albumin 3.9 g/dL | Fe 52 μg/dL | Neutrophils 48.8% | ALT 16 IU/L | Ferritin 553.7 mg/dL |
|---------------------------------|--------------------------|------------------------|-----------|------------------------|------------------|-------------|-----------------|------------|-------------------|
| Eosinophils                     | 1.8%                     | 16 U/L                 |           | F2131                  | 146 U/L          | 15 mg/dL    | T-Bil 0.57 mg/dL | pA 157 mg/dL |                  |
| Basophils                       | 1.8%                     | 14 U/L                 |           |                        |                  |             |                 |            |                   |
| Lymphocytes                     | 40.2%                    | 15 U/L                 |           |                        |                  |             |                 |            |                   |
| Monocytes                       | 8.2%                     | 3.8 mg/dL              |           |                        |                  |             |                 |            |                   |
| Red blood cells                 | 3.98 x 10^12/μL          | 16.8 mg/dL             |           |                        |                  |             |                 |            |                   |
| Hemoglobin                      | 11.9 g/dL                | 3.8 mg/dL              |           |                        |                  |             |                 |            |                   |
| Hemoglobinuria                  | 34.6%                    | 8.9 mg/dL              |           |                        |                  |             |                 |            |                   |
| Reticulocytes                   | 20%                      | 3.8 mg/dL              |           |                        |                  |             |                 |            |                   |
| Platelets                       | 14.8 x 10^12/μL          | 15 mg/dL               |           |                        |                  |             |                 |            |                   |

β2-MG = beta 2-microglobulin, γ-GT = gamma-guanosine triphosphate, ALT = alanine transaminase, AST = aspartate transaminase, CK = creatine kinase, CRP = C reactive protein, LD = lactate dehydrogenase, T-Bil = total bilirubin, UIBC = unsaturated iron binding capacity, UN = urea nitrogen.

Figure 1. (A) Preoperative 18F-deoxyglucose positron emission tomography/computed tomography scan shows atypical accumulation at the bilateral brachia and thigh bones and the paraabdominal aortic lymph nodes in addition to weaker accumulation in the stomach without accumulation in the gastric lymph nodes. (B) Histopathologic findings in a bone marrow clot. Large foamy histiocytes constitute sheet-like pattern (hematoxylin and eosin stain; original magnification, x 100). (C) Histopathologic findings in a bone marrow smear. The histiocytes have huge basophilic enlarged cytoplasm containing wrinkled structures (Wright–Giemsa stain; original magnification, x 1000). (D) Histopathologic findings in a bone marrow smear. The cytoplasm of the abnormal histiocytes is strongly positive for acid phosphatase stain (original magnification, x 1000).
stomach revealed that the gastric cancer was poorly differentiated paraabdominal lymphadenectomy were performed. cancer was considered as operative, so distal gastrectomy and nodes but no paragastric lymph nodes. Thus, the patient CT found a limited accumulation in the paraabdominal lymph proliferation and occupied most of the lymph node in the absence of gastric paraabdominal lymph node. Atypical large histiocytes with foamy cytoplasm cytokeratin staining, which suggested Gaucher cell phenotype, acid Schiff, and acid phosphatase but were negative for anti-pan cellularity that were stained positively for CD68, CD163, periodic abnormalities were found by head magnetic resonance imaging, which confirmed the classification of GD as type 1. The ERT with every-other-week intravenous infusion of 60 units per kilogram of velaglucerase α, a recombinant β-glucocerebrosidase that was most recently approved in Japan, was started after the diagnosis of GD. Hemoglobin and platelet levels were restored at 2 and 3 months after velaglucerase α administration, respectively. Bone marrow samples collected 10 months after velaglucerase α administration showed reduction of Gaucher cells in bone marrow to 2% of total cellularity. The patient has received ERT for 16 months without any side effect up to the present. This case report was approved by the ethics committee of Shiga University of Medical Science, Shiga, Japan and written informed consent was obtained.

### Table 2

| Diagnosis | Result | Normal range |
|-----------|--------|-------------|
| ACP       | 18.4 IU/L | ≤14.3 IU/L |
| ACE       | 20.5 IU/L | 7.7–29.4 IU/L |
| GBA       | 1.4 nmol/mg protein/h | 4.1–9.7 nmol/mg protein/h |

ACE = angiotensin converting enzyme, ACP = acid phosphatase, GBA = β-glucocerebrosidase activity.

adenocarcinoma and that paraabdominal lymph nodes included abnormal sheet-like proliferation of foamy cells, indicating a lysosomal disease, for example, GD, as was also suggested by the abnormal foamy cells of the aspirated bone marrow specimen (Fig. 2B).

As a result of those findings, GD was highly suspected. The diagnosis of GD was confirmed by the low activity of β-glucocerebrosidase and concomitant acid phosphatase elevation (Table 2). Additionally, after the diagnosis, we sent the patient’s blood sample to the Tottori University for the genotypic test of GBA. GBA gene was analyzed using direct sequencing according to the methods described by Mitsui et al. The analysis found the compound heterozygote mutations of K157R (c.387A>G) on exon 6 and RecNcl, including 1.444P (c.1444T>C), A456P (c.1483G>C), V460 (c.1497G>C), on exon 11 of the GBA gene (Fig. 3).

The patient did not develop any neurologic symptoms, and no abnormalities were found by head magnetic resonance imaging, which confirmed the classification of GD as type 1.

The ERT with every-other-week intravenous infusion of 60 units per kilogram of velaglucerase α, a recombinant β-glucocerebrosidase that was most recently approved in Japan, was started after the diagnosis of GD. Hemoglobin and platelet levels were restored at 2 and 3 months after velaglucerase α administration, respectively. Bone marrow samples collected 10 months after velaglucerase α administration showed reduction of Gaucher cells in bone marrow to 2% of total cellularity. The patient has received ERT for 16 months without any side effect up to the present. This case report was approved by the ethics committee of Shiga University of Medical Science, Shiga, Japan and written informed consent was obtained.

### 3. Discussion

To the best of our knowledge, this is the first report of the novel K157R (c.387A>G) mutation in GBA that caused GD and of the use of 18F-FDG PET/CT for suggestive diagnosis of GD in a patient with slight anemia and thrombocytopenia but no complaints.

The morbidity of GD in Japan is much less than in Western countries (1 per 3.3 × 10^5 and 1.16 per 1 × 10^5 live births, respectively), and the information about Japanese patients with GD is relatively limited. Tajima et al reported that 38% of Japanese patients with GD develop types 2 or 3 GD, which is accompanied by neurologic symptoms (24% and 34% of cases, respectively). In contrast, only 1% or 5% of patients with GD globally are diagnosed as types 2 or 3 GD, respectively, according to the worldwide registry reported by Charrrow et al. As the age at diagnosis for the patients with types 2 and 3 GD is usually younger than that of patients with type 1 GD, Japanese patients are diagnosed with GD at a relatively younger age. Thus, in Japan, it is quite difficult to diagnose GD in adult individuals who present with slight anemia and thrombocytopenia but exhibit no

![Figure 2. (A) Electron microscopy findings in the bone marrow. The abnormal histiocyte distorted by enlarged cytoplasm contained enlarged lysosomes (A-1, ×2000). Further magnification shows that the lysosomes are filled with various tubular structures (A-2, ×25,000). (B) Histopathologic findings in the excised paraabdominal lymph node. Atypical large histiocytes with foamy cytoplasm proliferated and occupied most of the lymph node in the absence of gastric cancer cell invasion (hematoxylin and eosin stain; original magnification, ×100).](image-url)
In our case, 18F-FDG uptake in the bone marrow was the important clue to suspect GD. The correlation between 18F-FDG accumulation in bone marrow and GD was previously reported by Erba et al.\[^{14}\] In their report, all 7 enrolled patients who had been diagnosed previously as type 1 GD had 18F-FDG accumulation in the bone marrow. Furthermore, the score based on the extension and magnitude of 18F-FDG uptake in the bone marrow was highly correlated to the clinical severity score. However, we believe that no case reports that actually utilized 18F-FDG PET/CT for GD diagnosis had been published before our present case. Moreover, our case is the first report of K157R mutation in GBA.

GBA is located on 1q21; its total length is 7kb, and it has 11 exons, and there is a highly homozygous pseudogene that easily recombines with GBA. Over 300 mutations causing GD have been reported. RecTL (c.1342G>C, c.1448T>C, c.1483G>C, and c.1497G>C) and RecNciI (c.1448T>C, c.1483T>G, and c.1497G>C) are the well-known recombinant mutations between GBA and that pseudogene.\[^{15}\]

The mutations causing GD are significantly associated with patient’s ethnicity. N370S (c.1226A>G) is common among Ashkenazi Jewish patients, in which it is found in approximately 70% of cases, whereas in an Asian cohort, this mutant allele has been found only in 12% of cases.\[^{15}\] The prevalence of F2131 (c.754T>A) and L444P (c.1448T>C) among Japanese patients is about 41% and 11%, respectively, whereas in Ashkenazi Jewish patients, those mutations are relatively rare.\[^{8}\] Genetic screening for 8 common mutations, including N370S, L444P, F2131, R463C (c.1504C>T), 84GG (c.84dupG), IVS2+1 (c.115+1G>A), D409H (c.1342G>C), and RecNciI, can identify causative mutations in most Ashkenazi Jewish patients. However, this gene screening cannot recognize mutations in 39% of Japanese patients.\[^{16}\] To detect mutations unidentified by the

**Figure 3.** Mutation analysis of the GBA gene in the patient shows heterozygous point mutation K157R (c.587A>G) on exon 6 (A) and heterozygous recombinant mutations of RecNciI, including L444P (c.1448T>C), A456P (c.1483G>C), and V460 (c.1497G>C) on exon 11 (B).
gene screening, comprehensive resequencing of the GBA gene for all 11 exons is required.

To verify that K157R was responsible for GD in the patient studied, the heterozygous mutations of K157R and RecNciI were confirmed by using DdeI, a restriction enzyme recognizing double stranded deoxyribonucleic acids. However, our case report has a limitation in that it remained unclear whether the mutation was de novo or inherited, as we could not obtain genetic material of the patient’s parents or other relatives. Assuming that K157R was inherited from the parents and was also passed to the patient’s offspring, there should be a population carrying K157R in Japan. For such population, which could potentially develop GD, further investigation of this gene is needed.

In conclusion, our case demonstrated the utility of $^{18}$F-FDG PET/CT for GD diagnosis and suggested the existence of a population carrying K157R allele that causes GD in Japan. For such patients, $^{18}$F-FDG PET/CT is valid as the first step of diagnosis.

**Author contributions**

Data curation: Kaori Adachi, Ryota Nakanishi, Suzuko Moritani, Eiji Nanba.

Writing – original draft: Sakura Hosoba.

Writing – review & editing: Katsuyuki Kito, Yukako Teramoto, Kaori Adachi, Ryota Nakanishi, Ai Asai, Masaki Iwasa, Rie Nishimura, Suzuko Moritani, Masahiro Kawahara, Hitoshi Minamiguchi, Eiji Nanba, Ryoji Kushima, Akira Andoh.

**References**

[1] Brady RO, Kanfer JN, Shapiro D. Metabolism of glucocerebrosides. II. Evidence of an enzymatic deficiency in Gaucher’s disease. Biochem Biophys Res Commun 1965;18:221–5.

[2] Cox TM, Schofield JP. Gaucher’s disease: clinical features and natural history. Baillieres Clin Haematol 1997;10:657–89.

[3] Thomas AS, Mehta A, Hughes DA. Gaucher disease: haematological presentations and complications. Br J Haematol 2014;165:427–40.

[4] Charrow J, Esplin JA, Gribble TJ, et al. Gaucher disease: recommendations on diagnosis, evaluation, and monitoring. Arch Intern Med 1998;158:1754–60.

[5] Luana Z, Li L, Higaki K, et al. The chaperone activity and toxicity of ambroxol on Gaucher cells and normal mice. Brain Dev 2013;35:317–22.

[6] Sawkar AR, Cheng WC, Beutler E, et al. Chemical chaperones increase the cellular activity of N370S beta-glucosidase: a therapeutic strategy for Gaucher disease. Proc Natl Acad Sci U S A 2002;99:15428–33.

[7] Sibille A, Eng CM, Kim SJ, et al. Phenotype-genotype correlations in Gaucher disease type I: clinical and therapeutic implications. Am J Hum Genet 1993;52:1094–101.

[8] Tajima A, Yokoi T, Ariga M, et al. Clinical and genetic study of Japanese patients with type 3 Gaucher disease. Mol Genet Metab 2009;97:272–7.

[9] Okuyama T. Gaucher disease. Brain Nerve 2015;67:1109–13.

[10] Mitsui J, Mizuta I, Toyoda A, et al. Mutations for Gaucher disease confer high susceptibility to Parkinson disease. Arch Neurol 2009;66:571–6.

[11] Poorthuis BJ, Wevers RA, Kleijer WJ, et al. The frequency of lysosomal storage diseases in The Netherlands. Hum Genet 1999;105:151–6.

[12] Ida H, Rennert OM, Kawame H, et al. Mutation prevalence among 47 unrelated Japanese patients with Gaucher disease: identification of four novel mutations. J Inherit Metab Dis 1997;20:67–73.

[13] Charron J, Anderson HC, Kaplan P, et al. The Gaucher registry: demographics and disease characteristics of 1698 patients with Gaucher disease. Arch Intern Med 2000;160:2835–43.

[14] Erba PA, Boni R, Sollini M, et al. Clinical application of $[^{18}]$F-FDG-PET/CT in Gaucher’s disease. J Nucl Med 2012;53(Supplement 1):2141.

[15] Hruska KS, LaMarca ME, Scott CR, et al. Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). Hum Mutat 2008;29:567–83.

[16] Ida H, Iwasawa K, Kawame H, et al. Characteristics of gene mutations among 32 unrelated Japanese Gaucher disease patients: absence of the common Jewish 84GG and 1226G mutations. Hum Genet 1995;95:717–20.