Clinical Case Report

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

A case report

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Abstract

Rationale: Gaucher disease (GD) is an autosomal recessive disorder that leads to multiorgan complications caused by β-glucocerebrosidase deficiency due to mutations in the β-glucocerebrosidase-encoding gene (*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}F-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 β-glucocerebrosidase activity test found decreased. Sequencing of the patient's *GBA* gene revealed a RecNciI recombinant mutation and the novel mutation K157R (c.587A>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 α was started after the diagnosis of type 1 GD.

Outcomes: The patient's β-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 α showed reduction of Gaucher cells in bone marrow to 2% from 4% of total cellularity.

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

Abbreviations: ERT = enzyme replacement therapy, FDG PET/CT = ^{18}F-deoxyglucose positron emission tomography/computed tomography, GBA = β-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 β-glucocerebrosidase, a lysosomal enzyme in monocytes and macrophages that catalyzes hydrolysis of β-glucocerebroside to glucose and ceramide.[1] The deficiency of β-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.[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.[3]

The definite diagnosis of GD must be based upon experimental proof of the low activity of β-glucocerebrosidase in total leukocytes or mononuclear cells or in fibroblasts cultured from skin biopsies.[4] Genotype identification of the β-glucocerebrosidase gene (*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 β-glucocerebrosidase activity and are sometimes difficult to diagnose by the latter parameter only. *GBA* genotype testing should be an essential clue to diagnose GD in such patients. Second, *GBA*

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Table 1

Laboratory findings at diagnosis.

White blood cell	3900/μL	Total protein	6.1 g/dL	CK	64 U/L
Leukocyte differential		Albumin	3.9 g/dL	Fe	52 μg/dL
Neutrophils	48.8%	AST	18 U/L	UIBC	212 μg/dL
Eosinophils	1.8%	ALT	16 U/L	Ferritin	553.7 ng/mL
Basophils	1.8%	LD	146 U/L	IgG	1054 mg/dL
Lymphocytes	40.2%	γ-GT	15 U/L	IgM	186 mg/dL
Monocytes	8.2%	T-Bil	0.87 mg/dL	IgA	157 mg/dL
Red blood cells	3.98×10^6/μL	UN	16.8 mg/dL		
Hemoglobin	11.8 g/dL	Creatinine	0.57 mg/dL		
Hematocrit	34.6%	Ca	8.9 mg/dL		
Reticulocytes	20%	P	3.8 mg/dL		
Platelets	14.8×10^4/μL	CRP	0.05 mg/dL		
		β2-MG	2.1 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.

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).[5,6] 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).[7] 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*.[8] 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.[9]

Here, we report a Japanese patient who presented with slight anemia and thrombocytopenia without major complaints and was initially diagnosed at the preoperative ^{18}F-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 14.8×10^4/μL), and high level of ferritin (553.7 ng/mL) (Table 1). Preoperative ^{18}F-FDG PET/CT found strong bilateral accumulations in the bone marrow of humeri and femora and in the paraabdominal aortic lymph nodes in addition to a weaker

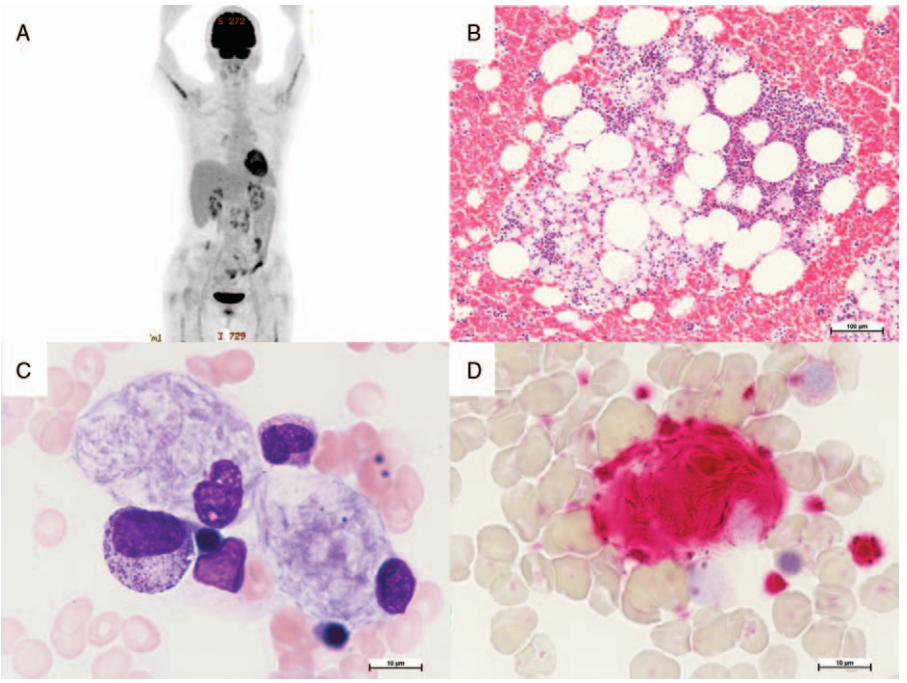

Figure 1. (A) Preoperative ^{18}F-deoxyglucose positron emission tomography/computed tomography scan shows atypical accumulation at the bilateral brachia and thigh bones and the paraabdominal aortic lymph nodes in addition to weaker accumulation in the stomach without accumulation in the gastric lymph nodes. (B) Histopathologic findings in a bone marrow clot. Large foamy histiocytes constitute sheet-like pattern (hematoxylin and eosin stain; original magnification, ×100). (C) Histopathologic findings in a bone marrow smear. The histiocytes have weekly basophilic enlarged cytoplasm containing wrinkled structures (Wright–Giemsa stain; original magnification, ×1000). (D) Histopathologic findings in a bone marrow smear. The cytoplasm of the abnormal histiocytes is strongly positive for acid phosphatase stain (original magnification, ×1000).

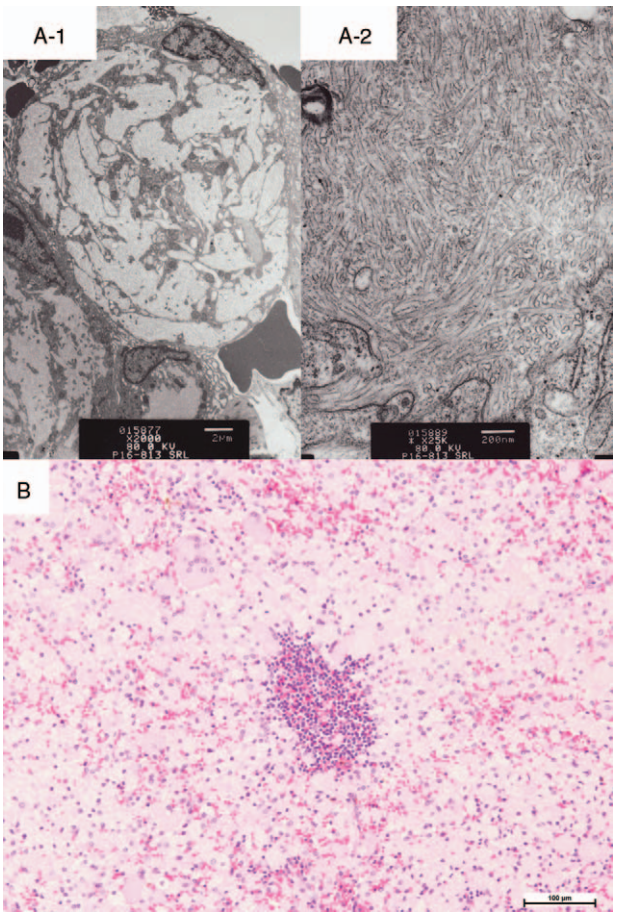

Figure 2. (A) Electron microscopy findings in the bone marrow. The abnormal histiocyte distorted by enlarged cytoplasm contained enlarged lysosomes (A-1, ×2000). Further magnification shows that the lysosomes are filled with various tubular structures (A-2, ×25,000). (B) Histopathologic findings in the 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).

accumulation in the stomach but without accumulations in the gastric lymph nodes or hepatosplenomegaly (Fig. 1A). Those findings were considered as comorbidity in the bone marrow. The subsequent bone marrow aspiration revealed that there were no pathologic cells that would indicate gastric cancer metastasis but abnormally large foamy histiocytes occupying 4% of total cellularity that were stained positively for CD68, CD163, periodic acid Schiff, and acid phosphatase but were negative for anti-pan cytokeratin staining, which suggested Gaucher cell phenotype, were found (Fig. 1B–D). Further electron microscopy investigation of the bone marrow specimen elucidated that the large foamy cells had irregular extended cytoplasm filled with abundant lysosomes, which were fully occupied by tubular-form structures, a feature commonly seen in lysosomal diseases (Fig. 2A).

The preoperative histopathology of gastric biopsy by upper endoscopy suggested moderately to poorly differentiated adenocarcinoma infiltrating into the gastric submucosa. ^{18}F-FDG PET/CT found a limited accumulation in the paraabdominal lymph nodes but no paragastric lymph nodes. Thus, the patient's gastric cancer was considered as operative, so distal gastrectomy and paraabdominal lymphadenectomy were performed.

Postoperative histopathologic examination of the extracted stomach revealed that the gastric cancer was poorly differentiated 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).

Table 2

Laboratory findings for definitive diagnosis.

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.

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.[10] The analysis found the compound heterozygote mutations of K157R (c.587A>G) on exon 6 and RecNciI, including L444P (c.1448T>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.587A>G) mutation in *GBA* that caused GD and of the use of ^{18}F-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.[11,12] Tajima et al reported that 58% of Japanese patients with GD develop types 2 or 3 GD, which is accompanied by neurologic symptoms (24% and 34% of cases, respectively).[8] 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 Charrow et al.[13] 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

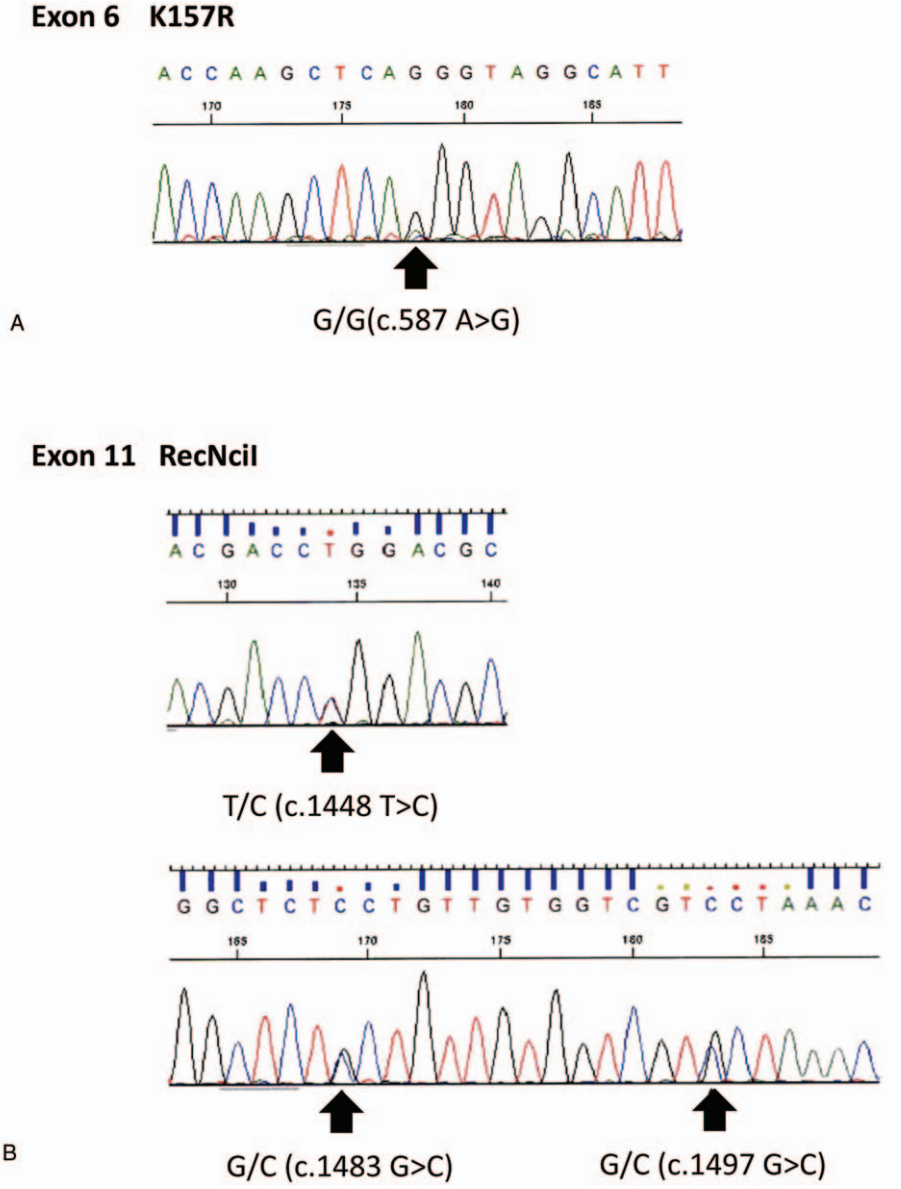

Figure 3. Mutation analysis of the *GBA* gene in the patient shows heterozygous point mutation K157R (c.587A>G) on exon 6 (A) and heterozygous recombinant mutations of RecNciI, including L444P (c.1448T>C), A456P (c.1483G>C), and V460 (c.1497G>C) on exon 11 (B).

neurologic manifestations, splenomegaly, bone pain, or other typical GD symptoms.

In our case, ^{18}F-FDG uptake in the bone marrow was the important clue to suspect GD. The correlation between ^{18}F-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 ^{18}F-FDG accumulation in the bone marrow. Furthermore, the score based on the extension and magnitude of ^{18}F-FDG uptake in the bone marrow was highly correlated to the clinical severity score. However, we believe that no case reports that actually utilized ^{18}F-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 7 kb, 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

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

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