ORIGINAL ARTICLE

Allele frequency of a 24 bp duplication in exon 10 of the *CHIT1* gene in the general Korean population and in Korean patients with Gaucher disease

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Plasma chitotriosidase activity is used for diagnosis and monitoring of Gaucher disease. However, homozygous duplication of a 24 bp region in exon 10 of the chitotriosidase gene (*CHIT1*) abolishes enzyme activity, limiting its use as a biomarker in Gaucher disease. This study investigates the allele frequency of the 24 bp duplication, in both the general Korean population and in patients with Gaucher disease. Fifteen Korean patients with Gaucher disease and 231 Korean normal individuals were enrolled. Genotyping was performed to identify the 24 bp duplication in exon 10 of *CHIT1* using DNA extracted from peripheral leukocytes or dried blood spots. Two patients with Gaucher disease (13.3%) had normal plasma chitotriosidase activity, and carried a homozygous 24 bp duplication of exon 10 of the *CHIT1* gene. Nine patients were heterozygote carriers (60.0%). Of the normal 231 Korean individuals, heterozygous duplication was detected in 109 individuals (47.2%) and homozygous duplication in 75 (32.5%). The allele frequency was 56.1% (95% confidence interval, 49.4–62.7%). The frequency of the 24 bp duplication was remarkably high in both Korean patients with Gaucher disease and in the normal population, limiting the efficacy of chitotriosidase as a biomarker in Gaucher disease in Korea. New biomarkers are required that consider the genetic characteristics of different populations.

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INTRODUCTION

Gaucher disease (OMIM #230800) is one of the most common lysosomal storage disorders. It is caused by a deficiency in β -glucocerebrosidase and is inherited in an autosomal recessive manner.¹ The causative gene, glucocerebrosidase, is located on chromosome 1q21.² Gaucher disease is characterized by hepatosplenomegaly, anemia, thrombocytopenia, osteopenia, osteonecrosis and growth retardation. A subgroup of patients suffer from neurological manifestations including seizure, gait abnormality, tremor, intellectual disability and abnormal eye movement.¹ Although most Caucasians with Gaucher disease are free of neurological manifestations, approximately half of East Asians with Gaucher disease present with neurological manifestations.^{3,4}

Since the advent of recombinant glucocerebrosidase in 1993, enzyme replacement therapy has markedly improved clinical outcomes in patients with Gaucher disease. In Korea, enzyme replacement therapy for Gaucher disease has been covered by national health

insurance since 1998. Currently, around 40 Korean patients have undergone enzyme replacement therapy.

Chitotriosidase (CHIT1; EC 3.2.1.14) is the human analog of chitinase, which breaks down glycosidic bonds in chitin, a component of yeast cell walls and a skeletal component in arthropods and worms.⁵ Despite high similarity to the chitinases of lower organisms, mammalian CHIT1 lacks chitinase activity.⁵ Instead, CHIT1 is thought to be involved in the degradation of chitin-containing pathogens, including fungi. CHIT1 is synthesized, stored and secreted by macrophages and neutrophils, which have a major role in maintaining host immunity.⁶ CHIT1 activity is highly elevated in patients with Gaucher disease,^{7,8} but gradually decreases with enzyme replacement therapy.^{3,9} Thus, its activity is widely used for diagnosis and monitoring of Gaucher disease. However, some patients display low CHIT1 activity despite having Gaucher disease.¹⁰ Most of these patients carry a homozygous 24 bp duplication in exon 10 of the *CHIT1* gene. Therefore, CHIT1 activity cannot be used as a

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biomarker of disease activity in patients with homozygous *CHIT1* mutations.

This study investigated the frequency of the 24bp duplication in exon 10 of the *CHIT1* gene in Korean patients with Gaucher disease. Follow-up data was acquired for patients receiving enzyme replacement therapy. In addition, we investigated the frequency of the 24bp duplication in the general Korean population.

MATERIALS AND METHODS

Subjects

Fifteen unrelated Korean patients with Gaucher disease undergoing enzyme replacement therapy in Asan Medical Center, Seoul, Korea were recruited.³ The frequency of a 24bp duplication in exon 10 of the *CHIT1* gene was investigated in all the patients. To investigate the duplications, genomic DNA was extracted from dried blood spots of 231 healthy Korean individuals. This study was approved by the Institutional Review Board of Asan Medical Center, Seoul, Korea, and written informed consent was obtained from all subjects or their parents.

CHIT1 assay

CHIT1 enzyme assays were performed according to the method of Hollak *et al.*⁷ with minor modifications. CHIT1 activity was determined by incubating 10 μ l of EDTA-treated plasma, 20 $\times$ or 50 $\times$ diluted with 100 μ l of 22 μ mol l⁻¹ 4-methylumbelliferyl β -D-N,N',N''-triacylchitotriosidase (Sigma-Aldrich, St Louis, MO, USA) in McIlvain's buffer (100 mmol l⁻¹ citric acid and 200 mmol l⁻¹ sodium phosphate, pH 5.2), for 15 min at 37 °C. The sample was diluted twofold in distilled water before incubation. The reaction was terminated by adding 300 μ l of 1 M Na₂CO₃ buffer, pH 10.7. Fluorescence was measured with an LS 55 Fluorescence Spectrometer, 230 V (PerkinElmer, Waltham, MA, USA).

CHIT1 genotyping

Genomic DNA from patients with Gaucher disease was extracted from peripheral blood leukocytes using PUREGENE DNA isolation kits (Gentra, Minneapolis, MN, USA). DNA from normal Korean individuals was extracted from two dried blood spot disks, 3 mm in diameter, using a Generation Capture Card Kit (Qiagen, Hilden, Germany). Exon 10 of the *CHIT1* gene was amplified using the primers listed in Table 1. Genotyping of the 24bp duplication in exon 10 of the *CHIT1* gene was performed. The amplified DNA was separated on a 6% polyacrylamide gel and visualized by ethidium bromide staining with 100 bp DNA ladder (Bioneer, Daejeon, Korea). The genotype was determined by the presence or absence of a 384 bp wild-type band or a 408 bp mutant band. For the subjects with the mutant band, sequencing analysis was done using an ABI3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

RESULTS

CHIT1 activity in patients with Gaucher disease

CHIT1 activity was measured in a total of 15 patients with Gaucher disease. Pretreatment data were available for six patients as CHIT1 activity has been measured in Korea since 2004. For the other nine patients, CHIT1 activity was measured after 8.4 $\pm$ 4.0 years (1–14 years) of enzyme replacement therapy (Table 2). The mean value for

CHIT1 activity at first measurement was 2270 $\pm$ 4095 nmol h⁻¹ ml⁻¹ plasma (normal range, 4–76 nmol h⁻¹ ml⁻¹ plasma). Figure 1 shows the change in CHIT1 activity over time for each patient receiving enzyme replacement therapy. In most patients, enzyme replacement therapy gradually reduced CHIT1 activity. However, CHIT1 activity was persistently below the normal range in two patients (subject 7 and 9; 13.3%) (Table 2 and Figure 1). We investigated the 24bp duplication in exon 10 of the *CHIT1* gene in all 15 patients (Figure 2). Homozygous duplications were found in subject 7 and 9 (13.3%), who also displayed low CHIT1 activity. Nine patients were heterozygote carriers (60.0%). The 24bp duplication was confirmed by sequence analysis in each patient. The allele frequency of the 24bp duplication was 43.3% (13/30 alleles) in the 15 patients with Gaucher disease.

Allele frequency of the 24bp duplication in exon 10 of the *CHIT1* gene in the Korean population

To investigate the allele frequency of the 24bp duplication in exon 10 of the *CHIT1* gene in the general Korean population, DNA was extracted from dried blood spots from a total of 231 healthy Korean individuals. Heterozygous duplication was detected in 109 individuals (47.2%) and homozygous duplication in 75 individuals (32.5%) (Figure 2). The allele frequency was 56.1% (95% confidence interval, 49.4–62.7%).

DISCUSSION

Accurate monitoring of disease activity is important for evaluation of the therapeutic efficacy of enzyme replacement therapy in Gaucher disease. Notably, some patients experience disease progression despite enzyme replacement therapy.⁴ Currently, blood tartrate-resistant acid phosphatase, angiotensin-converting enzyme and CHIT1 are used as markers for monitoring disease progression.¹ These markers are secreted by activated macrophages. Of these markers, CHIT1 is more specific to Gaucher disease. However, these markers can also be elevated in other disease conditions. For example, high levels of CHIT1 can be observed in patients

Table 2 Chitotriosidase activity in patients with Gaucher disease

	Chitotriosidase activity (4–76 nmol h ⁻¹ ml ⁻¹ plasma)	Time of measurement	
		(year of enzyme replacement therapy)	Genotype of 24 bp duplication
Pt. 1	15 918	0	WT/WT
Pt. 2	724	0	WT/WT
Pt. 3	805	0	WT/MUT
Pt. 4	5967	0	WT/MUT
Pt. 5	2543	0	WT/MUT
Pt. 6	2279	0	WT/WT
Pt. 7	2	1	MUT/MUT
Pt. 8	906	4	WT/MUT
Pt. 9	2	7	MUT/MUT
Pt. 10	1557	8	WT/WT
Pt. 11	111	10	WT/MUT
Pt. 12	38	10	WT/MUT
Pt. 13	225	10	WT/MUT
Pt. 14	344	12	WT/MUT
Pt. 15	2635	14	WT/MUT

Abbreviations: MUT, mutant; Pt., patient; WT, wild type.

Table 1 PCR primer pairs used for the amplification of exon 10 of the *CHIT1* gene.

Forward PCR primer 5' $\rightarrow$ 3'	Reverse PCR primer 3' $\rightarrow$ 5'
(<i>CHIT1_m10_F</i>)	(<i>CHIT1_m10_R</i>)
ccaaggctgtctccagaatc	gggcaaagtcatttcctcag

Figure 1 Changes in plasma chitotriosidase activity for patients with Gaucher disease receiving enzyme replacement therapy.

Figure 2 Polyacrylamide gel electrophoresis image for *CHIT1* genotyping. Genotype was determined by the presence or absence of a 384 bp wild-type band or a 408 bp mutant band. MUT, mutant; WT, wild type.

with nonalcoholic fatty liver disease, atherosclerosis or juvenile idiopathic arthritis.^{11–13} In addition, our study demonstrates the limitations of using *CHIT1* for monitoring Gaucher disease in Korean population. The *CHIT1* allele carrying the 24 bp duplication in exon 10 leads to aberrant messenger RNA splicing and an absence of *CHIT1* activity.¹⁰ In our study, the frequency of this variant was high at 43.3% in the 15 patients with Gaucher disease, including two (13%) homozygotes. Its allele frequency in the normal Korean population was also high at 56.1% (95% confidence interval, 49.4–62.7%).

A previous study described its frequency in a small number of Koreans (40 individuals) and reported an allele frequency of 58%.¹⁴ The present study is the first to describe its frequency in a large number of normal Korean individuals and confirmed that the allele frequency is high in the general Korean population. Moreover,

over 30% of the population are homozygous for this variant. High allele frequency for this duplication has also been reported for other East Asian countries including Taiwan, China, and Japan.^{14,15} However, the number of subjects in these previous studies is too small for the data to be considered an accurate representation of the allele frequency of the general population. Thus, validation using a large number of subjects is required, as is the case in the present study. On the other hand, the allele frequency of 24bp duplication in the *CHIT1* gene has been known as low in Caucasian (~20%), Hispanic (8–13%) or African ancestry (5–7%), indicating the ethnic diversities in the frequency of 24bp duplication.^{14,16}

As CHIT1 mediates innate immune responses against chitin-containing pathogens, candida, mycobacterium, plasmodium and other parasites,^{17–20} the 24bp duplication in the *CHIT1* gene was previously thought to increase susceptibility to these pathogens. However, most studies have failed to confirm this association.^{21,22} CHIT1 deficiency is now thought to convey an evolutionary advantage in humans,¹⁴ as natural selection has eliminated the activity of this enzyme.

As CHIT1 is of limited use as a marker for the diagnosis and monitoring of Gaucher disease, new surrogate biomarkers have been suggested. Pulmonary and activation-regulated chemokine (PARC or CCL18) is a chemokine that mediates leukocyte chemotaxis. Notably, plasma levels of PARC are elevated in patients with Gaucher disease.²³ Similarly, glucosylsphingosine accumulates in various tissues of patients with Gaucher disease. Plasma levels are significantly higher in patients with Gaucher disease than in the normal population, and can be reduced by enzyme replacement therapy. Although these new surrogate biomarkers can be used for disease monitoring, an important issue still remains that no biomarker reflects the neurologic status of Gaucher disease. Because neuronopathic Gaucher disease is highly prevalent in East Asian countries, more studies are required to investigate new biomarkers for neuronopathic Gaucher disease.

In conclusion, as the frequency of the 24bp duplication in exon 10 of *CHIT1*, a mutation conferring null enzymatic activity, differs widely according to ethnic group, care should be taken when using CHIT1 for the diagnosis and monitoring of Gaucher disease in different ethnic groups. Further investigations are required to identify new biomarkers tailored to the genetic characteristics of different populations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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