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Original article

Genotypes and phenotypes in 20 Chinese patients with type 2 Gaucher disease

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Abstract

Background: Gaucher disease (GD) is one of the most common lysosomal storage diseases resulting from a deficiency of glucocerebrosidase. Three main types have been described, with type 2 being the most rare and severe form. Here we investigated the clinical symptoms and mutation spectrum in 20 unrelated type 2 GD patients.

Method: The diagnosis of GD was based on the acid β-glucocerebrosidase (GBA) enzyme activity and direct sequencing of the *GBA* gene. GBA activity was measured in leukocytes and the *GBA* gene was amplified by a polymerase chain reaction (PCR). For patient 7, the *GBA* gene was analyzed by PCR as well as quantitative real-time PCR.

Results: The age of onset was under 12 months for all patients. All patients experienced severe neurological involvement. A total of 19 different *GBA* gene mutations were identified, including 6 novel mutations: two were exonic point mutations, c.1127T > C (p.Phe376Ser), c.1418T > G (p.Val473Gly); one was splicing error, ISV7-1G > C; one was insertion, c.717_718insACAG; and the other two were a gross deletion that involved exon 6 and a recombinant allele. The most prevalent mutation was Leu483Pro, which constituted 42.5% of all mutant alleles and was associated with a neurological form in Chinese GD patients as calculated by a Fisher's exact test.

Conclusion: The clinical characteristics of Chinese type 2 GD were consistent with reports from other ethnic populations. We identified 6 novel mutations that contribute to the spectrum of *GBA* gene mutations. Our study confirmed that GD patients with the Leu483Pro allele were prone to experience neurological symptoms.

Keywords: Gaucher disease; Type 2; GBA; L483P

1. Introduction

Gaucher disease (GD), the most common lysosomal storage disorder, is an autosomal recessive disease caused by β-glucocerebrosidase (GBA) deficiency. GBA can catalyze glucosylceramide into ceramide and glucose in the presence of an essential activator called saposin C [1]. Deficiency of GBA or saposin C lead to an accumulation of glucocerebroside in macrophages of the endothelial system, especially in the liver, spleen, bone marrow and even central nervous system [2]. Most GD cases are caused by mutations in the *GBA* gene, few cases are caused by saposin C deficiency [3].

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Three main types of GD have been described based on the clinical signs, age of onset and central nervous system involvement [4]. Type 1 (MIM 230800) is also called non-neuronopathic subtype and accounts for 90% of known GD cases worldwide [5]. Type 1 presents with varying degrees of symptoms, ranging from asymptomatic to a combination of hepatosplenomegaly, skeletal lesions and bone pain. Type 2 (MIM 230900), the rarest form, presents with early-onset nervous system involvement and progresses rapidly. Type 3 (MIM 231000) is considered the less acute subtype and is associated with slowly progressive neurological symptoms [2,6].

The incidence of type 2 GD differs in different ethnic groups, and is estimated to be 1 in 500,000 of the general population [7]. Type 2 GD was first described in 1927 and presents with manifestations either prenatally or during infancy [8]. Clinical signs of type 2 GD include hepatosplenomegaly, anemia, thrombocytopenia, hydrops fetalis, ichthyosis, ophthalmological impairment (such as strabismus and oculomotor apraxia), developmental delay, swallowing difficulties and respiratory distress. Many patients eventually develop opisthotonus and seizures as the disease progresses [8]. Patients with type 2 GD usually die before 3 years old [7].

The *GBA* gene is localized on chromosome 1q21 and is 7,600 bp in length. It consists of 11 exons and encodes 497 amino acids [2]. A pseudo-*GBA* (*GBAP*) gene is located 16,000 bp downstream of the functional *GBA* and they share 96% exonic homology [9]. The main differences between *GBA* and *GBAP* are small deletions in introns 2, 4, 6, and 7, some missense mutations in exons, and a 55-bp deletion in exon 9 of *GBAP* [10]. The *GBAP* gene is transcribed, but produces a nonfunctional protein [10]. Some complex recombination alleles that carry two or more disease-causing mutations develop due to gene conversion events between the *GBA* and *GBAP* genes [11]. Nearly 460 mutations including point mutations, nonsense mutations, deletions, insertions, splicing aberrations and recombination alleles in *GBA* have been described according to the Human Gene Mutation Database (HGMD), and more mutations continue to be discovered.

The correlation of genotypes and phenotypes of GD is complex. The Asn409Ser (also called N370S) is the most frequent mutation in the Ashkenazi-Jewish and Caucasian populations. Patients heterozygous or homozygous for Asn409Ser do not manifest type 2 GD. However, Leu483Pro (also called L444P), the common mutation of GD patients in Asian counties, occurs alone or as a part of a complex of recombinant mutations in all types of GD [7]. Additionally, mutations involving an arginine frequently occur in patients with type 2 GD [12].

Timely and accurate diagnosis is essential for appropriate treatment and genetic counseling of type 2 GD. In this study, we summarized the clinical and genetic features of 20 Chinese patients with type 2 GD. The aim of our paper was to report the spectrum of *GBA* mutations among Chinese patients with type 2 GD and to compare it with other populations.

2. Material and methods

2.1. Patients

Twenty patients from east (14), central (4), and south (2) of China of Han ethnicity were retrospectively reviewed. All subjects were from unrelated families and their parents had no consanguinity. Diagnoses of type 2 GD for 17 patients were based on clinical symptoms and reduced GBA enzyme activity in peripheral leukocytes or dry blood spots, which were further confirmed by one disease-causing mutation on each allele of *GBA* gene. Diagnoses of type 2 GD for another 3 patients were based on clinical symptoms and one disease-causing mutation at each separate allele where bloods samples for enzyme analysis were not available. Informed consent was obtained from patients. This study was approved by the Institutional Review Ethics Board of the Shanghai Xinhua Hospital, Shanghai Jiao Tong University School of Medicine.

2.2. GBA activity

GBA activity in peripheral blood leukocytes was measured by modifying the method reported by Chabás [13]. We prepared 0.2 mol/L citrate phosphate buffer (pH 5.8, containing 0.3% taurodeoxycholate and 0.15% Triton-X100) as the reaction buffer. 4-methylumbelliferyl-β-D-glucopyranoside was added to the reaction buffer to achieve a final substrate concentration of 5 mmol/L. Substrate (20 μL) was mixed with leukocyte homogenate (10 μL). After incubation for 2 h at 37 °C, the reaction was terminated with 200 μL of 0.5 mol/L glycine-carbonate buffer (pH 10.5, 0.17 mol/L). Fluorescence of the enzyme product was measured on a Wallac 1420 victor^2 micro plate reader (Perkin Elmer, USA) with an excitation wavelength of 366 nm and an emission wavelength of 460 nm. Fluorescence readings were corrected for blanks. Results were compared with the fluorescence from a 4-methylumbellyferone calibrator. GBA activity was expressed as nmol/h/mg protein. All chemicals were obtained from Sigma (St Louis, MO, USA) unless indicated otherwise. GBA activity on dry blood spots were performed as described previously [14].

2.3. Polymerase Chain Reaction (PCR)

The *GBA* gene was amplified in all patients using a PCR. Genomic DNA was isolated from peripheral

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