Beta-galactosidase deficiencies and novel *GLB1* mutations in three Chinese patients with Morquio B disease or GM1 gangliosidosis

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Background: **This paper aims to report GLB1 activities and mutation analysis of three patients from the mainland of China, one with Morquio B disease and two with GM1 gangliosidosis.**

Methods: **GLB1 activity and *GLB1* gene mutation were analyzed in the three patients who were clinically suspected of having Morquio B disease or GM1 gangliosidosis. Novel mutations were analyzed by aligning *GLB1* homologs, 100 control chromosomes, and the PolyPhen-2 tool.**

Results: **The enzymatic activity of GLB1 was found to be 5.03, 4.20, and 4.50 nmol/h/mg in the three patients, respectively. Patient 1 was a compound heterozygote for p.[Arg148Cys] and p.[Tyr485Cys] mutations in the *GLB1* gene. Patient 2 was a compound heterozygote for p.[Tyr270Phe] and p.[Leu337Pro] mutations. Patient 3 was a homozygote for p.[Asp448Val] mutation. Three mutations (p.[Tyr485Cys], p.[Tyr270Phe] and p.[Leu337Pro]) were novel variants and were predicted to damage GLB1 function.**

Conclusions: **The enzymatic activity and related gene analysis of β-galactosidase should be performed in clinically suspected individuals to confirm diagnosis. The three novel mutations, p.[Tyr485Cys], p.[Tyr270Phe], and p.[Leu337Pro], are thought to be disease-causing mutations.**

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Key words: **β-galactosidase; enzyme assays; GM1 gangliosidosis; Morquio B disease; mutation analysis**

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Introduction

GM1 gangliosidosis (MIM# 230500) and Morquio B disease (MIM# 253010) are autosomal recessive lysosomal storage disorders (LSDs) caused by deficiency of lysosomal β-galactosidase (GLB1; EC 3.2.1.23) due to mutations in the *GLB1* gene. GLB1 deficiency leads to accumulation of ganglioside GM1 and keratan sulfate.[1,2] The estimated incidence of GM1 gangliosidosis is 1 in 100 000-200 000 live births.[3] GM1 gangliosidosis has been classified into three major clinical forms.[4] The infantile form is the most severe one, which is characterized by rapid psychomotor deterioration beginning within three to six months of birth, skeletal dysplasia, and death typically within 24 months of age. The Morquio B disease is rare and characterized by short-trunk dwarfism, progressive and generalized skeletal dysplasia, keratansulfaturia, and normal intelligence, which is distinguished from GM1 gangliosidosis.

The human *GLB1* gene, localized to 3p21.33, contains 16 exons. To date, more than 160 mutations have been reported in the *GLB1* gene. In 2007, we conducted an enzymatic analysis of lysosomal storage disorders. In this study, we reported the results of enzyme assays and mutation analysis in the *GLB1* gene for two patients with GM1 gangliosidosis and one patient with Morquio B disease, who were diagnosed in the last three years.

Methods

Subjects

Patient 1

A male patient showed no symptoms or obvious skeletal signs until 13 years of age when he presented with

weakness in his lower limbs and pain in his hip joint, which gradually worsened. His mental development was normal, and he had no symptoms of central nervous system involvements. He visited our clinic at 14 years of age with short stature and difficulty in walking. Physical examination showed short stature (151.6 cm), clear cornea, pigeon breast, lumbar kyphosis, wrist joint laxity and a waddling gait. No neurological symptoms were noted during a two-year follow-up.

Patient 2

A male patient presented with difficulty in sucking, floppy, poor head control, and a large area of skin with Mongolian spots on his back and hip at two months of age. At the age of six months, he showed thoracolumbar kyphosis and gradually developed more serious signs of illness. By one year of age, he began to have five to six episodes of seizures every day. He visited our clinic at two years of age because of severe floppiness, lack of response to any stimuli, and severe mental retardation. Physical examination revealed mild hepatomegaly, kyphosis, and hypotonia. He died at the age of two years and one month due to fastidium and respiratory failure.

Patient 3

A male patient was healthy during the neonatal period. However, he had several Mongolian spots on the hip area. At six months of age, he presented with weakness and anorexia. His motion and mental development retrograded gradually with age. He suffered from seizures and his responses to the surroundings were reduced at the age of 20 months. Physical examination showed mild hepatomegaly, thoracolumbar kyphosis, hypotonia, and O-type legs. He died at the age of two years and five months.

Laboratory and radiological investigation

A quantitative dimethylmethylene blue test for urinary glycosaminoglycan showed a weak positive result in patient 1, and a negative result in patients 2 and 3. In all patients, only urinary chondroitin sulfate could be detected using mucopolysaccharide unidirectional electrophoresis.[5]

Radiological investigation or magnetic resonance imaging of the skeleton showed ribbon-like ribs, thoracolumbar kyphosis, and bullet-like changes in the anterior end-plate of the thoracolumbar vertebral bodies in all three patients.

Based on the above information, patient 1 was suspected of having Morquio disease, and both patients 2 and 3 were suspected of having GM1 gangliosidosis.

Enzyme analysis

Leukocytes were isolated from peripheral blood of the three patients using dextran. Activities of leukocyte galactosamine-6-sulfate sulfatase (GALNS) and β-galactosidase (GLB1) were measured using specific artificial fluorescent substrates.[6]

Mutation analysis of the *GLB1* gene

This study was approved by the Ethics Committee of Xinhua Hospital, Shanghai Jiaotong University School of Medicine. Written informed consent was obtained from the parents of the three patients before DNA analysis. Genomic DNA was extracted from the peripheral white blood cells of these patients and their parents. All 16 exons and the exon-intron boundaries of the *GLB1* gene were amplified from the genomic DNA of the patients by PCR using 15 pairs of primers designed using the software Primer Premier 5. Direct DNA sequencing was performed routinely. The reference *GLB1* gene sequence used was GenBank NM_000404.

Analysis of novel gene variant

Novel gene variations were analyzed by comparing direct sequencing results of corresponding exons in the patient's parents and 100 control chromosomes.

Clustal (1.81) software was used to assess amino acid conservation for novel missense mutations in six different vertebrates. To predict the possible impact of an amino acid substitution (missense mutations) on the structure and function of the human GLB1 protein by PolyPhen-2 tool.[7]

Results

Enzyme diagnosis

Patient 1 had normal GALNS activity. The GLB1 activity was found to be 5.03, 4.20, and 4.50 nmol/h/mg (normal: 118-413 nmol/h/mg) in three patients, respectively. So patient 1 was diagnosed as having Morquio B disease, and both patients 2 and 3 were diagnosed with GM1 gangliosidosis.

Analysis of *GLB1* gene mutation

The compound heterozygote mutations in the *GLB1* gene, c.442C>T (p.[Arg148Cys]) and c.1454C>T (p.[Tyr485Cys]), were identified in patient 1. The latter is a novel mutation. The compound novel heterozygote mutations, c.809A>T (p.[Tyr270Phe]) and c.1010T>C (p.[Leu337Pro]), and one polymorphism (p.P10L) were identified in patient 2. A previously reported homozygote mutation, c.1343A>T (p.[Asp448Val]),

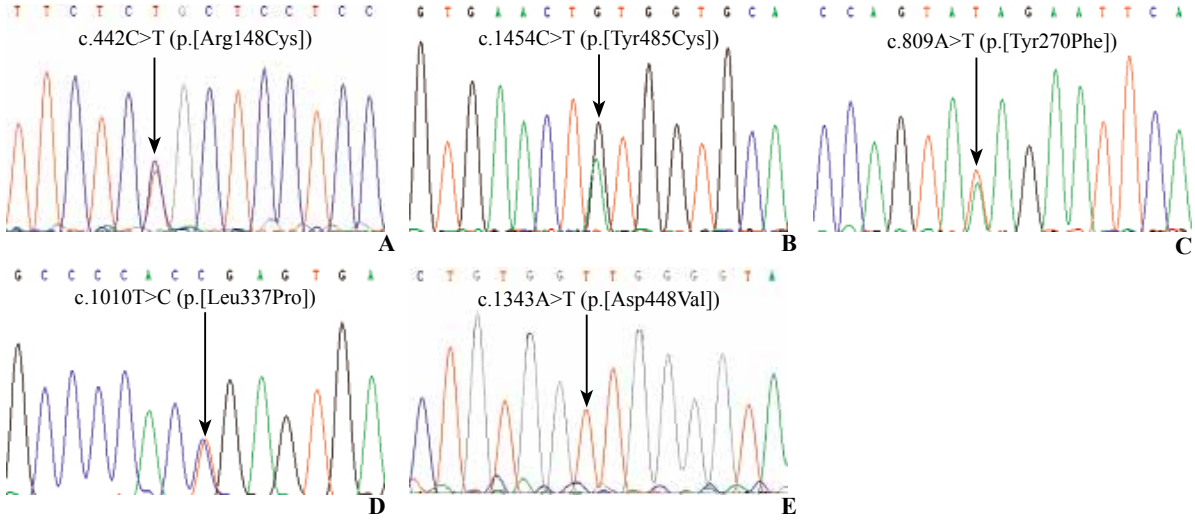

Fig. Nucleotide sequences of the neighboring regions of the mutations in the *GLB1* gene of the three patients. Patient 1: A&B; Patient 2: C&D; Patient 3: E.

was identified in patient 3 (Fig.).

Analysis of novel gene variants

All mutations detected in the three patients were inherited from their parents. Three novel variants, p.[Tyr270Phe], p.[Leu337Pro] and p.[Tyr485Cys], were not identified among the 100 control chromosomes tested. The p.270 tyrosine and p.337 leucine were highly conserved, and the p.485 tyrosine was a relatively conserved position in six different vertebrates. The three novel mutations were predicted to cause damage to the structure and function of human GLB1 protein with scores of 1. 000, 1.000, and 0.999, respectively, according to the PolyPhen-2 tool.

Discussion

LSDs are a group of genetic disorders that result from the deficient activity of a specific lysosomal enzyme. LSDs are individually rare, but together they have an incidence of approximately 1 to 7000-8000 live births.

Morquio disease is also named as mucopolysaccharidos (MPS) type IV which is relatively common. However, Morquio B disease is very rare. It is difficult to distinguish between the two diseases clinically because of similar symptoms, but they can be easily distinguished using enzyme assays and genetic testing. GM1 gangliosidosis and Morquio B disease are both caused by a deficiency in GLB1. Their clinical manifestations have many similarities; neurological symptoms are useful markers for differential diagnosis between the two diseases. However, some studies have reported that patients initially diagnosed as having Morquio B disease subsequently developed neurological impairment and were re-diagnosed with GM1 gangliosidosis.[8] Patient 1 presented with severe skeletal malformation and motion disability, but he had no neurological symptoms after follow-up to 16 years of age. Infantile GM1 gangliosidosis shows rapid psychomotor deterioration beginning within six months after birth, and dies at two to three years of age. The clinical histories in our patients 2 and 3 were consistent with symptoms of infantile GM1 gangliosidosis and the diagnosis was confirmed by GLB1 activity.

Only a few mutations have been reported to be specifically associated with Morquio B disease or GM1 gangliosidosis, but some common mutations have been observed, such as p.I51T, p.R201C, p.R208C, and p.R482H for GM1 gangliosidosis and p.W273L and p.Y83H for Morquio B disease.[9] p.[Arg148Cys] detected in patient 1 has also been previously reported in an adult patient with GM1 gangliosidosis,[10] and p.[Arg148Cys] mutated vector is transiently expressed in COS-1 cells showing no residual GLB1 enzyme activity.[11] Thus, p.[Arg148Cys] may be related to two different phenotypes of Morquio B disease and GM1 gangliosidosis. p.[Tyr485Cys], p.[Tyr270Phe] and p.[Leu337Pro] are all not polymorphic by analysis for controls and alignment of *GLB1* homologs. p.[Tyr270Phe] and p.[Leu337Pro] detected in patient 2 resulted in an infantile type of GM1 gangliosidosis and early death, indicating that the two mutations are disease-causing mutations. Paschke et al[8] found that the region between codons 400 and 530 of the *GLB1* gene is very important in the molecular structure of the enzyme. Therefore, p.[Tyr485Cys] may affect the functionally sensitive domain and may be

relevant to the pathogenesis of Morquio B disease. In conclusion, the three novel mutations, p.[Tyr485Cys], p.[Tyr270Phe], and p.[Leu337Pro], are thought to be disease-causing mutations.

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