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Molecular analysis of the α-N-acetylglucosaminidase gene in seven Japanese patients from six unrelated families with mucopolysaccharidosis IIIB (Sanfilippo type B), including two novel mutations

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Abstract Molecular analysis of the α-N-acetylglucosaminidase gene in seven Japanese patients with Sanfilippo syndrome type B from six unrelated families was carried out, and six disease-causing mutations were found. The parents of Patient 2 had a consanguinous marriage, but other families did not have any record of consanguinity. Two families were from Okinawa Island, where more patients with Sanfilippo syndrome were found than in other areas in Japan. Patients 1 and 6 showed the most severe phenotype with rapid progression. Patients 2, 5, and 7 were moderate. Patients 3 and 4 (sib cases) showed an attenuated form compared with other patients. Patients 1, 2, and 6 were homozygous for R482W, R565W, and R565P, respectively. Patients 3 and 4 were compound heterozygous for F314L and R565P. Patient 5 had delTG2171–2172 in exon 6 in one allele, and the other allele was unknown. Patient 7 was compound heterozygous for V241M and R482W. The family of Patients 3 and 4 and that of Patient 6 are unrelated, although both families are from Okinawa Island, and the patients have the same mutation, R565P; thus, R565P might be a common mutation in the Okinawa district. F314L and V241M are novel mutations.

Key words Sanfilippo syndrome type B · Mucopolysaccharidosis type IIIB · α-N-acetylglucosaminidase · Molecular analysis · Japanese patients · Okinawa district

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Introduction

Sanfilippo syndrome, mucopolysaccharidosis type III, is characterized by severe central nervous system degeneration, but shows only mild somatic disease. Such disproportionate involvement of the central nervous system is unique among mucopolysaccharidoses. Patients with Sanfilippo syndrome are classified into four types based on deficient enzymes: heparan N-sulfatase (type A), α-N-acetylglucosaminidase (type B), acetyl CoA: α-glucosaminide acetyltransferase (type C), and N-acetylglucosaminide 6-sulfatase (type D) (Neufeld and Muenzer 2001). Type A is the most common subtype, and type B is the second most common among Sanfilippo syndrome patients in northern Europe (OMIM #252900 and #252920) and in Australia (type A:type B = 1.9:1) (Weber et al. 1999). However, among Japanese patients, type B is more frequent than type A (type A:type B = 1:1.2) according to the registered members of the Japanese Society of the Patients and the Families with Mucopolysaccharidoses.

The α-N-acetylglucosaminidase gene has been isolated and characterized (Zhao et al. 1996; Weber et al. 1996), and many disease-causing mutations have been reported (Bunge et al. 1999; Coll et al. 2001; Emre et al. 2002). Type B is reported to be phenotypically heterogenous with severe and mild forms (Zhao et al. 1998; Weber et al. 1999) compared with type A, although most of the disease-causing mutations reported so far have been for the severe phenotype.

This is the first report of mutation analysis of the α-N-acetylglucosaminidase gene in Japanese patients with Sanfilippo type B.

Patients and methods

Patients

Patient 1 was an 8-year-old boy. He showed speech delay at the age of 2 years, and the diagnosis of Sanfilippo type B

was made when he was 4 years old. He showed coarse facial features, joint stiffness, and hepatomegaly. He deteriorated rapidly: He could speak only one-word sentences when he was 3 years old, he could not speak at all when he was 5 years old, and he could not walk at the age of 6 years.

Patient 2 was a male patient who died of respiratory infection at the age of 26 years. He was noted to have hyperactivity and speech delay at the age of 3 years. He was diagnosed with Sanfilippo type B at the age of 4 years. He showed coarse facial features and joint stiffness. He could not talk at the age of 9 years, seizures appeared when he was 13 years old, he became bedridden at the age of 15 years, and nasal tube feeding was instituted at the age of 19 years.

Patients 3 and 4 were siblings aged 20 and 18 years, respectively. Neither patient showed abnormal facial features. Patient 3, a brother, was noted to have delay of speech at the age of 3 years, and showed hyperactivity at the age of 5 years. He could talk and walk until the age of 8 and 14 years, respectively. Seizures appeared at the age of 10 years. Patient 4, a sister, was noted to have hyperactivity at the age of 6 years. She could not talk when she was 14 years old, and sleep disorders appeared at the age of 16 years. She could walk and had no seizures at the age of 18 years.

Patient 5 was a 9-year-old boy. He showed coarse facial features and developmental delay at the age of 3 years, and showed hyperactivity at the age of 6 years. He could walk but could not talk at the age of 9 years.

Patient 6 was a 5-year-old girl. She was noted to have speech delay and hyperactivity at the age of 2 and 3 years, respectively. She showed coarse facial features. She could speak one-word sentences at the age of 5 years.

Patient 7 was a 5-year-old boy. He had a large skull and hepatomegaly, and showed hypertrichosis. He showed a delay of speech but no hyperactivity at the age of 5 years.

The parents of Patient 2 had a consanguinous marriage, but other families did not have any record of consanguinity.

α-N-acetylglucosaminidase assay

The activity of α-N-acetylglucosaminidase was analyzed in cultured skin fibroblasts (Patients 1, 3, 4, and 6) or in lymphocytes (Patients 2, 5, and 7) by the method described previously (Marsh and Fenson 1985) using the fluorogenic substrate of 4-methylumbelliferyl-α-N-acetylglucosaminide (Sigma-Aldrich, Tokyo, Japan).

Molecular analysis

Cultured fibroblasts were used in Patients 1, 3, 4, and 6, and peripheral white blood cells were used in Patients 2, 5, and 7. Genomic DNA was extracted by the standard method. Each exon of the α-N-acetylglucosaminidase gene was amplified by polymerase chain reaction (PCR) according to the method of Schmidtchen et al. (1998) with some modifications. PCR products were sequenced by the direct sequencing method using a capillary sequencer ABI PRISM 310 Genetic Analyzer (Perkin Elmer Japan/ABI, Chiba, Japan) with a dRhodamin Terminator Cycle Sequencing Kit from the same company.

Table 1. α-N-acetylglucosaminidase activity in six patients with MPS IIIB

Subject	Activity (nmol/mg/h)	
	Fibroblasts	Lymphocytes
Normal control (n)	9.02 ± 2.29 (n = 6)	3.01 ± 0.90 (n = 6)
Patient 1	0.595	NA
Patient 2[a]	NA	0.673
Patient 3[b,c]	0.734	NA
Patient 4[b,c]	0.854	NA
Patient 5	NA	0.449
Patient 6[c]	0.169	NA
Patient 7	NA	0.109

MPS, Mucopolysaccharidoses; NA, not available
[a] Parents are cousins
[b] Siblings
[c] From Okinawa district

Results

The activity of α-N-acetylglucosaminidase in each patient as analyzed in cultured skin fibroblasts or peripheral blood lymphocytes is shown in Table 1. No significant difference of enzyme activity among the patients was seen. The results of the molecular analysis are summarized in Table 2. Patients 1, 2, and 6 were homozygous for R482W (CGG→TGG; exon 6), R565W (CGG→TGG; exon 6), and R565P (CGG→CCG; exon 6), respectively. Patients 3 and 4 were compound heterozygous for F314L (TTC→TTG; exon 5) and R565P (CGG→CCG; exon 6). Patient 5 had a two-nucleotide deletion in exon 6 (2171–72 TG) in one allele, and the mutation of the other allele was unknown. Patient 7 had V241M (GTG→ATG; exon 4) in one allele, and R482W (CGG→TGG; exon 6) in the other. Four polymorphisms were found in the noncoding region. They included g1352insC (5′ end of the gene; 14/14 alleles), a2259A→C (intron 1; 10/14 alleles), g2304insA (intron 1; 14/14 alleles), and g2739G→C (intron 2; 12/14 alleles).

Discussion

In patients with Sanfilippo syndrome, type B occurs more frequently than type A (type A:type B = 1:1.2) among the Japanese, according to the registered members in the Japanese Society of the Patients and the Families with Mucopolysaccharidoses. Moreover, type B occurs more frequently in the Okinawa district in Japan. We examined molecular defects of the α-N-acetylglucosaminidase gene in seven patients with Sanfilippo type B from six unrelated families, including two families from Okinawa. We found six distinct disease-causing mutations including five missense mutations and one two-base deletion.

The mutations R482W, R565W, R565P, and delTG2171–2172 have been reported previously (Weber et al. 1999; Bunge et al. 1999; Coll et al. 2001) in patients from other ethnic groups with severe phenotypes of type B. The mutations R482W and R565W were caused by a C-to-T transi-

Table 2. Mutations and polymorphisms in the α-N-acetylglucosaminidase gene

Subject	Mutation	Polymorphism (frequency in normal individuals)			
		g1352insC (14/14)	a2259A→C (8/14)	g2304insA (14/14)	g2739G→C (11/14)
Patient 1					
Allele 1	R482W	(+)	(+)	(+)	(+)
Allele 2	R482W	(+)	(+)	(+)	(+)
Patient 2					
Allele 1	R565W	(+)	(−)	(+)	(+)
Allele 2	R565W	(+)	(−)	(+)	(+)
Patient 3					
Allele 1	R565P	(+)	(+)	(+)	(+)
Allele 2	F314L	(+)	(+)	(+)	(+)
Patient 4					
Allele 1	R565P	(+)	(+)	(+)	(+)
Allele 2	F314L	(+)	(+)	(+)	(+)
Patient 5					
Allele 1	delTG2171–2172	(+)	(−)	(+)	(−)
Allele 2	Unknown	(+)	(−)	(+)	(−)
Patient 6					
Allele 1	R565P	(+)	(+)	(+)	(+)
Allele 2	R565P	(+)	(+)	(+)	(+)
Patient 7					
Allele 1	V241M	(+)	(+)	(+)	(+)
Allele 2	R482W	(+)	(+)	(+)	(+)

tion at a CpG hot spot. R482W has been reported in a Turkish patient with the severe phenotype as a homozygote (Bunge et al. 1999). Weber et al. (1999) reported that R565W accounted for 6% of mutant alleles in Australian patients with Sanfilippo type B. Each of R565P and delTG2171–2172 has been found in 1 allele of 50 mutant alleles among Australian patients (Weber et al. 1999).

R565P was found in two unrelated families from the Okinawa district, and the parents of Patient 6, who was homozygous for R565P, had no record of consanguinity, although both parents come from Okinawa. This mutation was a nucleotide substitution from CGG to C<u>C</u>G, which was neither a Cp<u>A</u> nor a <u>T</u>pG change although it was at a CpG site. Moreover, four polymorphisms studied in this report were completely the same in these three alleles carrying R565P. Thus, R565P might be a common mutation in Okinawa. However, these polymorphisms did not necessarily indicate that R565P in these patients originated from a common ancestor, because each polymorphism was frequent in the Japanese population.

F314L and V241M are novel mutations. F314L may cause an attenuated phenotype in Patients 3 and 4, because the other allele in these patients was R565P, which was known to cause a severe Sanfilippo disease. Patient 6 was homozygous for R565P and showed a severe phenotype. Although a wide clinical spectrum and allelic heterogeneity of Sanfilippo type B has been reported, only a few mutations were shown to cause attenuated clinical forms so far. For example, R643C was shown by a clinical survey in Dutch patients to cause the attenuated phenotype (Weber et al. 1999), and F48L was shown to have significant residual enzyme activity by in vitro gene expression study (Yogalingam et al. 2000).

Four polymorphisms were found. Two of them, g2304insA and g2739G→C, were also reported in non-Japanese individuals (Zhao et al. 1998; Tessitore et al. 2000). The frequencies of g2304insA and g2739G→C were 14/14 and 11/14 in the Japanese, and 2/36 and 20/36 in non-Japanese (Zhao et al. 1998), respectively. Two other polymorphisms, g1352insC (14/14) and a2259A→C (8/14), were frequently in the Japanese, and have not been reported in non-Japanese; thus, these polymorphisms might have originated in Japan.

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