Genotype-Phenotype Correlations in Filipino Patients with Type 3 Gaucher Disease

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ABSTRACT

Gaucher disease is an inherited glycolipid storage disorder caused by a deficiency of the lysosomal enzyme glucocerebrosidase. Clinical manifestations include hepatosplenomegaly, skeletal abnormalities, anemia and thrombocytopenia. We present here the corresponding genotypes and the genotype-phenotype correlations of 3 Filipino patients. Clinical phenotypes and genotypes were documented by reviewing the charts of 3 Filipino patients with Gaucher disease. Clinical parameters such as liver and spleen sizes, hematologic variables, disease types and response to enzyme replacement therapy were compared. Likewise, quantitative enzyme assays and mutation analysis were reviewed.

All have the type III neuronopathic Gaucher disease. Patients 1 and 2 are twin sisters who both have mild mental retardation with Patient 1 having a concomitant seizure disorder. They have the corresponding genotype of p.L444/p.P319A. Patient 3 has global developmental delay, oculomotor apraxia, pyramidal tract signs and carries the p.L444P/p.G202R/p.G202R genotype. Genotype-phenotype correlations for the 3 patients showed that their genotypes are compatible with the severe neuronopathic type of disease.

Key Words: genotype – phenotype, Gaucher Disease, Filipinos

Introduction

Gaucher disease (GD), inherited in an autosomal recessive manner, is the most common of the glycolipid storage disorders. It is caused by mutations in the gene coding the enzyme B glucosidase (glucocerebrosidase). This enzyme defect leads to accumulation of its substrate glucocerebroside in cells of monocyte/macrophage lineage. In turn, these cells infiltrate the liver, spleen, bone marrow and other tissues leading to their dysfunction.[1]

Clinically, the disease has been classified into three types on the basis of severity and the presence of primary neuronopathic effects: non-neuronopathic (Type 1), infantile acute neuronopathic (Type 2), and chronic juvenile neuronopathic (Type 3). Type 1 is the most common and is characterized by hepatosplenomegaly, pancytopenia, bone abnormalities and absence of neurologic symptoms. Type 2 is a rare and lethal form of the disease. Type 3 is characterized by early onset of visceral impairment and a later appearance of CNS symptoms.[2]

The isolation and cloning of the glucocerebrosidase gene on chromosome 1q21 has facilitated DNA-based testing for specific mutations and more precise carrier testing in at risk family members. To date, more than 250 mutant glucocerebrosidase alleles have been identified and genotype-phenotype correlations have been established for Gaucher disease.[3] Prevalence of different mutations studied and genotype-phenotype inferred are useful in genetic counseling and allow better understanding of the molecular basis of the disease.[4]

The worldwide incidence for Gaucher disease is 1:57,000-100,000.[5,6] In the Philippines, there were 5 confirmed cases of Gaucher disease since year 2000[7] but only the 3 patients included in this report completed molecular testing. This is the first Filipino study that correlates the genotypes of Gaucher patients with their corresponding phenotypes.

We present, herein, the clinical features and the corresponding mutations in the glucocerebrosidase gene of 3 Filipino patients with Gaucher disease.

Clinical Reports

Patient 1

Patient 1 is a female patient, first of twins, born to non-consanguineous Filipino parents. She was well until the onset of abdominal enlargement along with pallor, occasional nose bleeding and bone pains at 2½ years of age. Bone marrow aspirate and biopsy revealed hypercellular

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marrow with numerous histiocytes, strongly suggestive of Gaucher disease.

When seen at $3^{1}/_{2}$ years old, her height of 93 cm and weight of 13.5 kg were both within the 10^{th}-25^{th} percentile for age. She was pale and had hematomas on the lower extremities and petecchiae over the eyelids. The liver was palpable 10 cm below the right costal margin and the spleen was 14.5 cm below the left costal margin. Neurologic examination was essentially normal. Further investigations showed Erlenmeyer flask deformity in both femurs. Liver enzymes were elevated. Her initial hemoglobin was 68 g/L and platelet count was $31{,}000X10^{9}$/L (Table 1).

Enzymatic analysis done on peripheral leucocytes (National Referral Laboratory at Women's and Children's Hospital, Adelaide, Australia) showed a level of 390 pmol/min/mg protein (NV: 600-3200). Molecular analysis showed that the patient was a compound heterozygote. One allele had the common mutation p.L444P and the other allele had the p.P319A mutation. The latter was a single base substitution at c.1072 of exon 8 of the acid β-glucosidase gene (NYU Langone Medical Center New York, Neurogenetics Laboratory). Chitotriosidase activity was below the detection limit which could be due to the duplication polymorphism in exon 10 of the chitotriosidase gene (National Taiwan University Hospital, Department of Medical Genetics, Taiwan) (Table 2).

She was started on enzyme replacement therapy (ERT) at $3^{8}/_{12}$ years old with Imiglucerase (Cerezyme) at a dose of 60 units/kilogram body weight every 2 weeks. After 5 years of ERT, the liver and spleen sizes have markedly decreased and her hematologic parameters have normalized. Her bony abnormalities however remain, but have not worsened. She has had significant gains in weight and height and there has been a remarkable improvement in her quality of life. However, on her 5^{th} year of ERT, at 9 years of age, she had 3 episodes of generalized tonic clonic afebrile seizures that were easily controlled by barbiturates. Her EEG and cranial CT scan were normal. She had no gait problems, cerebellar, pyramidal or bulbar signs and no oculomotor involvement was observed. She is currently a grade 1 student and is performing below average. Her psychometric examination revealed that she has mild mental retardation (Table 3).

Patient 2

Patient 2 is the twin sister of Patient 1. She was evaluated at 3 years of age and noted to have hematoma on the lower extremity, a liver palpable 4.5 cm below the right costal margin and a spleen palpable 5 cm below the left costal margin. Neurologic examination was normal. A presumptive diagnosis of Gaucher disease was made, however, it was only at the age of 4 years that the diagnostic evaluation was completed. By this time, she was anemic and thrombocytopenic, with hemoglobin of 84 g/L and platelet count of $49{,}000X10^{9}$/L. Liver and spleen were markedly enlarged to 9 cm and 14 cm below the right and left costal margins, respectively. Liver function tests were normal. Skeletal x-rays showed Erlenmeyer flask deformities in both distal femurs. Bone marrow aspiration showed the characteristic "Gaucher cells". She had a low leukocyte acid β-glucosidase activity of 0.88 nmol/mg/prot/hr (normal range 4.7- >5.10, National Taiwan University Hospital, Department of Medical Genetics, Taiwan) compatible with Gaucher disease. Similar to her twin sister, molecular analysis showed that the patient was a compound heterozygote for the common mutation p.L444P and p.P319A mutations (University of Washington, Department of Pediatrics, Molecular Development Laboratory). Chitotriosidase activity was also below the detection limit (National Taiwan University Hospital, Department of Medical Genetics, Taiwan). She was started on ERT at 6 years of age and three years after the start of ERT at 60 units/kg/day given every two weeks, the anemia and thrombocytopenia resolved. Liver and spleen sizes have decreased; however, bony abnormalities have remained, but have not deteriorated. She has had a significant improvement in growth parameters. Neurologic

Table 1. Summary of demographic and clinical features

	Patient 1	Patient 2	Patient 3
Disease type	Type 3	Type 3	Type 3
Sex	Female	Female	Female
Age at onset of symptoms	2 years 6 months old	3 years old	1 year old
Age at confirmation of diagnosis	3 years 6 months old	4 years old	2 years old
Age at start of ERT	3 years 8 months old	6 years old	2 years 6 months old
Treatment status	Ongoing ERT	Ongoing ERT	Ongoing ERT
Splenectomy	No	No	No

Table 2. Summary of biochemical and molecular genotypes

	Patient 1	Patient 2	Patient 3
Enzyme activity	390 pmol/min/mg protein (n.v.600-3200)	0.88 nmol/mg/prot/hr (n.v.4.7->5.1)	3.26 nmol/mg/prot/hr (n.v.4.7->5.1)
Molecular genotype	p.L444P/p.P319A	p.L444P/p.P319A	p.L444P/p.G202R/p.G202R

Table 3. Summary of clinical manifestations at the time of diagnosis and after ERT

	Patient 1		Patient 2		Patient 3	
	At dx	**After 5 yrs of ERT (60U/kg/2 wks)**	**At dx**	**After 3 yrs of ERT (60U/kg/2 wks)**	**At dx**	**After 9 mos of ERT (60U/kg/2 wks)**
Hematological						
Anemia	Present	Absent	Present	Absent	Present	Improved
Thrombocytopenia	Present	Absent	Present	Absent	Present	Improved
Bleeding (epistaxis, hematoma, petecchiae)	Present	Absent	Present	Absent	Present	Present
Visceral						
Spleen size	14.5 cm	Absent	5 cm	Absent	14 cm	10 cm
Liver size	10 cm	Absent	4.5 cm	Absent	7 cm	6 cm
Bone manifestations						
Bone pain	Absent	Absent	Absent	Absent	Absent	Absent
Bone crisis	Absent	Absent	Absent	Absent	Absent	Absent
Radiologic bone disease [Erlenmeyer flask deformity (EFD), avascular necrosis, osteopenia]	EFD	EFD but has not worsened	EFD	EFD but has not worsened	No EFD, osteopenia	No EFD, osteopenia
Growth	p10-25 for age	p 25 for height; p75 for weight	p5 for age	p25 for age	p10 for age	p25 for age
Ophthalmologic evaluation	Normal	Normal	Normal	Normal	Oculomotor apraxia	Oculomotor apraxia
Neurologic evaluation						
Mental retardation/Global delay	No formal testing done	Mild mental retardation; 50% by DQ	No formal testing done	Mild mental retardation	GDD Pyramidal tract signs	GDD Pyramidal tract signs
Seizures	Absent	Present	Absent	Absent	Present	Present
CT scan/MRI	Not done	Normal	Not done	Not done	Normal	
EEG	Not done	Abnormal	Not done	Not done	Abnormal	

examination is currently normal. She is currently an average grade 2 student; however, formal psychometric examination showed that she also has mild mental retardation (Tables 1, 2, and 3).

Patient 3

Patient 3 is a female who presented with developmental delay and abdominal enlargement during the first year of life. At 1½ years of age, she started to have blank stares which eventually developed to generalized tonic-clonic seizures. While being investigated for the cause of the seizures, she was noted to have hepatosplenomegaly and thrombocytopenia. Bone marrow aspiration biopsy showed "Gaucher cells". When seen at 2 years of age, she had pallor, petecchiae on the chest and hepatosplenomegaly. Initial hemoglobin was 108 g/L and platelet count was 39,000 $X10^9$/L. Liver was 7 cm below the right costal margin and spleen was 14 cm below the left costal margin. Neurologic examination showed oculomotor apraxia, brisk reflexes with 2-3 beats clonus. She walked with an unsteady gait. There was no report of bone pains. Investigations showed anemia and thrombocytopenia. Skeletal survey showed no Erlenmeyer flask deformity or osteopenia. Electroencephalogram showed bifrontal dysfunction and epileptiform discharges. Cranial MRI was normal. β-glucocerebrosidase assay showed 3.26 nmol/mg prot/hr (normal range 4.7- >5.10, National Taiwan University Hospital, Department of Medical Genetics, Taiwan) which confirmed the diagnosis of Gaucher disease. Chitotriosidase activity was high at 5013.82 nmol/ml/hr (nv 0.1-98.0, National Taiwan University Hospital, Department of Medical Genetics, Taiwan). Molecular analysis which involved sequence analysis of the entire coding region of the glucocerebrosidase gene showed that one of her alleles had the common mutation p.L444P, the other allele was homozygous for a rare mutation p.G202R/p.G202R. Mutation testing of the parents' samples showed that the father carried the p.L444P mutation while the mother had the p.G202R mutation. She was started on ERT at 2.5 years of age at 60 units/kg/day given every two weeks. After 4 months of ERT, the liver and spleen sizes have decreased to 5 and 11 cm below the costal margins, respectively. Her hemoglobin has improved although she continues to be thrombocytopenic leading to occasional epistaxis and appearance of petecchiae. Her energy level has improved. Formal neurologic and developmental assessment showed gaze initiation deficit and global developmental delay (Tables 1, 2 and 3).

Discussion

Patients 1 and 2 were initially classified as having type I GD until one of them presented with a seizure disorder. This development to type III Gaucher disease is part of the phenotypic variation and evolution seen in this type of GD.[8] The neurologic manifestations of patients with type III Gaucher disease are likewise varied and may be in the form of developmental delay, seizures, oculomotor apraxia, laryngeal spasms and myoclonus. Not all of them will present with the characteristic oculomotor apraxia seen generally in patients with type III GD.[8] Patients 1 and 2, based on careful neurologic examination, had no signs of cerebellar, pyramidal, extrapyramidal and ocular involvement. Although they only presented with mental retardation and seizures, these two have been reported as part of the phenotypic spectrum of type III GD.[8] Although the impact of ERT on the visceral manifestations and quality of life were clinically significant, the onset of neurologic symptoms may not be prevented by ERT as seen in these two patients.[8]

Patient 3 was classified to have a neuronopathic type of GD on the basis of the subacute presentation in first year of life that slowly progressed to considerable organomegaly and deterioration of neurologic symptoms associated with developmental delay, seizures, hyperactive reflexes and gaze initiation deficit. Patient 3, compared to Patients 1 and 2 because of the presence of neurologic signs and oculomotor apraxia was classified at the outset to have type III GD.

Mutations of the glucocerebrosidase gene are classified on the basis of the severity of the observed phenotypic effects. Severe mutations that can produce enzyme, but when inherited with a null or another severe mutation are usually associated with a neuronopathic type II or III disease. Mild mutations are those that are not associated with a neuronopathic disease.[9] Mutations that prevent the formation of any enzyme such as complete deletion of the gene are considered to be lethal.

The L444P has been classified as severe[9]. The L444P allele is the most prevalent mutation identified in patients with the neuronopathic forms of GD, where it is present in 50% of mutated alleles.[10] Although this allele has been reported across all types of GD including type I disease,[3,11] its presence predicts a neuronopathic phenotype. The absence of oculomotor apraxia in Patients 1 and 2, precluded a neuronopathic classification early on. However, in the background of the L444P allele, the presence of seizures in Patient 1 and mild mental retardation in both, a neuronopathic form was not entirely dismissed. Thus, although it is true that the distinction between type I and III GD is not obvious in early childhood, long term follow up and careful neurologic observation are essential because the neurologic abnormalities of type III GD may not develop until later in life.[8,12,13]

The other allele P319A found in the twins has been reported to be a rare mutation with unknown severity.[14] However, it is assumed that this could be a severe type of mutation resulting to the neuronopathic phenotype of Patients 1 and 2. (personal communication with CR Scott).

The other rare mutation p.G202R, present in Patient 3 has been reported to give rise to a severe neuronopathic phenotype in different ethnic populations.[8,9,14,15,16] Two patients homozygous for the mutation have been reported twice in type II patients.[15,16] The presence of 2 neuronopathic mutations in Patient 3 correlate well with the clinical presentation, that of having an obvious type III disease.

The most likely explanation for the homozygous state of the G202R allele in the patient could be explained by post zygotic meiotic events involving a somatic gene conversion during pairing of homologous alleles. (personal communication with CR Scott) Thus, L444p/G202R is a recombinant allele.

The results of the mutation analysis helped better define the phenotypic manifestation of our 3 patients. The mutations that were identified are all considered to be severe types of mutations, thus all of them presented with a neuronopathic phenotype. Even though all the mutations will predict a neuronopathic phenotype, the severity of the neurologic manifestations may still vary as seen in Patients 1 and 2 which presented mildly compared to Patient 3. Genetic counseling was given to both families and the results of the mutation analysis were able to provide them with a better understanding of the disease manifestations of their children.

In summary, we have discussed the genotypic characteristics of our 3 Filipino patients with Gaucher disease and correlated these with their corresponding phenotypes. Our genotype-phenotype correlations confirmed the previously reported severe phenotypic presentation of the p.L444P allele. The severity of the rarer mutation p.P319A remains unknown but seems to be also severe leading to a neuronopathic phenotype. The other rare mutation p.G202R also gives rise to a neuronopathic phenotype. Our findings further emphasize the importance of genotyping and their clinical consequences. These ultimately allow better understanding of this lifelong disease and aids physicians in therapeutic decision making, better patient monitoring and genetic counseling.

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