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Coinheritance of Gaucher disease and α -thalassemia resulting in confusion between two inherited hematologic diseases

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Abstract

Gaucher type 1 disease has a wide spectrum of phenotypes ranging from asymptomatic individuals to patients with massive hepatosplenomegaly and bone involvement. In most, anemia, thrombocytopenia and splenomegaly are the primary manifestations at diagnosis, findings shared by the hemoglobinopathies. Here we report the co-inheritance of α -thalassemia and Gaucher disease in a consanguineous family followed in Iran, which resulted in confusion regarding the diagnosis. This report emphasizes the need to independently establish the diagnosis of every affected member of a family to ensure appropriate management and therapeutic decisions.

Keywords

Gaucher disease; α -Thalassemia; Splenomegaly; Hemoglobinopathies

Introduction

Gaucher disease (GD) (type 1: OMIM #230800, type 2: OMIM #230900, type 3: OMIM #230100), the most common lysosomal disorder, is caused by a deficiency in the lysosomal enzyme glucocerebrosidase, which cleaves glucocerebroside, a membrane glycolipid, to ceramide and glucose. The disease manifests with hepatosplenomegaly, anemia, thrombocytopenia, growth retardation, bone pain and in some cases neurologic symptoms (types 2 and 3) [1]. The gene for glucocerebrosidase (*GBA*, OMIM #606463) was cloned and sequenced two decades ago and is located on 1q21, a gene-rich region encompassing 7 genes and 2 pseudogenes within 85 kb. The 7.6 kb *GBA* gene consists of 11 exons and 10 introns. A highly homologous 5.7 kb pseudogene (*GBAP*) is located 16 kb downstream and has the same organization of exons and introns [2]. Mutations in *GBA* include point mutations, insertions, deletions, frame-shift changes, splice site alterations and recombinant alleles, found with different frequencies in patients with Gaucher disease from differing ethnicities [3]. Patients with the identical *GBA* genotype can exhibit considerable clinical

heterogeneity. To date, there are approximately 300 mutations and polymorphisms in the *GBA* that are identified in patients with varying presentations of Gaucher disease.

The α -thalassemias (OMIM #141800) are among the most common inherited single-gene disorders worldwide [4]. Allele frequencies for α -thalassemia vary between 0.01 and 0.98 in tropical and subtropical regions of the world [5]. The North American Thalassemia Clinical Research Network (TCRN) study showed that 85% of populations with α -thalassemia are Asian. Originally, α -thalassemias were most frequent in regions where malaria was endemic. About of 95% recognized mutant α -thalassemia alleles have a deletion in one or two of the duplicated α -globin genes on chromosome 16p13.3 [5,6]. Patients with single and double gene deletions have mild anemia while carriers of three gene deletions have severe hemolytic anemia. Deletion of all four α genes results in hydrops fetalis [7]. Different deletional and nondeletional forms of α -thalassemia have been reported worldwide. In Iran, thalassemia is endemic in northern regions near the Caspian Sea, and in southern regions near the Persian Gulf where malaria is endemic.

In this study we demonstrate how the co-inheritance of two genetic disorders with shared clinical manifestations can obscure the definite diagnosis of each disease.

Case report

An Iranian couple, residing in southern Iran with two healthy children, gave birth to five additional offspring, each presumed to have type 1 Gaucher disease. Samples from these family members were referred to our laboratory for further investigations (Fig. 1). The parents (G106 and G107) were consanguineous and came from the southeast of Iran (Sistan and Baluchestan province) near the Persian Gulf and Oman Sea. At birth, their first affected child (G101) was noted to have anemia, an enlarged spleen and thrombocytopenia. The preliminary workup did not lead to a diagnosis, and the spleen was removed to improve the low platelet count. Pathology results on the removed spleen revealed the diagnosis of Gaucher disease. The second and third children born subsequently (G102 and G103) developed the same symptoms (Table 1). To confirm the diagnosis Gaucher disease, enzymatic activity was measured using a fluorometric assay [8] in a laboratory in Europe, demonstrating glucocerebrosidase deficiency in all three children. Enzyme replacement therapy (ERT) with recombinant glucocerebrosidase (Imiglucerase) was begun on the three children, resulting in an improvement of clinical symptoms. In subsequent years, the parents gave birth to two additional children (G104, G105). At age three, anemia was also noted in each of these children. Enzymatic activity was measured in another laboratory in the Middle East and was reported to be deficient, and the two younger children were also begun on ERT. However, in these two, no clinical improvement was noted on treatment, and the cases were referred to us for further investigation.

At this point, peripheral blood samples were obtained from all five children and their parents with informed consent. DNA was extracted using a standard method and was shipped to the National Institutes of Health for genotyping. Sequencing of the all exons and flanking intronic regions of the glucocerebrosidase gene (*GBA*) was performed on the seven genomic DNA samples using a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) as previously described [9]. The data was analyzed using Sequencing Analysis v.5.1.1 and SeqScape v.2.1.1 (Applied Biosystems, Foster City, CA). Additionally, the intronic regions of *GBA*, known to have a high prevalence of polymorphisms, were sequenced to confirm the amplification of both alleles.

Results

The results of sequencing showed that the first three children were homozygous for *GBA* mutation G377S, while parents and the younger two children were heterozygous (Fig. 2, Table 1). Thus DNA analysis confirmed the diagnosis of Gaucher disease in the first three children, while only one mutant allele was identified in the two younger children, who were, therefore, Gaucher carriers.

The G377S mutation, located on exon 9, has been previously identified in patients from different parts of the world. It is considered as a mild rare allele [10] and homozygotes have been reported with type 1 Gaucher disease. However, when this mutation appears with a null or severe *GBA* allele, patients commonly have neurologic symptoms associated with type 3 disease [11]. This mutation is also the third most common mutation in patients of Spanish ancestry [12,13].

Further studies were subsequently performed to identify the underlying cause of the symptoms in the two younger children. Their most significant finding was microcytic anemia. Iron studies and hemoglobin electrophoresis were reported as normal. Since the family lives in a malaria endemic zone where α -thalassemia is common, the patients were analyzed for defects in their α -globin genes. Deletions in α -globin gene were evaluated using multiplex PCR, as previously described [14], revealing that all family members were carriers of the $-\alpha^{3.7}$ deletion. This thalassemia allele is a 3.7 kb deletion in the α -globin DNA sequence introduced by homologous recombination between misaligned chromosomes causing a fusion allele, and is the most common cause of α -thalassemia. Among the family members, four cases were homozygous (G101, G104, G105, G107) and three cases were heterozygous (G102, G103, G106) for the $-\alpha^{3.7}$ deletion, as shown in Table 2. Thus the symptoms encountered in the youngest two family members were likely due to α -thalassemia rather than Gaucher disease, and ERT was superfluous.

Discussion

While there is a temptation to assume that siblings sharing the same disease manifestations have the same disorder, this is not necessarily the case. Actually, in a recessive disorder like Gaucher disease, the likelihood of five affected out of five consecutive live born children is quite low. Moreover, the results of in vitro enzyme activity assays developed for the diagnosis of Gaucher disease including fluorescence and chromogenic assays need to be interpreted carefully. Although the fluorescence-based assay is simple, sensitive and routinely used in diagnostic laboratories, results may vary based on different pro-fluorescent substrates and experiment conditions [8]. It is important to evaluate the results provided together those from normal controls and carriers simultaneously evaluated in the same assay. In this family, the analysis was performed at two separate times and in two different laboratories, and the values should not have been compared. Here, genetic studies to find disease-causing mutations in *GBA* clarified the diagnosis. In Gaucher disease establishing a definite diagnosis is necessary before starting enzyme replacement therapy, as the treatment is very costly and inconvenient [15]. This study showed how inaccurate diagnostic assays can lead to a misdiagnosis in cases with co-inheritance of two disorders with similar hematologic manifestations.

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Fig. 1. Pedigree of affected family showing Gaucher and α -thalassemia status. Filled- symbols indicate cases homozygous for the G377S mutation and half-filled cases are heterozygotes for the same mutation. The written genotype for each case reflects the α -thalassemia genotype.

a) Heterozygote

b) Homozygote

Fig. 2.
GBA sequencing analysis of two of the siblings. The sequence shown is from exon 9, encompassing the site of the G377S (G>A) mutation.

Table 1

Demographic and clinical characteristics of the family members.

Patient	Age (year)	GC activity	GBA genotype	Physical examination findings				
				Splenomegaly	Blood counts	Skeletal involvement	Neurological findings	Height (cm)
G101	13	0.6 ^a	G377S/G377S	Splenectomy	Anemia	Mild osteopenia/bone pain	None	157
G102	8	0.7 ^b	G377S/G377S	Mild	Anemia	Mild osteopenia/bone pain	None	125
G103	6	0.2 ^b	G377S/G377S	Mild to moderate	Anemia	Mild osteopenia/bone pain	None	117
G104	3.5	1.7 ^b	G377S/wt	None	Anemia	None	None	95
G105	2	3.7 ^b	G377S/wt	None	Anemia	None	None	85
G106	54	Not tested	G377S/wt	–	–	–	–	
G107	35	Not tested	G377S/wt	–	–	–	–	

GC: glucocerebrosidase. Wt: wild type.

^aNormal range: 1.4–20 (nmol/spot/21 h).

^bNormal range: > 4 (μmol/l h), G106 and G107 are parents.

Table 2

Hematologic investigation of the family members.

Case	Hematologic Index				Hb Electrophoresis				Coagulation Tests			Mutations in globin chains	
	Hb (g/dl)	RBC×10 ⁶ /mm ³	MCV (fl)	MCH (pg)	WBC/mm ³	Platelet/mm ³	Hb A	Hb A ₂	Hb F	PT	PTT	β mutation	α mutation
G 101	10.9	4.66	66	23	11,200	364,000	96.3	2.8	0.9	N ^a	N	None	-α ^{3,7} /-α ^{3,7}
G 102	11.9	4.91	68	24	6,500	172,000	96.3	3.2	0.5	N	N	None	-α ^{3,7} /αα
G 103	11.4	4.62	71	25	8,000	144,000	96.2	3.1	0.7	N	N	None	-α ^{3,7} /αα
G 104	10.4	4.99	64	21	8,600	289,000	96.9	2.5	0.6	N	N	None	-α ^{3,7} /-α ^{3,7}
G 105	11.1	4.65	72	25	6500	305,000	96.4	2.7	0.9	N	N	None	-α ^{3,7} /-α ^{3,7}
G 106	13.4	5.27	65	24	5,400	323,000	97.8	1.8	0.4	N	N	None	-α ^{3,7} /αα
G 107	11.2	4.55	69	26	6,700	370,000	97.6	2.1	0.3	N	N	None	-α ^{3,7} /-α ^{3,7}

^aN: in normal range.