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Gaucher disease: when molecular testing and clinical presentation disagree-the novel c.1226A>G(p.N370S)–RecNcil allele

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Abstract

We report a 31 year old woman who had prenatal carrier screening for Ashkenazi Jewish (AJ) genetic diseases and was found to have two acid β -glucosidase (GBA) mutations, c.1226A>G(p.N370S) and c.1448T>C(p.L444P), consistent with the diagnosis of Type 1 Gaucher disease (GD1). This genotype typically manifests in late-adolescence with hepatosplenomegaly and early-onset bone involvement. The Proband had a normal physical examination, no organomegaly, and normal blood counts, skeletal survey, and bone density. Leukocyte acid β -glucosidase and plasma chitotriosidase activities were normal. To investigate these unexpected results, her GBA alleles were RT-PCR amplified and sequenced. Five RT-PCR clones were negative for both mutations, while five clones had the c.1226A>G(p.N370S) and c.1448T>C(p.L444P) mutations, along with c.1483G>C (p.A456P), and c.1497G>C(p.V460V) mutations, the latter three lesions composing the rare GBA pseudogene-derived RecNcil allele. Genetic testing misdiagnosed the asymptomatic Proband as affected with Type 1 Gaucher disease; however, molecular studies revealed a novel allele with the two common GBA mutations on the RecNcil background. This allele presumably arose by crossing-over between a c.1226A>G allele and the pseudogene, gene conversion, or a new c.1226A>G mutation on the RecNcil background. This novel complex allele highlights a limitation of carrier screening for GD.

Introduction

Type 1 Gaucher disease (GD1), an autosomal recessive lysosomal storage disease, results from deficient, but not absent activity of acid β -glucosidase (β -Glu), and the lysosomal accumulation of its glycosphingolipid substrate, glucosylceramide (GL-1), primarily in the monocyte-macrophage cells of the liver, spleen, and bone marrow (Grabowski and Beutler 2001). GD1 is most prevalent in the Ashkenazi Jewish population with an incidence of ~1 in 1000 based on a carrier frequency of 1 in 15.2 (Scott et al. 2010). Clinical manifestations include hepatosplenomegaly, anemia, thrombocytopenia, and bone disease, and the absence

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of primary neuronal involvement. Four acid β -glucosidase (GBA) gene mutations c. 1226A>G(p.N370S), c. 84dupG(84GG), c.1448T>C(p.L444P) and c. 115+1G>A(IVS2+1) account for ~96% of the disease-causing lesions in AJ patients. The most common mutation, c.1226A>G(p.N370S), a missense mutation, is present in over 70% of the AJ Type 1 GD patients with a carrier frequency of 1 in ~17 (Scott et al. 2010). The next most common mutation, L444P occurs infrequently (<3%) in AJ GD1 patients (Grabowski 1997).

GD1 is clinically heterogenous, ranging from the childhood onset of severe manifestations to asymptomatic adults with mild disease (Grabowski and Beutler 2001; Balwani et al. 2010). Although there is considerable variability within each genotype, genotype/phenotype correlations have been established for the most common genotypes (Sibille et al. 1993). Patients homozygous for the c.1226A>G(p.N370S) mutation usually present in adulthood with less severe disease. In contrast, characterization of patients with the c. 1226A>G(p.N370S)/c.1448T>C(p.L444P) genotype revealed that they present at an average age of ~17 years with significant hepatosplenomegaly and bone involvement, although about 47% patients may not come to medical attention till adulthood (ICGG Gaucher Registry, data on file).

In the United States, prenatal carrier testing for GD1 by screening for the common GBA mutations is well accepted in the AJ population (Eng et al. 1997). The current expanded GBA screening panel includes eight common mutations [c.1226A>G(N370S), c. 1448T>C(L444P), c.84dupG (84GG), c.115+ 1G>A(IVS2 + 1), c.1263_1317del55, c. 1297G>A(V394L), c.1343G>C(D409H) and c.1604G>A(R496H) (Scott et al. 2010). The American College of Medical Genetics recommends that AJ patients should be offered GD screening to make appropriate reproductive decisions (Gross et al. 2008). Previous experience indicates that the majority of patients chose testing for GD as part of AJ screening after genetic counseling (Eng et al. 1997; Scott et al. 2010) Here, we report a 31 year old AJ female who had routine prenatal carrier counseling and screening as part of her pregnancy planning visit. The patient was screened at a commercial laboratory and was found to have two common GD mutations, c.1226A>G(p.N370S) and c.1448T>C(p.L444P), which were reported as diagnostic of homozygosity for GD1.

Case report

The patient was asymptomatic, had 100% AJ ancestry (i.e., all four grandparents were AJ), and was tested for 11 AJ diseases as part of pregnancy care by her obstetrician. She was screened at a commercial laboratory and was found to have the two common GD mutations, c.1226A>G(p.N370S) and c.1448T>C(p.L444P) [Table 1].

Her husband did not carry any GBA mutation. She was told she had GD1 and was referred to the Comprehensive Gaucher Disease Treatment Center at the Mount Sinai School of Medicine for evaluation, counseling, and treatment.

On review of systems, the patient reported occasional aches in her arms and legs and thought that she bruised easily. Her medical history was otherwise non-contributory with no history of easy bleeding, bone or joint pain, fatigue, or fractures. There was no known history of GD1 in the family and her 35 year old sister who had been previously screened for GD as part of carrier screening did not have a GBA mutation. Her father had died in his 30's in an accident and her 61 year old mother had arthritis and osteopenia.

On examination, the patient had no palpable organomegaly or any other physical findings suggestive of GD1. Her hematologic counts, chemistries (including serum ferritin and HDL cholesterol), skeletal survey, bone density, and echocardiogram were all normal (Table 1). The patient refused skeletal and abdominal MRI's due to claustrophobia. Her acid β -

glucosidase enzymatic activity in isolated peripheral leukocytes was in the low normal range at 2.9 nmol/hr/mg (normal 1.6–8.5 nmol/hr/mg). Chitotriosidase activity, a biomarker for GD1, was normal at 20 U/ml (normal <180U/ml); she had a wild-type CHIT1 genotype (Aerts et al. 2005; Grace et al. 2007). Other GD1 biomarkers, including angiotensin converting enzyme and tartrate resistant acid phosphatase, also were normal.

Diagnostic molecular and enzyme testing

Based on the unexpectedly normal clinical, laboratory, and imaging studies, peripheral blood samples were obtained from the Proband and her mother with informed consent for GBA molecular and/or enzyme assays (Grabowski et al. 1982; Grace et al. 1999).

RT-PCR and sequencing studies

RT-PCR studies of the Proband's leukocyte total RNA were performed and their sequences were determined. For these studies, total leukocyte RNA was reverse transcribed (RT) and PCR-amplified and the entire 1556 nt cDNA was sequenced. RNA was isolated from 15 ml of the Proband's peripheral blood (using the Qiagen RNeasy Midi kit (Valencia, CA). First-strand cDNA was synthesized using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlesbad, CA). The GBA cDNA was PCR-amplified using forward (5'-ATCATGGCTGGCAGCCTCACA-3') and reverse (5'-CCATCACTGGCGATGCCACAG-3') primers, and the cDNA amplicons were subcloned into the pCR 2.1 vector using the TA Cloning kit, Ver 7 (Invitrogen, Carlesbad, CA). The plasmid GBA inserts from 10 individual subclones were sequenced as previously described using an ABI Prism 3730xl Capillary Array Sequencer and the ABI Prism™ BigDye™ Terminator Ready Reaction Mix (Perkin-Elmer-Cetus Norwalk, CT) (Grace et al. 2007).

Results

Molecular genetic testing in our laboratory confirmed the presence of the two disease-causing mutations (Scott et al. 2010). Repeat GBA leukocyte enzymatic activity was in the low normal range (3.2 nmol/hr/mg; normal: 1.6–8.5 nmol/hr/mg protein).

As her clinical presentation was not consistent with GD1, especially for the c.1226A>G/c.1448T>C genotype (Sibille et al. 1993), it was hypothesized that the mutations may be on the same GBA allele. Therefore, family studies were recommended. However, the patient's mother was not initially available and her father was deceased. As the Proband was anxious to establish a diagnosis and start her family, molecular studies were undertaken to determine if both mutations were on the same allele.

RT-PCR of her GBA alleles was performed and 10 cDNA amplicons were sequenced. Five did not have the mutations encoding either p.1226A>G(p.N370S) or p.1448T>C(p.L444P) and had a normal GBA sequence. In contrast, the other five cDNA amplicons carried both mutations as well as two additional lesions, c.1483G>C(p.A456P) and c.1497G>C(p.V460V), the latter three mutations identifying the GBA pseudogene-derived RecNcil GBA allele [Table 2] (Eyal et al. 1990). The novel GBA allele was designated the c.1226A>G(p.N370S)–RecNcil allele.

Subsequently, the Proband's healthy 61 year old mother was tested. Her mother was heterozygous for the c.1226A>G(p.N370S)–RecNcil allele, and had normal acid β-glucosidase enzyme and chitotriosidase activities, further documenting that the mutations were in *cis* on one GBA allele. The Proband's 35 year old sister was previously tested and did not have a GBA mutation, confirming that the mother also had a wild-type allele.

Discussion

Here, we describe a novel GBA allele, c.1226A>G(p.N370S)–RecNcil, identified in an asymptomatic heterozygote for GD1 who was initially misdiagnosed as an affected homozygote based on molecular genetic testing performed as part of routine AJ prenatal carrier screening. Previously, undiagnosed affected GD1 homozygotes who had the c.1226A>G(p.N370S) and c.1448T>C(p.L444P) mutations in *trans* have been identified by prenatal carrier screening programs (Balwani et al. 2010). However, these patients were symptomatic in the first or second decades of life with significant organomegaly and bone disease requiring therapeutic intervention (Sibille et al. 1993). Even homozygous patients on the milder end of this genotype's clinical spectrum have hematologic abnormalities, organomegaly, bone disease and elevated GD1 biomarker levels (Balwani et al. 2010).

Comprehensive clinical, laboratory, and imaging studies of the Proband did not reveal any symptoms of GD. Specifically, she did not have anemia, thrombocytopenia, hepatosplenomegaly, or bone disease. Her acid β -glucosidase activity determined on two occasions was in the low normal range, consistent with heterozygosity (Grabowski et al. 1982). In addition, she had a normal chitotriosidase activity, a biomarker which is significantly elevated in GD1 homozygotes (Aerts et al. 2005). These results led to the notion that the Proband was heterozygous for GD1 and that the c.1226A>G(p.N370S) and c.1448T>C(p.L444P) missense mutations were in *cis* on the same allele. Molecular analysis of her subcloned GBA RT-PCR amplicons confirmed that the Proband had a normal allele and a novel multi-mutated allele, containing the c.1226A>G(p.N370S) mutation and the three lesions [c.1448T>C(p.L444P), c.1483G>C(p.A456P), and c.1497G>C(p.V460V)] in the previously described RecNcil allele (Eyal et al. 1990). The RecNcil allele resulted most probably from non-homologous recombination between the functional GBA allele and the highly homologous, non-functional GBA pseudogene, which is 16 kb downstream, or by gene conversion (Horowitz et al. 1989; Eyal et al. 1990). Thus, the Proband is heterozygous for GD1, having a very rare allele with the most common GD mutation in the Ashkenazi Jewish population, c.1226A>G(p.N370S) (Grabowski and Beutler 2001), present on the RecNcil allele containing the other common GD allele, c.1448T>C(p.L444P).

Recombination events are known to occur with the GBA pseudogene resulting from non-homologous recombination, gene conversion, fusion or duplication (Hruska et al. 2008). Data from The International Collaborative Gaucher Group Gaucher Registry identified 27 of over 3,580 GD patients with known genotypes who had one GBA allele with multiple mutations and the second allele with a single mutation (ICGG Gaucher Registry, data on file). Most of the multi-mutated alleles contained rare mutations which are not included in the GBA targeted mutation panels used for carrier screening and would not be detected unless the alleles were sequenced. Of these 27 affected GD patients, four had two or more mutations on one allele in *cis*, and a pathogenic mutation on the other allele. These included two affected patients with c.1226A>G(p.N370S) and c.1448C>T(p.L444P) in *cis* on one allele, a third patient having an allele with c.1226A>G(p.N370S) and RecTL [i.e., c.1343G>C (p.D409H), c.1448C>T (p.L444P), c.1483G>C (p.A456P), and c.1497G>C(p.V460V)], and the fourth having one allele with RecTL and c.1263_1317del55. Most commercially available GBA screening panels include the c.1226A>G(p.N370S), c.1448C>T(p.L444P), c.1343G>C(p.D409H) and c.1263_1317del55 mutations. Among these four patients, three had the p.1226A>G(p.N370S) on the other allele, while the fourth had the c.597delT mutation, which predicted the neuropathic phenotype. These presumably all resulted from non-homologous recombination or gene conversion with the GBA pseudoallele (Hruska et al. 2008).

In 2000, Beutler and colleagues first reported an affected GD1 patient with three mutations, the c.1226A>G(p.N370S) missense mutation on one allele and the c.1226A>G(p.N370S) and c.1448T>C(p.L444P) missense mutations on the other (Beutler et al. 2000). They recognized the implications for a false-positive prenatal diagnosis for such patients if the fetus had inherited the c.1226A>G(p.N370S)–c.1448C>T(p.L444P) allele and was diagnosed as affected, but was actually a GD heterozygote.

To our knowledge, our Proband is the first reported case who was misdiagnosed as being affected with GD1 when actually she was a heterozygous carrier. This initial misdiagnosis had serious implications for genetic counseling and prenatal testing as our patient was planning a pregnancy. Without determination of the carrier status of the mother by enzyme and molecular studies, the Proband's pregnancy may have been misdiagnosed as affected by molecular testing using commercially available panels if the fetus had inherited the multi-mutated allele. Prenatal diagnosis by GBA enzyme assay is not readily available due to the enzyme assay's lack of accuracy and difficulty in diagnosing carriers who may have intermediate or low normal enzymatic activities (Grabowski et al. 1982). Similarly, if prenatal testing by amniocentesis or chorionic villus sampling was offered to the Proband's mother, it could have incorrectly identified our patient as a compound heterozygote affected with GD1 and predicted a moderate to severe phenotype, which may have resulted in an erroneous pregnancy termination.

In summary, this study illustrates the pitfalls of molecular genetic testing for the common GD mutations for prenatal carrier screening or diagnostic studies. All screen-positive individuals should be referred to an expert center for confirmation by acid β -glucosidase enzyme assay and a comprehensive clinical evaluation, as well as for genetic counseling including reproductive risks.

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References

- Aerts JM, Hollak CE, van Breemen M, Maas M, Groener JE, Boot RG. Identification and use of biomarkers in Gaucher disease and other lysosomal storage diseases. *Acta Paediatr Suppl.* 2005; 94:43–46. [PubMed: 15895711]
- Balwani M, Fuerstman L, Kornreich R, Edelmann L, Desnick RJ. Type I Gaucher disease: Significant disease manifestations in “asymptomatic” homozygotes. *Arch Intern Med.* 2010; 170:1463–1469. [PubMed: 20837833]
- Beutler E, Liebman H, Gelbart T, Stefanski E. Three Gaucher-disease-producing mutations in a patient with Gaucher disease: mechanism and diagnostic implications. *Acta Haematol.* 2000; 104:103–105. [PubMed: 11154983]
- Eng CM, Schechter C, Robinowitz J, et al. Prenatal genetic carrier testing using triple disease screening. *JAMA.* 1997; 278:1268–1272. [PubMed: 9333269]
- Eyal N, Wilder S, Horowitz M. Prevalent and rare mutations among Gaucher patients. *Gene.* 1990; 96:277–283. [PubMed: 2269438]
- Grabowski GA. Gaucher disease: gene frequencies and genotype/phenotype correlations. *Genet Test.* 1997; 1:5–12. [PubMed: 10464619]
- Grabowski, GA.; Beutler, E. Gaucher disease. In: Scriver, C.; Beaudet, A.; Sly, W.; Valle, D., editors. *The Metabolic and Molecular Bases of Inherited Diseases*. 8. McGraw-Hill; New York, NY: 2001. p. 3635-3668.

- Grabowski GA, Dinur T, Gatt S, Desnick RJ. Gaucher Type 1 (Ashkenazi) disease: a new method for heterozygote detection using a novel natural substrate. *Clin Chim Acta*. 1982; 124:123–133. [PubMed: 6889929]
- Grace ME, Ashton-Prolla P, Pastores GM, Soni A, Desnick RJ. Non-pseudogene-derived complex acid beta-glucosidase mutations causing mild type 1 and severe type 2 Gaucher disease. *J Clin Invest*. 1999; 103:817–823. [PubMed: 10079102]
- Grace ME, Balwani M, Nazarenko I, Prakash-Cheng A, Desnick RJ. Type 1 Gaucher disease: null and hypomorphic novel chitotriosidase mutations-implications for diagnosis and therapeutic monitoring. *Hum Mutat*. 2007; 28:866–873. [PubMed: 17464953]
- Gross SJ, Pletcher B, Monaghan K, Practice P, Committee G. Carrier screening in individuals of Ashkenazi Jewish descent. *Genet Med*. 2008; 10:54–56. [PubMed: 18197057]
- Horowitz M, Wilder S, Horowitz Z, Reiner O, Gelbart T, Beutler E. The human glucocerebrosidase gene and pseudogene: structure and evolution. *Genomics*. 1989; 4:87–96. [PubMed: 2914709]
- Hruska KS, LaMarca ME, Scott CR, Sidransky E. Gaucher disease: mutation and polymorphism spectrum in glucocerebrosidase gene (GBA). *Hum Mutat*. 2008; 29:567–583. [PubMed: 18338393]
- ICGG Gaucher Registry, Data on File
- Scott SA, Edelmann L, Liu L, Luo M, Desnick RJ, Kornreich R. Experience with carrier screening and prenatal diagnosis for sixteen Ashkenazi jewish genetic diseases. *Hum Mutat*. 2010; 31:1240–1250. [PubMed: 20672374]
- Sibille A, Eng CM, Kim SJ, Pastores G, Grabowski GA. Phenotype/genotype correlations in Gaucher disease type I: clinical and therapeutic implications. *Am J Hum Genet*. 1993; 52:1094–1101. [PubMed: 8503443]

Table 1

Clinical evaluation of the proband

Test	Proband	Normal values
Initial diagnostic genotype	c.1226A>G(p.N370S)/c.1448T>C(p.L444P)	—
Leukocyte GBA activity	2.9 nmol/hr/mg	1.6–8.5 nmol/hr/mg
Chitotriosidase	20 U/mL	<180 U/mL
Hemoglobin	13.3 g/dL	12–15 g/dL
Platelets	344,000/uL	150–450,000/uL
Skeletal survey	Normal	—
Bone density	Normal at hip and spine for age	—
MRI abdomen and femurs	Deferred	—

Table 2

Sequencing results for the GBA RT-PCR subclones

Number of clones	c.1226A>G	c.1448C>T	Other DNA changes
5	c.1226A (wt)	c.1448C (wt)	—
5	c.1226A>G (p.N370S)	c.1448C>T* (p.L444P)	c.1483G>C* (p.A456P); c.1497G>C* (p.V460V)

* c.1448C>T(p.L444P), c.1483G>C(p.A456P) and c.1497G>C(p.V460V) are all pseudogene-derived and known as the RecNcil allele