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Alleles with more than one mutation can complicate genotype/phenotype studies in Mendelian disorders: Lessons from Gaucher disease

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Abstract

Autosomal resessive Mendelian disorders usually result from two inherited disease-causing mutations. However, this is not always the case. Focusing on Gaucher disease, which results from mutations in *GBA1*, we found that more comprehensive genotyping revealed important exceptions. For example, patients with uniparental disomy or new mutations do not inherit a mutation from each parent. Furthermore, we identified patients found to carry more than one *GBA1* mutation on the same allele. It is essential to examine the entire *GBA1* gene in order to establish an accurate genotype. Missing the second mutation can complicate genotype/phenotype studies and result in improper genetic counseling.

Keywords

Gaucher disease; Genotype/phenotype correlation; Glucocerebrosidase; Sequencing

In the current era, the diagnosis of Mendelian disorders, including lysosomal storage disorders, is increasingly performed by DNA-based methods. When DNA diagnostics are utilized to test for an autosomal recessive genetic disorder, the clinician is generally assured of a diagnosis when two disease-causing mutations are identified in a given gene. However, in certain situations, this can be an over-simplification, and the genotype is more complex despite the Mendelian pattern of inheritance. Studying Gaucher disease (GD) as a prototype disorder, we have encountered patients who actually have more than one *GBA1* mutation on the same allele. Sometimes, this can be due to a recombination event with the highly homologous nearby pseudogene. However, we also provide examples of alleles carrying two descrete point mutations in *cis*, complicating accurate genetic diagnosis. Such cases emphasize the need to carefully examine the entire coding regions prior to assigning a genotype, especially when attempting to determine prognosis or establish genotype/phenotype correlations.

Gaucher disease (GD), a disorder characterized by the deficiency of the enzyme glucocerebrosidase, ordinarily results when an individual inherits a *GBA1* mutation from

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each parent. This deficiency causes the accumulation of glycolipids in the lysosomes of cells, leading to a range of symptoms [1]. Traditionally, GD is divided into three types depending upon the presence, severity and duration of neurological symptoms. Type 1 (GD1) is characterized by the absence of neurological manifestations, although the recent association between *GBA1* mutations and parkinsonism has complicated this designation [2]. Patients with GD1 can experience a variety of symptoms, including fatigue, skeletal involvement, thrombocytopenia, and hepatosplenomegaly. Patients with type 3 (GD3) can develop these same symptoms, but also have neurological involvement, ranging from an isolated eye movement disorder to epilepsy and ataxia. The most severe form is type 2 (GD2), a lethal disorder with progressive neurodegeneration and death in the first decade of life resulting from brainstem involvement [3,4].

However, within each type, there is still significant clinical heterogeneity and limited correlation between genotype and phenotype. Gaucher disease is a pan-ethnic disorder seen more frequenty among Ashkenazi Jews, where the carrier frequency is 1 in 14–18. Multiple genotypes have been found in Ashkenazi and non-Ashkenazi patients and in each GD type [5]. Moreover, patients with the same genotype, even affected siblings, display phenotypic discordance [6]. Thus, efforts are ongoing to explain how the genetic findings correlate with specific phenotypes.

Genotyping *GBA1* can be challenging because of the presence of a nearby pseudogene, whose exons share > 96% homology with the gene. While various screening panels have been utilized for genotyping, direct sequencing of all exons is desirable, and care must be taken to identify alleles resulting from recombination with the pseudogene. Adding to the complexity in interpreting genotypic results in GD are cases of patients found to carry multiple mutations on the same allele. Table 1 summarizes the genotypes of several patients (taken from data on approximately 400 patients with GD) with at least two mutations on the same allele. We do not include the individual base pair changes found in recombinant alleles known to have several pseudogene-derived nucleotide changes, unless they are found in *cis* with another unrelated non-pseudogene-derived *GBA1* mutation [7–9].

Doubly mutated *GBA1* alleles were encountered in patients with all three types of GD. The patient with GD1 with genotype N188 K/N188 K+R463C (p.N227 K/p.N227 K+p.R502C) is puzzling since R463C has been observed in GD1 and GD3, while N188 K was also found in GD2 [10–12]. A second patient with GD1 had three known *GBA1* mutations N370S (p.N409S), G377S (p.G416S) and a recombinant allele (RecTL). Had only two of the mutations, RecTL and G377S, been first identified, a neurological phenotype may have been falsely predicted.

Another interesting genotype, c.(−150)A > G+S125R/R131H+*RecNciI*, was detected in a patient of Indian descent with GD2 who had congenital ichthyosis and a rapidly progressive phenotype with organomegaly and neurological deterioration. She died at age three months. Genotyping first revealed two mutations, R131H (p.R170H) and S125R (p.S164R), both in exon 5, but then the recombinant allele, Rec*NeiI* was noted. Mutation R131H, present in *cis* with this known severe recombinant allele, was previously identified in a newborn with GD together with L444P (p.L483P) [13]. Homozygosity for the second exon 5 mutation, S125R,

was reported in a patient with GD from India [14]. Thus, the affected infant likely carried three severe mutations. Another patient with GD2 had two mutations, R257Q (p.R296Q) and L444P, in addition to a fusion recombinant allele that results from a crossover between intron 10 and exon 11. Several of our patients, including four with GD3, carry two point mutations, in addition to a recombination allele (Rec 7b) with a crossover site between the 3' UTR and the nearby gene for metaxin [7].

As previously reported, approximately 5% of patients with Gaucher disease with two identified *GBA1* mutations carry the alterations E326K (p.E365K) or T369 M (p.T408 M), which are also found both in controls and subjects with Parkinson disease [15–17]. However, homozygosity for either of these changes does not appear to result in GD. In addition, both the K(−)27R, located in the leader peptide signal sequence, and c.(−203)A > G mutations have been reported together with other *GBA1* mutations, although the consequence of these 5' nucleotide changes is not clear. Moreover, it has previously been established that a mutant allele with both D409H (p.E448H) and H255Q (p.H294Q) is a founder allele present in patients from Greece and Croatia [18]. Awareness of this doubly mutated allele is essential, as genotype D409H/D409H is associated with a specific form of GD3 associated with cardiac calcifications, whereas D409H+H255Q/ D409H+H255Q generally results in GD2.

Searching through the published literature, we did find several other instances of GD with two mutations on the same allele. Remarkably, Beutler et al. described a patient with genotype N370S/ N370S who also carried mutation L444P [19]. Additionally, Filcamo et al. reported a patient, with GD2 and genotype G202R/G202R (p.G241Rp./G241R), who also carried M361I (M400I) [8]. M361I was also reported as a third mutation W179X/M361I +N370 (p.W218X/p.M400Ip.N409S) in a patient from Brazil [20].

Finally, another phenomenon that can occur in Mendelian disorders is somatic mosaicism. This was described in a patient with GD2 who was initially found to have genotype L444P/ N370S. Further investigation revealed that the actual genotype was L444P/N370S+S488P where S488P (p.S527P) was found to be a somatic mutation [21].

Together, these findings suggest that when either an appropriate or an unexpected genotype is first identified, it is necessary to completely evaluate the entire locus, as the genotype may indeed be more complex than initially reported. Incomplete screening could result in an incorrectly predicted phenotype, or complicate future genotype-phenotype correlation studies. Also, care must be taken when using genotyping to establish the diagnosis of GD, as there is at least one published case where a carrier with two mutations on the same allele was mistaken as a patient with GD [9]. This consideration is deeply relevant when performing genetic counseling or direct-to-consumer genetic testing.

Since most patients with GD carry only two *GBA1* mutations, these findings do not explain the vast degree of phenotypic heterogeneity encountered in GD. However, they do provide another possible explanation in individual cases, and demonstrate the need to perform complete genotyping, even when a seemingly appropriate genotype has already been reported. There have also been recent reports of possible germline mutations and uniparental disomy in patients with GD [22,23]. Thus, multiple molecular and cellular events can

complicate genetic diagnosis in a Mendelian disorder. In addition, current next-generation sequencing (NGS) strategies often fail to accurately identify all nucleotide changes and recombinant alleles when genes like *GBA1* have non-processed pseudogenes. Therefore, new and improved methodologies are needed to enable the detection of recombinant alleles by NGS [24]. The use of quantitative real-time PCR methods to detect and identify recombinant *GBA1* alleles, including other genetic events such as *GBA1* fusions, duplications, and crossover sites can complement sequencing [25]. Overall, a more complete and comprehensive genetic analysis of patients with GD is necessary, especially when attempting to establish reliable genotype-phenotype correlation.

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Table 1

Genotypes of patients with Gaucher disease with > 2 *GBA1* mutations.

GD Type	Genotype	Reference
Genotypes found in our patient cohort		
1	N188 K/N188 K+R463C (g.4357 T > G/ g.4357 T > G+ g.7375C > T)	This study
1	RecTL+N370S/G377S or RecTL+ G377S/ N370S (RecTL+ g.6728A > G / g.6748G > A)	This study
1	V352 L/Recla+Rec7b* (g.6273G > C/ Recla+Rec7b*)	[7]
1	R170C/L444P+Rec 7b* (g.4301C > T/ g.7319T > C+ Rec7b*)	[7]
2	c.(−150)A > G+S125R/R131H+*RecNciI* (c.(−150) A > G+g.3958C > G/ g.3975G > A+*RecNciI*)	This study
2	L444P/L444P+A456P (g.7319T > C/ g.7319 T > C+g.7354G > C)	[22]
2	R257Q/L444P+Rec 6b* (g.5118G > A/ g.7319 T > C+Rec 6b*)	[7]
2	L444P+E326K/L444P+Rec 7b* (g.7319T > C+g.6195G > A/ g.7319 T > C+Rec 7b*)	[7]
3	R463C/Rec 1a*+Rec 7b* (g.7375C > T/ Rec 1a*+Rec 7b*)	[7]
3	L444P/D409H+Rec 7b* (g.7319 T > C/ g.6844G > C+Rec 7b*)	[7]
3	L444P/L444P+Rec 7b* (g.7319 T > C/g.7319 T > C+Rec 7b*)	[7]
3	G202R/L444P+Rec 7b* (g.4397G > A/ g.7319 T > C+Rec 7b*)	[7]
Genotypes described in the literature		
1	N370S/N370S+L444P (g.6728A > G/ g.6728A > G+g.7319 T > C)	[19]
1	N370S/L461P+IVS10+1G > T (g.6728A > G/ g.7370 T > C+IVS10+1G > T)	[20]
1	W179X/M361I+N370S (g.4329G > A/ g.6302G > A+g.6728A > G)	[20]
1	N370S/W184R+K(−)27R (g.6728A > G/ g.4343 T > C+g.l864A > G)	[26]
1	N370S / IVS4-2A > G+c.(−203)A > G (g.6728A > G/ IVS4-2A > G+g.l256A > G]	[27]
2	G202R/G202R+M361I (g.4397G > A/ g.4397G > A+g.6302G > A)	[8]
2	L444P/N370S+S488P (g.7319 T > C/ g.6728A > G+g.7144 T > C)	[21]

Recombinant alleles are designated as described in Tayebi et al., 2003 [7].