

SHORT REPORT

A germline or *de novo* mutation in two families with Gaucher disease: implications for recessive disorders

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Gaucher disease (GD) is an autosomal recessive storage disorder that most commonly results from the inheritance of one identifiable mutant glucocerebrosidase (*GBA1*) allele from each parent. Here, we report two cases of type 2 GD resulting from the inheritance of one identifiable paternal mutant allele and one allele that likely resulted from a maternal germline mutation. Germline mutations or mosaicism are not generally associated with autosomal recessive disorders. The probands from the two unrelated families had the same maternal mutation, leu444pro, that we propose resulted from a *de novo* maternal germline mutation occurring at this known 'hotspot' for mutation. This first report of a germline mutation for a common point mutation leu444pro (c.1448 T>C;p.leu483pro) in GD has significant implications for molecular diagnostics and genetic counseling in recessive disorders.

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INTRODUCTION

Gaucher disease (GD), the most common lysosomal storage disorder, results from the autosomal recessively inherited deficiency of glucocerebrosidase (GCCase, EC 3.2.1.45).^{1,2} GD is subdivided into three types based on the presence and severity of CNS involvement. Type 2, acute neuronopathic GD, is the most severe form with early and progressive neurologic involvement, leading to death prenatally, in infancy or by early childhood. The gene encoding GCCase (*GBA1*, MIM no. 606463) has a highly homologous pseudogene located 16 kb downstream on chromosome 1q21, sharing 96% exonic sequence homology. This pseudogene has, over time, given rise to recombinant mutant alleles.³ Over 300 unique mutations have been identified in *GBA1*. Mutation leu444pro (c.1448 T>C;p.leu483pro) is a common mutant allele encountered in many different populations.^{4–10} The high frequency and worldwide distribution of the leu444pro mutation indicates that the site may be a 'mutational hotspot' for a T→C nucleotide transversion on c.1448 of *GBA*. This T→C substitution occurs both as a single point mutation and as part of a recombinant allele with other pseudogene sequence, further implicating this site as a mutational hotspot.¹¹

Studies in sperm have made it possible to estimate the distribution and frequency of new human germline mutations at specific sites, providing evidence that premeiotic gonadal cells carrying a mutation might have a selective advantage, resulting in an increased likelihood of germline mosaicism or a new mutation in germline cells.^{12,13} This phenomenon may also contribute to the high frequency of the leu444pro mutation among different populations. We encountered two families, each with an affected child with type 2 GD, where the paternal mutant allele was identified in their respective fathers.

However, in both cases, the second mutant allele, leu444pro, was not detected in the mother. This prompted us to explore whether the leu444pro mutation might have resulted from a maternal germline mutation in these families. One other case of a presumed germline recombinant mutation in GD has been reported¹⁴ that includes the leu444pro alteration, but not as a point mutation.

PATIENTS AND METHODS

Family no. 1:

The proband, a female born at term to non-consanguineous parents of non-Jewish, European (German, British and Swedish) and Puerto Rican and Native American descent, had an early course complicated by stridor, copious secretions, poor feeding, gastroesophageal reflux, swallowing difficulties, ophthalmoplegia, paroxysmal apneic episodes, failure to thrive and developmental delay. At 5 months of age, a chest MRI revealed a vascular ring/double aortic arch that was surgically corrected at 7 months of age. A Nissen fundoplication and gastrostomy were performed at 8 months of age to address worsening reflux and persistent feeding difficulties. Laboratory findings demonstrated anemia and thrombocytopenia, and a bone marrow biopsy revealed lipid-laden macrophages characteristic of GD. A lysosomal enzyme panel demonstrated diminished GCCase activity. The early onset of disease, coupled with apneic spells, ophthalmoplegia, stridor, swallowing difficulties, poor gross motor development and failure to thrive strongly suggested a diagnosis of type 2 GD. The patient died at 11 months of age.

Family no. 2:

This proband, born to a non-consanguineous Ashkenazi Jewish father and a Sephardic Jewish mother, has been described previously.¹⁵ He was diagnosed at the age of 7 months after a bone marrow aspiration revealed Gaucher cells.

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The diagnosis was confirmed by deficient GCCase activity, and he died at 10 months secondary to respiratory failure.

Genotyping:

GBA1 gene was sequenced in genomic DNA samples from both probands and their family members. Genomic DNA was extracted from leukocytes, and/or fibroblast and buccal cells. The *GBA1* gene was amplified from genomic DNA in three fragments encompassing exons 1–5, 5–7 and 8–11 as previously described.¹⁶ Amplified fragments were purified using a QIAquick PCR Purification Kit (Qiagen, Santa Clarita, CA, USA), and Cycle sequencing was performed using the DyeTerminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) with both forward and reverse primers,¹⁶ using an ABI Prism 3730XL DNA Analyzer (Applied Biosystems). The sequencing data was analyzed by SeqScape v2.6 using GeneBank NG_009783.1 as the reference sequence. To confirm the mutation, and exclude any chance of nucleotide variation at the primer site in the mother, a 700-bp amplified region of *GBA1* surrounding the leu444pro mutation was digested with the restriction enzyme *Nci* I (New England Biolabs, Ipswich, MA, USA) as previously described.¹⁷ Long-range PCR of *GBA* also was performed on maternal DNA to rule out deletions or nucleotide variations at the primer sites.¹⁸

Maternity testing

DNA samples from the parents and probands in both families were sent to the DNA diagnostics Center (Fairfield, OH, USA). The probability of maternity was evaluated using 32 markers for Family no. 1 and with 16 markers for Family no. 2.

Enzymatic activity and western blotting

Enzyme activity was determined using a 4-methylumbelliferyl- β -D-glucopyranoside substrate (Sigma, St Louis, MO, USA). Western blot analysis was performed on fibroblast extracts from proband no. 1, her parents, and two controls as previously described.^{19,20}

RESULTS

The genotype of proband no. 1 was thr323ile (c.1085C>T;p.thr362ile)/leu444pro (c.1448T>C;p.leu483pro; Figure 1a) based on genomic DNA sequencing data. Sequencing of paternal genomic DNA identified a heterozygous mutation, thr323ile, which was previously reported in a patient with type 1 GD.²¹ Surprisingly, no mutation was detected in the mother. The proband had a maternal half sibling, and genomic sequencing of her DNA also failed to demonstrate the leu444pro mutation. Prenatal testing of a subsequent pregnancy demonstrated that the fetus carried thr323ile, but not the leu444pro mutation. Evaluation of 32 different DNA markers in the parents and the child confirmed maternity with over 99.9% certainty. Leu444pro mosaicism was deemed unlikely, based upon documentation of normal *GBA1* sequence in different maternal tissues, including blood, skin fibroblasts and buccal cells. Restriction digestion with the enzyme *Nci* I and sequencing of the long-range PCR product confirmed the absence of leu444pro in the mother. A western blot demonstrated normal expression of GCCase in maternal cells, whereas the affected infant displayed markedly decreased GCCase expression (Figure 1b). Finally, GCCase activity assayed in the proband, mother and two controls confirmed that levels of activity in maternal samples were similar to controls, while the proband showed severely deficient GCCase activity typical of type 2 GD (Figure 1c). Therefore, the results of *GBA1* sequencing, restriction digests, western blots and GCCase activity all provided no evidence of a somatic maternal mutation.

In proband no. 2, genomic sequencing of *GBA1* demonstrated the genotype leu444pro/leu444pro + ala456pro (c.1483G>C;p.ala495pro). His father was heterozygous for the leu444pro + ala456pro allele, but no mutation was detected in DNA samples from the

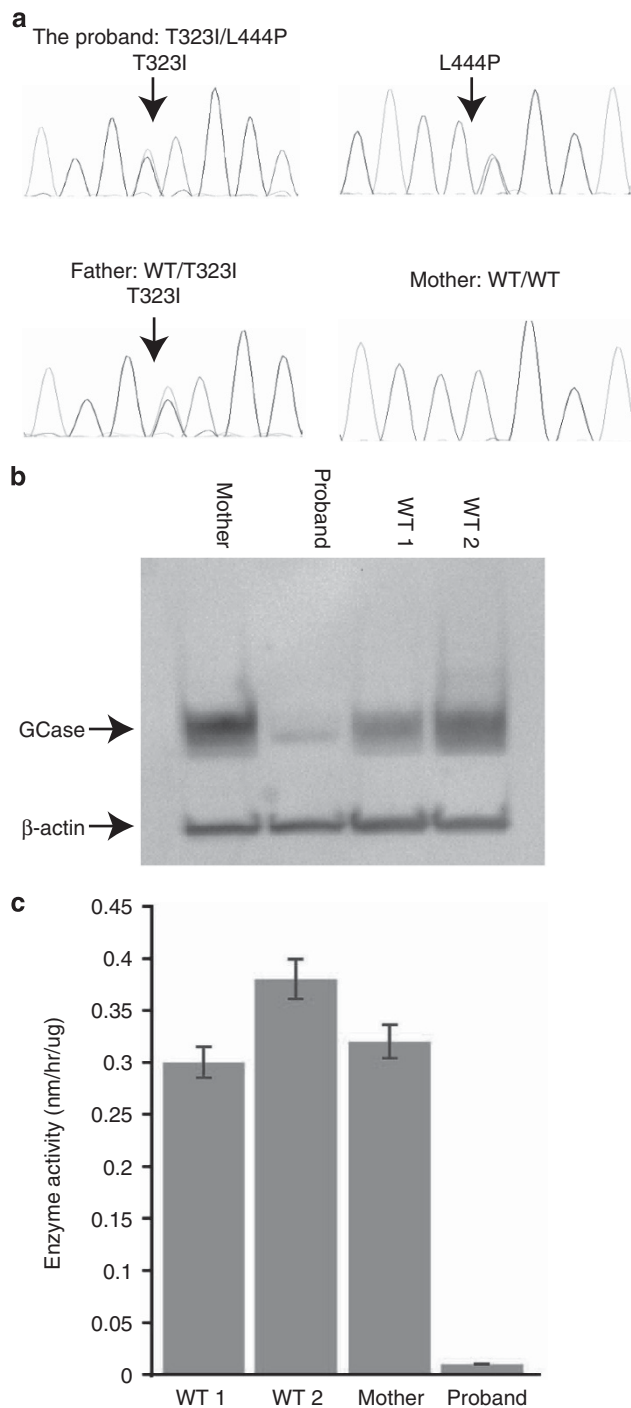


Figure 1 Studies in Family no. 1. (a) Chromatograms showing *GBA1* sequence including the proband, mother and father. Each individual genotype has been labeled. (b) Western blot probed with antibody to GCCase performed on total protein extracted from the proband, mother and two WT controls fibroblast cell lines. GCCase levels were compared with β -actin. (c) GCCase activity measured in fibroblast extracts from the proband, mother and two WT controls.

mother, sister or maternal grandmother. Maternity was confirmed with 99.7% certainty.

In both families, the heterozygosity of polymorphic sites surrounding position c.1448 was confirmed by the complete amplification of

the maternal *GBA1* allele. In addition, sequencing of *GBA1* amplified by long-range PCR confirmed that there were no deletions or nucleotide variations at the primer sites.

DISCUSSION

On the basis of results described, we propose that the leu444pro mutation in both families originated in the mothers' ova, either as a result of germline mosaicism or a *de novo* mutation in one ovum that took place during cell division (mitosis/meiosis). Multiple affected offspring from non-carrier parents is usually attributed to germline mosaicism. In our study, none of the other offspring was a carrier for leu444pro, enhancing the probability that the leu444pro mutation resulted from a *de novo* mutation rather than a germline mosaicism. Unfortunately, these possibilities cannot be definitively confirmed without ovarian biopsies. A less likely alternative possibility is that the mutation arose as an early post-zygotic event in the proband, leading to mosaicism. The high frequency of the leu444pro mutation among different populations, along with evidence that this site has been a mutational hotspot, could explain the nucleotide transversion originating at this site. Although common in different types of cancers, germline mutations are rare in autosomal disorders. There have been reports of germline mutations in several dominant disorders such as achondroplasia, Apert syndrome and multiple endocrine neoplasia,²² and in one patient with Ataxia Telangiectasia, an autosomal recessive disorder.²³ However, this is the first report of the presence of the same maternal germline mutation in two unrelated families with GD, with significant implications for molecular diagnostics and genetic counseling. Such germline mutations may not always be evident, as parental DNA is not consistently evaluated. This finding has implications for genetic counseling, for if only one parent is a carrier for a recessive disorder, the chance of having an affected child may not be zero and the possibility of germline mosaicism or a *de novo* mutation should be considered. The identification of the two cases reported suggests that these events may occur more often than appreciated in GD and other recessive disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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