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Type 2 Gaucher Disease in an Infant Despite a Normal Maternal Glucocerebrosidase gene

Ermias Hagege[#], Richard J Grey[#], Grisel Lopez, Tamanna Roshan Lal, Ellen Sidransky^{*}, and Nahid Tayebi

Medical Genetics Branch, National Human Genome Research Institute, NIH, Bethesda MD

Abstract

Gaucher disease (GD) is a recessively inherited autosomal lysosomal storage disease, the most severe of which is type 2, an acute neuronopathic form. We report an affected infant who inherited one mutant allele, Arg257Gln (c.887G>A; p.Arg296Gln) from his father, while the second, Gly202Arg (c.721G>A; p.Gly241Arg) arose by either maternal germline mosaicism or as a *de novo* mutation. This is the first time mutation Gly202Arg has been reported to be inherited non-traditionally. This report is part of a growing literature suggesting that Gaucher disease can be inherited via germline or *de novo* mutations, and emphasizes that it is critical for clinicians to consider such inheritance when making diagnostic decisions or providing genetic counseling.

Keywords

Gaucher disease; glucocerebrosidase; germline mosaicism; neuronopathic; genetic counseling

Introduction

Gaucher disease (GD, MIM 606463) is a lysosomal storage disorder resulting from a deficiency of the enzyme glucocerebrosidase (GCase, EC 3.2.1.45) (Sidransky et al, 2004; Beutler & Grabowski, 1995). This disease, typically acquired in an autosomal recessive manner, is caused by mutations in the gene *GBA1*, and has been categorized into three types based on the extent of central nervous system involvement. Type 2, acute neuronopathic GD, is the most lethal form of the disease, with mortality generally occurring in the first months to years of life (Stone et al, 2000; Tezuka et al. 2017).

GBA1 is located in a gene-rich region on chromosome 1q21 containing seven genes and two pseudogenes (Winfield et al, 1997). A highly homologous *GBA1* pseudogene (p*GBA*), with the same organization of exons and introns, is located only 16 kb downstream (Horowitz et al, 1989). In addition, *MTX*, a contiguous gene just downstream of p*GBA*, is convergently transcribed and encodes metaxin, a protein located on the outer mitochondrial membrane. *MTX* also has a homologous pseudogene, located downstream of *GBA* (Armstrong et al,

^{*}Corresponding author: Ellen Sidransky M.D., Chief, Section on Molecular Neurogenetics, Medical Genetics Branch, National Human Genome Research Institute, NIH, Building 35, Room 1E623, 35 Convent Drive, MSC 3708, Bethesda, MD 20892-3708, Phone: 301-451-0901 or 301-496-0373, Fax: 301-402-6438, sidranse@mail.nih.gov.

[#]These authors contributed equally

1997; Long et. al, 1996). The close proximity of these genes and their homologous pseudogenes leads to relatively frequent recombination events (Mazzarella & Schlessinger, 1998; Tayebi et al. 2003). Today, more than 300 unique mutations in *GBA1* have been described, including both point mutations and more complex recombinant alleles (Hruska et. al, 2008).

Traditionally, Gaucher disease has been understood to be inherited in an autosomal recessive manner. However, that model has been expanded to include recent cases with non-traditional inheritance. In one example, a child with paternal uniparental disomy of chromosome 1 was found to have both Gaucher disease and Charcot-Marie-Tooth disease (Benko et. al, 2008). *De novo* germline mutations at amino acid Leu444 (p.Leu483) were reported in two other infants with type 2 Gaucher disease, where no mutations were identified in either mother (Saranjam et. al, 2013). These studies have spurred physicians to consider alternative means of inheritance when presented with a challenging case. In this report, we present a child with type 2 Gaucher disease whose phenotype likely arose from a different germline mutation.

Materials and Methods

Blood and/or fibroblast samples were collected from the proband and his parents with informed consent under an NHGRI Institute Review Board-approved clinical protocol.

Case Report

The proband was full-term male born to non-consanguineous Caucasian parents by normal home delivery with a birth weight of 3.40 kgs. He was noted to have peeling skin, as well as inspiratory stridor and tachypnea immediately after birth, and required observation in the hospital. Both symptoms resolved, and the infant was discharged home. He was breastfed for two months, but due to poor weight gain breast milk was fortified with formula to increase calories. At 2.5 months of age his increasing stridor and poor weight gain necessitated evaluations by multiple specialists, including gastroenterology and otolaryngology. He was diagnosed with laryngomalacia and the parents were told that the condition was expected to resolve. At 3.5 months, he was hospitalized for failure-to-thrive and received nasogastric feeds. At this time, he also had an abnormal swallow study. While awaiting discharge he went into respiratory arrest and was intubated. After several repeated failed attempts at extubation, laryngoscopy was performed, demonstrating paradoxical vocal chord movement, and botox was administered to the vocal cords twice. However extubation was still unsuccessful, and both a tracheostomy and gastrostomy tube placement were performed. By age 4 months, he lost the ability to move his eyes, and he developed generalised myoclonic jerks which initially did not show as seizure activity on the EEG. Two abdominal ultrasounds showed an enlarged spleen. By age 5 months, the EEG demonstrated seizure activity. The parents requested evaluation by an independent neurologist to consider a lysosomal storage disease. The diagnosis of type 2 Gaucher disease was confirmed by whole exome sequencing when the proband was 6 months old. He was started on Ambroxol at 6 months of age, at 12mg/kg twice daily. However his seizures and myoclonus worsened and became difficult to control, and he became ventilator-dependent.

His parents opted to place the proband in hospice care, and despite excellent supportive care, he passed away at the age of 17 months due to respiratory failure from status epilepticus.

Genomic DNA and cDNA Sequencing

GBA1 sequencing was performed on genomic DNA from the proband and his parents. DNA was extracted from leukocytes of the parents, and from fibroblasts from the proband. *GBA1* was amplified from genomic DNA in three fragments encompassing exons 1–5, 5–7 and 8–11 as previously described (Stone et. al, 2000), and individual exons were amplified as well. Amplified fragments were purified using a QIAquick PCR Purification Kit (Qiagen, Santa Clarita, CA, USA), and cycle sequencing was performed using both forward and reverse primers with a DyeTerminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA), on 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 findings in the mother and proband, cDNA was made from fibroblast RNA using Superscript IV (Life Technologies, Carlsbad, CA, USA) to synthesis the first strand. The 1.7 kb cDNA was amplified using exon 1 forward and exon 11 reverse primers (Stone, et. al, 2000), cleaned and then sequenced.

Protein Analysis

Frozen fibroblast pellets were homogenized in 100 uL of Citrate-phosphate buffer at pH 4.2, with 0.25% Triton X-100, with a protease-inhibitor tablet (Roche Diagnostics, Indianapolis, IN, USA). Cells were disrupted by two ten-second sonication pulses at 50% amplitude, and cell debris was removed by centrifugation at 5000 rpm for five minutes. Western blotting was performed and glucocerebrosidase levels were quantified as previously described (Westbroek et. al, 2016), using β -actin (abcam 20272, 1:4000) as the loading control.

Enzyme Activity Assay

Glucocerebrosidase activity in the mother and proband was analyzed using 4-methylumbelliferyl- β -D (4-MU) glucopyranoside substrate, as previously described (Westbroek et. al, 2016; Raghavan et. al, 1980), with the following modifications. Reactions were performed in pH 4.2 citrate-phosphate buffer and incubated for 2.5 hours at 37°C. Fluorescence was measured at λ_{ex} =365 nm and λ_{em} =448 nm.

Genomic Realtime PCR

Quantitative realtime PCR was performed on genomic DNA isolated from cultured fibroblasts from the proband, the mother, and a control as described (Velayati et. al, 2011).

Results

Analysis of the proband's DNA revealed two mutations, Arg257Gln (c.887G>A; p.arg296gln) and Gly202Arg (c.721G>A; p.gly241arg). The Arg257Gln mutation was identified in the paternal DNA. However, the Gly202Arg mutation, presumed to be from the mother, was not detected in the maternal genomic DNA. While the initial genotyping was performed using next-generation technology by a commercial laboratory, genomic DNA isolated from blood and fibroblasts was Sanger-sequenced, confirming the initial results

(Fig. 1A). The proband and mother's results were also confirmed by sequencing cDNA made from fibroblast RNA (results not shown). Recombination events between *GBA1* and its highly homologous pseudogene located 16 kb downstream are relatively common and are often found in patients with type 2 GD (Hruska et. al, 2008). To confirm there were no recombinations in the proband that were not detected by sequencing, quantitative real-time PCR was performed on genomic DNA. No deletions or duplications were detected in any of the regions targeted by our probes (Fig. 1B).

GCcase levels and activity were analyzed in fibroblasts from the mother and proband (Fig. 2). Protein levels and activity in the mother's fibroblasts were in the control range, while the proband had almost undetectable levels, as typically seen with type 2 Gaucher disease.

Discussion

In a study conducted by Saranjam and colleagues (Saranjam et. al, 2013), *de novo* or germline mutation of Leu444Pro (c.1448T>C;p.leu483pro) was reported in two patients with type 2 GD from two unique families. It was noted that since Leu444 was a mutational hotspot, this site may have been more susceptible to nucleotide transition. This current case differs from those described as the child did not carry the Leu444Pro mutation, suggesting other sites in *GBA1* may also be susceptible to *de novo* mutation.

Despite the differences in genotype, we propose that in this case, the maternal mutation similarly arose by germline mosaicism in the mother's ova or by a single *de novo* mutation in one ovum. The maternal mutation, Gly202Arg, is not encountered frequently. However this baby's genotype, Arg257Gln/Gly202Arg has previously been identified in type 2 GD.

The possibility of germline mutations in patients with rare Mendelian disorders has now been appreciated for several decades. In 1987 it was shown that germline mosaicism could explain the inheritance of the X-linked disorder Duchenne muscular dystrophy in families of affected individuals where the mothers showed no evidence of the somatic mutation (Bakker et al 1987). Subsequently, this was observed in other X-linked disorders including Hunter disease (Froissart et al 1997) and Hemophilia A (Luer et al 2001). Cohn et al (1997) demonstrated that paternal germline mosaicism could account for recurrence of lethal osteogenesis imperfecta by showing that the mutation was present in roughly one in eight of the father's sperm. Multiple examples of *de novo* and germline mosaicism in different genetic disorders are reviewed by Samuels and Friedman (2015), including autosomal dominant and X-linked disorders. It is likely that new mutations will be appreciated further in additional recessive disorders as next generation sequencing is increasingly utilized to identify disease-causing mutations in probands.

Also of note, in all three cases described with type 2 GD, the father was a *GBA1* mutation carrier, while the *de novo* mutation occurred in the maternal germline DNA. This is interesting considering that 80% of *de novo* germline point mutations occur on the paternal allele (Rahbari et. al, 2016; Acuna-Hidalgo et. al, 2016). Since the parents of patients with Gaucher disease are not always studied to confirm the presence of their child's mutant alleles, it is possible that this event occurs more frequently than appreciated, and that other

de novo cases may have been missed. This case demonstrates that germline mosaicism in Gaucher disease can occur at sites other than L444. Since this is now the third reported case with *de novo* or germline mutations on the maternal allele associated with type 2 Gaucher disease, this situation should be considered in future cases, as it has considerable relevance for genetic counseling. Furthermore, it may be interesting to evaluate the factors underlying this disease mechanism and to explore why this seems more frequent in type 2 Gaucher disease.

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Figure 1. Genotyping results

A. Chromatograms showing DNA sequencing results for proband and parents. Genomic DNA isolated from fibroblasts (proband) and leukocytes (mother and father) demonstrated mutations Gly202Arg and Arg257Gln in the proband, no mutations in the mother, and the Arg257Gln mutation in the father **B. Quantitative** genomic realtime PCR of *GBA1* and *GBAP*, looking for possible recombination between *GBA1* and its pseudogene. The probes are shown in order, beginning from the region upstream of *GBA1* to the 3' end of *GBAP*. WT: Control sample with no known recombinations, used to normalize the data.

Red lines indicate range of variation in controls.

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Figure 2. Reduced glucocerebrosidase in fibroblasts from the proband

A. Western blot demonstrating GCase levels in fibroblasts from three adult controls, the proband and his mother, as measured with a MDW 933 probe assay. **B.** Quantification of GCase levels in Western blot using ImageJ 1.50i. **C.** Enzyme activity measured with a 4-MU β -glu assay. Three adult controls were assayed and error bars represent the mean standard error. The experiment was repeated twice.