# Prenatal Testing of a Complex Pathogenic Variant following Positive Carrier Screening for Gaucher Disease

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## CASE DESCRIPTION

A pregnant woman was referred to our laboratory for prenatal GBA1 full-gene analysis after both she and her reproductive partner (hereafter referred to as the mother and father, respectively) were identified as Gaucher disease carriers by an external laboratory, which utilizes a hybridization capture-based next-generation sequencing (NGS) assay, followed by long-range polymerase chain reaction PCR (LR-PCR) and/or Sanger sequencing as needed. The father was reportedly positive for the GBA1 (NM_000157.4):c.1226A>G (p.Asn409Ser) pathogenic variant. The mother's report, however, only described her as positive for a pathogenic gene conversion of GBA1. After contacting the external laboratory, they verbally clarified that she carried the pathogenic RecNciI allele, defined as 3 single-nucleotide variants (SNVs) in cis (c.1448T>C, c.1483G>C, and c.1497G>C) in exon 10 of GBA1.

The ordered service from our laboratory for this case was LR-PCR followed by nested Sanger sequencing of *GBA1* exons. LR-PCR primers were designed in uniquely mappable regions to avoid pseudogene interference (Fig. 1A), yielding an 8960 bp amplicon. We first tested the parental specimens via this assay to confirm the ability to detect the relevant variants. The paternal c.1226A>G variant was identified. However, none of the Rec*Nci*I-defining SNVs, or any other pathogenic variant, were detected in the mother. Notably, this assay had previously detected the Rec*Nci*I allele, supporting its analytical sensitivity.

Given the discrepant result we obtained from what was reported by the external laboratory, and because Sanger sequencing cannot detect copy-number variants, we performed *GBA1* short-read NGS, including copy-number analysis, on the maternal specimen as follow-up. Briefly, this assay uses Integrated DNA Technologies xGen Exome probes for whole-exome capture, followed by Illumina NGS, with tertiary analyses limited to *GBA1*. Copy-number analysis was conducted by normalizing the sample's coverage relative to concurrently sequenced samples. The mean coverage of *GBA1* exons for this sample was 233.0x, with no regions below the minimum requirement of 20x. This assay also did not detect any of the Rec*Nci*I-defining SNVs (Fig. 2A, short-read NGS). Nonetheless, it did reveal a pathogenic deletion of *GBA1* exons 10–11 (Fig. 2B).

Because compound heterozygosity for a Rec*Nci*I allele and a deletion of exons 10–11 would result in perinatal lethal Gaucher disease or be incompatible with life, we interpreted the deletion detected in the mother by our short-read NGS assay as representing the same variant reported by the external laboratory. After confirming the ability to detect both parental variants, we tested the fetal specimen using both Sanger sequencing and short-read NGS. Fortunately, neither pathogenic variant was detected in the fetus.

We further characterized the maternal specimen using Oxford Nanopore Technologies long-read sequencing. Interestingly, this analysis identified a Rec*Nci*I allele not generated by gene conversion but by a large structural variant, specifically an approximately 20.6 kb deletion, that spans the region between

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*GBA1* and *GBA1LP* (Figs. 1A and 2C; *GBA1LP* is also known as *GBAP1*). This deletion, likely mediated by nonallelic homologous recombination (NAHR), produced a fusion gene composed of the first nine exons of *GBA1* and the 3'-exons of *GBA1LP* that correspond to *GBA1* exons 10–11 (Fig. 1C). According to the Human Genome Variation Society's 3'-rule, the deletion coordinates were chr1:155 215 100–155 235 726 (GRCh38/hg38). This finding reconciles the discrepancy between our short-read NGS assay and the external laboratory's report.

| QUESTIONS TO CONSIDER | |
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| 1. | How can the same pathogenic allele arise through multiple molecular mechanisms, and what are the implications for choosing an appropriate variant detection method? |
| 2. | How does high-sequence homology between a gene and its pseudogene(s) complicate analysis by short-read NGS, and what are some mitigation strategies? |
| 3. | Beyond *GBA1*, in which other medically relevant genes might you encounter similar issues due to pseudogene homology? |
| 4. | What are the advantages of long-read sequencing for variant detection in challenging medically relevant genes (CMRGs)? |
| 5. | What is the value of including positive controls (e.g., parents, relatives) when testing for known familial variants reported by an external laboratory? |

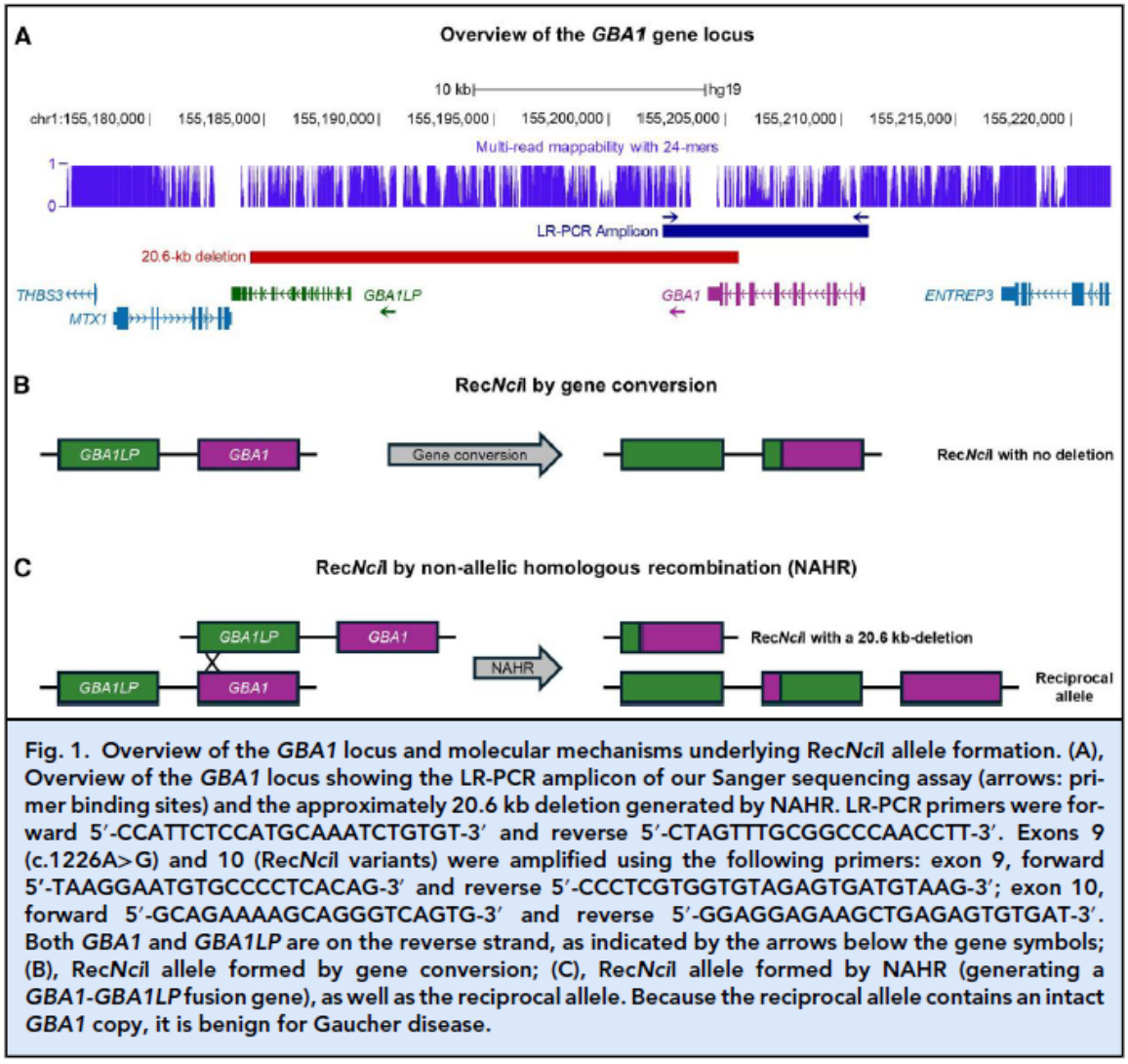


**Fig. 1. Overview of the *GBA1* locus and molecular mechanisms underlying Rec*Ncil* allele formation. (A), Overview of the *GBA1* locus showing the LR-PCR amplicon of our Sanger sequencing assay (arrows: primer binding sites) and the approximately 20.6 kb deletion generated by NAHR. LR-PCR primers were forward 5′-CCATTCTCCATGCAAATCTGTGT-3′ and reverse 5′-CTAGTTTGCGGCCCAACCTT-3′. Exons 9 (c.1226A>G) and 10 (Rec*Ncil* variants) were amplified using the following primers: exon 9, forward 5′-TAAGGAATGTGCCCCTCACAG-3′ and reverse 5′-CCCTCGTGGTGTAGAGTGATGTAAG-3′; exon 10, forward 5′-GCAGAAAAGCAGGGTCAGTG-3′ and reverse 5′-GGAGGAGAAGCTGAGAGTGTGAT-3′. Both *GBA1* and *GBA1LP* are on the reverse strand, as indicated by the arrows below the gene symbols; (B), Rec*Ncil* allele formed by gene conversion; (C), Rec*Ncil* allele formed by NAHR (generating a *GBA1-GBA1LP* fusion gene), as well as the reciprocal allele. Because the reciprocal allele contains an intact *GBA1* copy, it is benign for Gaucher disease.**

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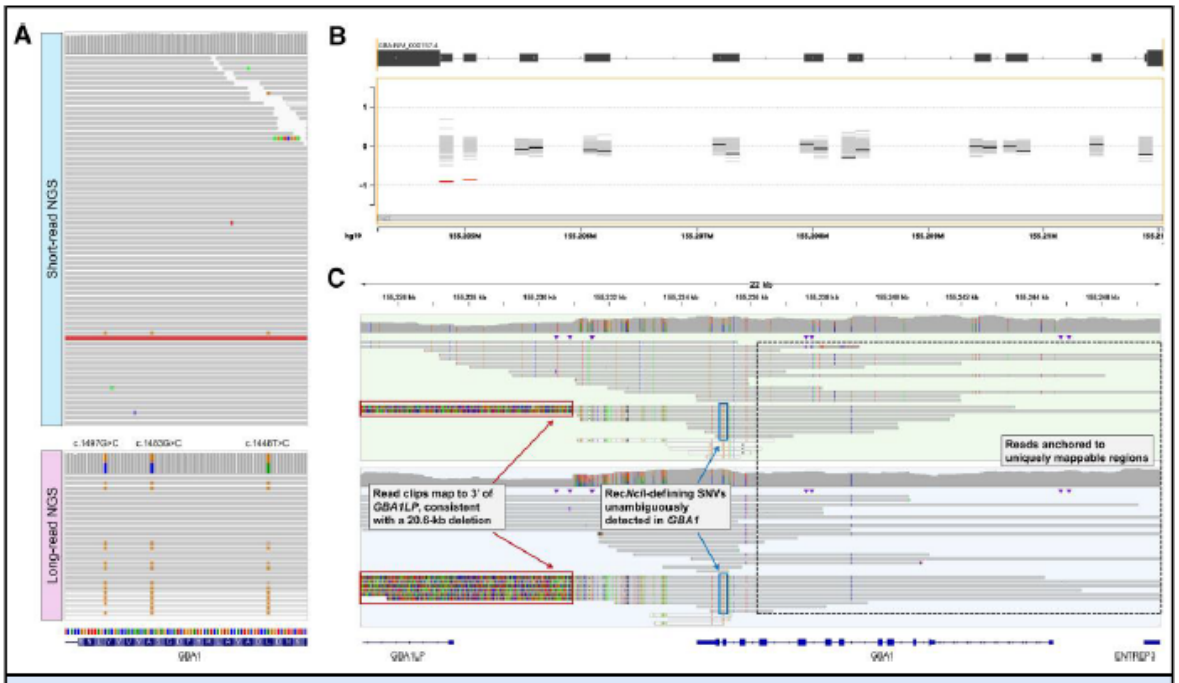


**Fig. 2. Detection of the NAHR-mediated Rec*Ncil* allele by short-read and long-read sequencing. (A), Comparison of short-read and long-read NGS results in the maternal sample. The 3 Rec*Ncil*-defining SNVs (c.1448T>C, c.1483G>C, c.1497G>C) in *GBA1* exon 10 were not detected by short-read NGS but were clearly identified in *GBA1* using long-read NGS; (B), Copy-number analysis of short-read NGS data showing reduced coverage of *GBA1* exons 10 and 11 in the maternal sample (red lines), suggesting a heterozygous deletion. Gray lines represent coverage from concurrently sequenced samples; (C), Long-read NGS alignments from the maternal sample (green background) and a second sample also carrying a NAHR-mediated Rec*Ncil* allele (blue background).**

## Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the January 2026 issue of *Clinical Chemistry*. To view the case and comments online, go to https://academic.oup.com/clinchem/issue/72/1 and follow the link to the Clinical Case Study and Commentaries.

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