An Ambiguous *NUDT15* Signal in a Child with B-cell Acute Lymphoblastic Leukemia

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CASE PRESENTATION

A pharmacogenetic test for variants in the *TPMT* and *NUDT15* genes, which encode thiopurine methyltransferase (TPMT) and nudix hydroxylase 15 (NUDT15), respectively, was ordered on a 6-year-old female to guide thiopurine treatment. DNA was extracted from the patient's whole blood specimen using a MagNA Pure system (Roche Diagnostics), followed by genotyping for 8 *TPMT* variants and 3 *NUDT15* variants by real-time PCR on a Quant Studio 12K Flex Open Array (Thermo Fisher Scientific). Initial testing revealed a genotype of *TPMT*1/*3A* and an inconclusive *NUDT15* c.415C > T (*3) assay signal shifted between the heterozygous and reference zones (Fig. 1A), which was still present upon repeat testing of the sample (data not shown). Next, Sanger sequencing was performed to troubleshoot the *NUDT15*3* signal and revealed a weak *NUDT15* c.415C > T peak (corresponding with *3), not consistent with the expected peak height for a heterozygote (Fig. 1B). Additionally, no nearby variants that could interfere with binding of the genotyping primers or probes were identified. The Sanger sequencing results suggested that the original genotyping signal was real (not artifact) and not due to allele drop-out (Fig. 1B).

The laboratory next considered whether the patient could have a known underlying genetic condition or mosaicism causing the skewed signal, or whether it could potentially be due to the patient's malignancy or the result of a treatment. The laboratory contacted the ordering physician for additional clinical information. The physician indicated that the patient had B-cell acute lymphoblastic leukemia (B-ALL), and more specifically, a hyperdiploid B-ALL. She had received a red blood cell (RBC) transfusion the day before the sample was drawn and had no other known underlying medical or genetic conditions. Her peripheral blast count at the time of draw was 58%. The physician and laboratory decided to re-test the patient after 2 weeks, prior to the receipt of any additional transfused products and after initiation of non-thiopurine chemotherapy. In addition, the blood bank was contacted, confirmed that the patient had received 2 units of leukocyte-reduced RBCs, and sent the segments for testing to rule out the possibility of filter failure. DNA extraction was attempted on the segments, with minimal DNA yield (0.21 ng/µL). Testing was attempted despite the low yield, and the *TPMT/NUDT15* genotyping assay showed no amplification, including the *NUDT15*3* assay (Fig. 1C). Testing performed on the specimen drawn 2 weeks later still showed a *TPMT*1/*3A* genotype, but the *NUDT15* genotype was now clearly **1/*3* (Fig. 1D). When the new sample was drawn, the patient had 0% peripheral blasts.

Put together, the results suggested that the patient's B-ALL clone had impacted results. To further confirm this unusual finding, whole genome chromosomal microarray (CMA) was performed on the diagnostic bone marrow cell pellet that had initially been used for chromosome banding analysis and interphase fluorescence in situ hybridization to characterize the patient's malignancy as a classic hyperdiploid B-ALL clone. *NUDT15* is located at 13q14.2, while *TPMT* is found at 6p22.3. In addition to confirming the hyperdiploidy, there was a copy-neutral loss of heterozygosity involving chromosomes 1, 13, and 22, along with single-copy gains involving chromosomes 8, 9, 10, 14, 17, 18, and X, a two-copy gain involving chromosome 21, and a secondary clone with a gain of 10q23.2q26.3 (Fig. 2A and B). While no gain of

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chromosome 13 was observed (2 copies present), interestingly, CMA identified copy- neutral loss of heterozygosity (cnLOH) across the whole of chromosome 13 (including *NUDT15*) (Fig. 2A). No anomalies were identified involving the *TPMT* locus.

QUESTIONS TO CONSIDER	
1.	What can cause an inconclusive signal for a genotyping assay?
2.	Can transfusion impact the results of genetic testing? If so, how?
3.	What modifications to blood products can help minimize their potential impact on genetic testing?

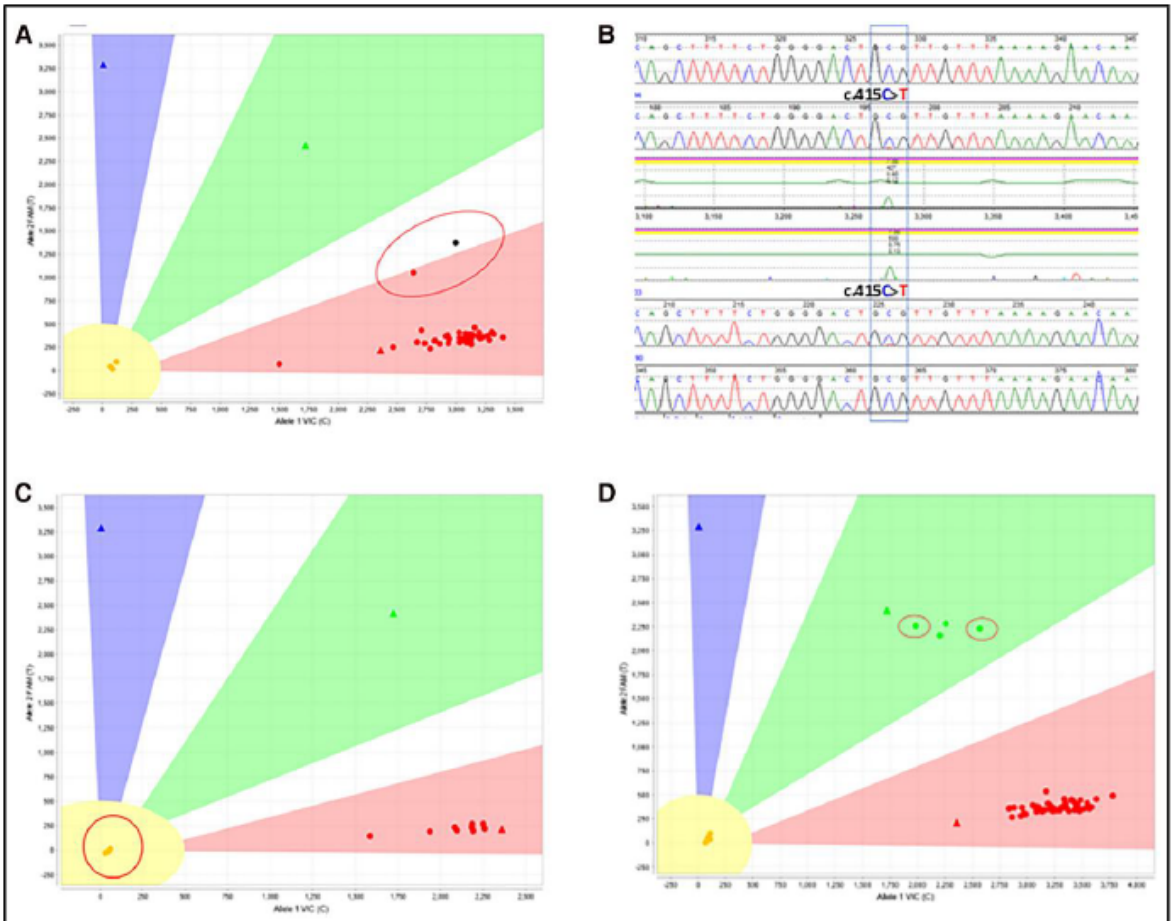

Fig. 1. *NUDT15* genotyping and Sanger sequencing. (A) Real-time PCR assay to genotype the *NUDT15*3* (c.415C > T) locus. The patient's sample was run in duplicate, with both data points circled. Both duplicates are falling away from the cluster in the bottom right corresponding to other patients on this run with a C/C genotype. The middle and left zones correspond to C/T and T/T genotypes, respectively. (B) Sanger sequencing of *NUDT15* c.415 and surrounding region. The patient sample (traces second from top and second from bottom) show a strong peak corresponding to the reference nucleotide, C (as seen in the top and bottom traces), and a small peak corresponding to the variant T within the blue box. The small variant peak can also be visualized in the difference plots (innermost traces) that highlight changes from reference present in the patient. (C) DNA extraction was attempted from the segments from the transfused RBC units, followed by genotyping. The duplicates (circled) appeared in the "no amplification" zone (bottom left zone on chart). (D) Genotyping results from the new sample drawn 2 weeks later (duplicates circled) are present within the middle zone, corresponding to a heterozygous, C/T (*1/*3) genotype. Color figure available at https://academic.oup.com/clinchem.

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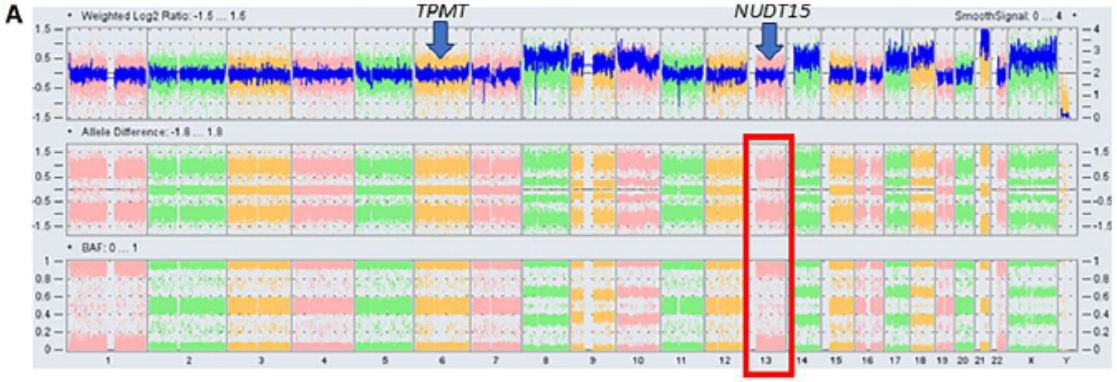

B **Result:** CN=Copy Number

Change (CN)	Region	Genome Coordinates	Size (Mb)
cnLOH (2)	Chromosome 1	chr1:882802-2469198692	248.3
Gain (3)	Chromosome 8	chr8:158048-146295771	146.1
Gain (3)	Chromosome 9	chr9:203861-141020389	140.8
Gain (3)	10p15.3q23.2	chr10:100026-88134257	88.0
Gain (2-3)	10q23.2q26.3	chr10:88135673-135427143	47.3
cnLOH (2)	Chromosome 13	chr13:19438806-115095705	95.7
Gain (3)	Chromosome 14	chr14:20511672-107285437	86.8
Gain (3)	Chromosome 17	chr17:525-81041938	81.0
Gain (3)	Chromosome 18	chr18:136226-78014123	77.9
Gain (4)	Chromosome 21	chr21:15168392-48097372	32.9
cnLOH (2)	Chromosome 22	chr22:16877134-51177928	34.3
Gain (3)	Chromosome X	chrX:168546-155233731	155.1

Fig. 2. Whole genome chromosomal microarray (CMA). (A) Results from the CMA performed using the residual cell pellet from the diagnostic cytogenetic studies. The genomic region including *NUDT15* (13q14.2), which is indicated by the right arrow, shows no copy number change (top row of figure). The *NUDT15* genomic region shows two genotype clusters on the allele difference and B allele frequency plots (middle and bottom rows, within the box) corresponding to homozygous reference and variant genotypes with absence of the expected heterozygous cluster. No abnormalities are apparent at the *TPMT* (6p22.3) locus, marked by the left arrow. (B) Summary of CMA results, including the identified copy-neutral loss of heterozygosity (cnLOH) across whole chromosome 13 (including *NUDT15* at 13q14.2). Color figure available at https://academic.oup.com/clinchem.

Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the August 2025 issue of *Clinical Chemistry*. To view the case and comments online, go to https://academic.oup.com/clinchem/issue/71/8 and follow the link to the Clinical Case Study and Commentaries.

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