Maternal iAMP21 acute lymphoblastic leukemia detected on prenatal cell-free DNA genetic screening

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Key Points

- cfDNA sequencing for fetal aneuploidy may detect chromosomal abnormalities representative of maternal malignancy.
- Maternal malignancy must be considered when abnormal cfDNA sequencing for fetal aneuploidy is associated with normal fetal karyotype.

Introduction

Recently, cell-free plasma DNA (cfDNA) sequencing for fetal aneuploidy has become the standard method for screening women at high risk for fetal aneuploidy and is now increasingly applied in low-risk pregnancies. Here, we present a case where a focal, chromosome arm-level gain on chromosome 21 detected via cfDNA screening did not reflect fetal aneuploidy, but in fact, represented the molecular driver of the patient's subsequently diagnosed iAMP21 B-cell acute lymphoblastic leukemia (ALL). This report highlights a technological evolution in maternal medicine that is of increasing relevance to hematologists and oncologists who may need to investigate possible cases of maternal malignancy.

Case description

A 21-year-old woman with a history of α -thalassemia trait was referred by her obstetrician during her first pregnancy for a hematologic evaluation because of a persistent, mild pancytopenia. At the time of referral, the patient was at 26 weeks of gestation and was noted to have a leukocytosis with circulating blasts, anemia, and thrombocytopenia with clinical concern for acute leukemia. She was transferred to our center for further management.

Upon presentation, a complete blood count revealed a white blood cell count of $13.1 \times 10^9/L$ with 68% blasts, hemoglobin of 7.6 g/dL, and a platelet count of $35 \times 10^9/L$. Flow cytometry of peripheral blood demonstrated an immature population positive for CD45 (dim), CD34, HLA-DR, CD123 (dim), TdT, and the B-lymphoid markers CD10, CD19, CD20 (variable), and CD22. The immature cells were negative for T lymphoid, myeloid, and monocytic markers. This pattern was consistent with a diagnosis of B-cell ALL. A bone marrow biopsy revealed a hypercellular marrow with 94% of the cellularity composed of small- to intermediate-sized blasts.

Methods

Genetic characterization of the leukemia was notable for a complex karyotype 46,X,add(X)(p22.1),del(9)(p21),add(10)(q25),-21,+mar[cp19]/46,XX[1]. Our institution's 95-gene hematologic malignancy-focused targeted DNA sequencing panel¹ revealed an insertion in *IL7R* (p.L242_L243delinsFGLRGCP) as well as mutations in *CSF3R* (p.R809fs*) and *LUC7L2* (p.R205*). Read count analysis (Figure 1A) was significant for loss of *IKZF1* (on 7p), loss of *CTCF* (16q), multiple copy gain of *RUNX1* (21q), deletion of *U2AF1* (21q), as well as gain of *CRLF2*, *PIGA*, *ZRSR2*, and *BCOR* (all on Xp). In addition, a *P2RY8-CRLF2* fusion was detected in bone marrow RNA via a multiplex polymerase chain reaction assay. Metaphase fluorescence in situ hybridization analysis confirmed amplification of *RUNX1* (estimated 10 copies per cell; Figure 1B). In sum, these genetic findings are consistent with a diagnosis of iAMP21 B-cell ALL (based on the multiple additional *RUNX1* copies), a provisional entity in the World Health Organization classification of acute leukemia.²⁻⁴

After diagnosis of B-cell ALL, it was recognized that the patient had undergone routine prenatal cfDNA screening (QNatal Advanced, Quest Diagnostics) from a maternal blood sample drawn ~14 weeks

Figure 1. Detection of iAMP21 leukemia by sequencing leukemia cells, fluorescence in situ hybridization analysis, and sequencing cell-free DNA. (A) Estimated DNA copy number via targeted capture sequencing of ~400 known cancer genes from the patient's bone marrow, showing amplification (~6-7 copies) of 3 genes located on 21q22 (*RUNX1*, *ERG*, *TPRSS2*) as well as copy number gain of Xp, including *CRLF2*, *FANCB*, *ZRSR2*, and *NR0B1*. (B) Bone marrow metaphase fluorescence in situ hybridization demonstrating multiple signals from a *RUNX1* probe (21q22, red) on the marker chromosome and 2 normal (green) signals for *ETV6* (12p13). (C) Normalized DNA copy number estimations on chromosome 21 from cfDNA derived from maternal plasma demonstrating an amplification spanning ~12 Mb of chr21q22 containing *RUNX1*, *DYRK1A*, *ERG*, and *HMGN1*.

prior, at 12 weeks and 2 days of gestation. A complete blood count ~4 weeks prior to cfDNA screening showed mild leukopenia (white blood cell count of $2.9 \times 10^9/L$ with normal differential) without anemia, thrombocytopenia, or abnormal cells. The test had returned an indeterminate result for chromosome 21 prompting an amniocentesis, which showed a normal fetal karyotype (46,XX).

Results and discussion

Prenatal cfDNA screening uses massively parallel shotgun sequencing of cfDNA isolated from maternal plasma to detect common fetal aneuploidies of chromosomes 13, 18, 21, X, and/or

Y, with a reported sensitivity of >99% and specificity ~100%.⁵⁻⁷ The positive predictive value of cfDNA screening has been reported to be 98%, 92%, and 69% for trisomies of 21, 18, and 13, respectively.⁷ This assay takes advantage of the fact that 10% to 20% of the cfDNA in maternal plasma in the late first to early second trimester is derived from fetal tissue, most from placental trophoblastic cells. Despite excellent screening test characteristics, discordant results from cfDNA screens are well recognized. False positive results can be attributed to diverse etiologies, including confined placental mosaicism, cotwin demise, maternal chromosomal mosaicism, maternal organ transplant from a male donor,⁸⁻¹² and, as in this case, maternal malignancy.¹³⁻¹⁵ Here, review of the patient's

sequencing confirmed that some of the major genetic alternations documented in her tumor sample, specifically amplification of a limited region of distal chromosome 21 as well as gain of genes on Xp, were detectable in the sequenced cfDNA.

Normalized DNA copy number estimations from the patient's cfDNA demonstrated an ~12-Mb amplification of chr21q22 (Figure 1C). This region of amplification is consistent with that seen in iAMP21 B-cell ALL cases^{3,16} and contains several genes implicated in B-ALL pathogenesis, including *RUNX1*,¹⁷ *DYRK1A*,¹⁸ *ERG*,¹⁹ and *HMGNI*.²⁰ The specific driver or drivers of leukemogenesis in iAMP21 are not yet clearly defined, but the amplification event is thought to occur via chromothripsis, a phenomenon of extensive genomic rearrangement usually confined to a single chromosome.²¹ Copy number inference via targeted capture DNA sequencing of ~400 cancer genes²² from the patient's diagnostic bone marrow showed amplification of 3 genes on 21q22 (*RUNX1*, *ERG*, *TMPS2*; Figure 1A). Gain of Xp was also detected, in a region that included *CRLF2*, which is often detected in B-cell ALLs with the intra-chromosomal *P2RY8-CRLF2* fusion.²³ *P2RY8-CRLF2* rearrangements also frequently cooccur with iAMP21.¹⁶

Detection of asymptomatic maternal malignancy during pregnancy by cfDNA screening has been reported.¹³⁻¹⁵ Bianchi et al described 10 cases of maternal cancer identified by cfDNA screening out of a tested cohort of 125 426 women associated with 3757 positive results (3%).¹⁴ Most of the cases associated with maternal cancer (7 of 10) contained the otherwise rare cfDNA finding of ≥ 2 whole chromosomal aneuploidies. The authors estimated the risk of maternal cancer among women with multiple aneuploidies in cfDNA to be between 20% and 44%.

There are not yet consensus guidelines for identification and management of cfDNA results suggestive of maternal malignancy. Therefore, interpretation of cases with discordant findings between cfDNA results and diagnostic testing for fetal aneuploidy requires expert individualized evaluation. Referral to a genetic counselor with experience evaluating common and uncommon etiologies of false positive cfDNA results is ideal. Communication with the diagnostic laboratory to review the primary sequencing data may provide

additional insight, as in this case where trisomy 21 was suspected, but an indeterminate result for chromosome 21 was issued on the cfDNA report.

The association between maternal malignancies and chaotic cfDNA results (ie, multiple aneuploidies) is gaining recognition. Our finding of a single chromosomal abnormality associated with maternal cancer is not well described and may be less likely to raise clinical suspicion for malignancy. However, given the specific association between chromosome 21 and hematologic malignancies, we would propose a heightened suspicion for malignancy in cases of discordant results involving chromosome 21, even if it is the only detected abnormality.

This report illustrates a notable presentation of the uncommon scenario of maternal malignancy during pregnancy. These cases will increase because cfDNA screening is now recommended in all pregnancies at high risk for fetal aneuploidy and is quickly becoming ubiquitous in low-risk pregnancies.^{5,6,24} Hematologists and oncologists will be called upon more often to investigate cases of possible or confirmed maternal malignancy. In addition, this case is unique in the literature in that a focal, chromosome arm-level copy gain detected on cfDNA screening was representative of a maternal malignancy defined molecularly by that exact DNA abnormality. In this patient, cfDNA screening detected amplification of a limited region of chromosome 21q22, which itself is thought to represent the primary driver of the patient's disease, iAMP21 B-cell ALL.

Authorship

Contribution: M.R.L. and A.A.L. wrote the manuscript; M.N.D., S.R.E., P.D.C., R.O., B.I., M.M., and A.A.L. performed diagnostic tests and analyzed the data; and all authors reviewed the manuscript.

Conflicts-of-interest disclosure: R.O., B.I., and M.M. are employees of Quest Diagnostics. The remaining authors declare no competing financial interests.

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