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CASE REPORT Open Access

False-positive XXY results by interphase FISH in cytogenetically normal XX individuals: two cases highlighting the necessity of additional laboratory follow-up

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Abstract

Background Interphase fluorescence in situ hybridization (FISH) is commonly used for rapid aneuploidy detection in clinical settings. While FISH-based aneuploidy detection provides rapid results desirable for patient management, it usually only utilizes one probe per chromosome, which may lead to rare false-positive findings.

Case presentation Here we report two interphase FISH results, which were false-positive for XXY in cytogenetically normal XX individuals. Both false-positive cases were due to hybridization of the Y chromosome centromeric probe DYZ3 to the pericentromeric region of chromosome 15. In both cases, chromosomal microarray revealed no detectable Y chromosome material, suggesting the hybridizations of the DYZ3 probe to chromosome 15 likely represent benign heterochromatic variants of no clinical significance. In one case, the DYZ3 hybridization was also identified in the phenotypically unaffected mother, further suggesting this is likely a rare variant of no clinical significance.

Conclusions This report marks the first documentation of hybridization of the DYZ3 probe to another chromosome in cytogenetically normal individuals. Our report has important clinical implications, because DYZ3 is widely used by clinical laboratories for Y chromosome detection. Our findings underscore the necessity of confirming abnormal aneuploidy detection FISH results with additional laboratory methods such as chromosomal microarray analysis.

Keywords Aneuploidy, Interphase FISH, DYZ3, XXY, Prenatal diagnosis

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Background

Aneuploidy detection assays, commonly based on interphase fluorescence in situ hybridization (FISH), are routinely employed in prenatal and neonatal settings to guide patient management [1–3]. One major advantage of aneuploid detection FISH is its rapid turnaround time, which is desired for time-sensitive patient management decisions. In our clinical laboratory, the turnaround time for this assay is as short as five hours. However, one significant limitation of aneuploidy detection FISH is that it usually only utilizes one probe per chromosome in interphase cells. For example, the FDA-cleared Abbott AneuVysion® kit targets the 13q14 region for chromosome 13, the 21q22.13-q22.2 region for chromosome 21, and the chromosome-specific centromeric alpha-satellite DNA for chromosomes 18, X, and Y. Thus, FISH-based aneuploidy detection is unable to distinguish between numerical chromosome abnormalities (i.e., aneuploidies) and structural chromosome abnormalities (e.g., copy-number variants and translocations) involving the FISH target region.

Inaccurate results caused by the above limitation are rare but have been previously reported. Five cases of false-positive trisomy 18 by interphase FISH have been reported due to the chromosome 18 centromeric probe (D18Z1) hybridizing to chromosomes 1, 2, 9, 15, and 22, respectively [4–8]. One case of the X chromosome centromeric probe (DXZ1) hybridizing to chromosome 19 has also been reported [9]. To date, no false-positive cases have been documented involving the Y chromosome centromeric probe DYZ3.

In this case report, we describe two cases of false-positive XXY results by interphase FISH. In both cases, chromosome microarray analysis was consistent with a normal XX result. Metaphase FISH showed hybridization of DYZ3 to the pericentromeric region of chromosome 15, likely representing a benign heterochromatic variant. These two cases provide additional data for genetic counseling of aneuploidy testing and underscore the need for follow-up laboratory studies to confirm abnormal aneuploidy detection FISH results.

Case Presentation

Case 1

An amniotic fluid specimen from a 30-year-old pregnant individual was referred to our laboratory for chromosomal microarray analysis. Ultrasonography showed that the fetus had a unilateral club foot but was otherwise normal. However, prior testing revealed conflicting information on fetal sex. Prenatal cell-free DNA screening (cfDNA screening) and ultrasound both suggested a female fetus. However, aneuploidy detection FISH performed at an external laboratory using the Abbott AneuVysion® kit revealed mosaic Klinefelter syndrome. Out of the 100 scored interphase cells, 83%, 14%, and 3% showed XXY, XX, and XY patterns, respectively.

At our laboratory, chromosomal microarray analysis was performed using DNA extracted from cultured amniocytes. The Thermo Fisher CytoScan™ HD array contains about 2.6 million copy-number probes across the genome. Notably, due to the repetitive nature of alpha-satellite DNA, the pericentromeric regions are generally not well-covered by chromosomal microarray. A normal XX profile was observed, with no detectable Y chromosome material.

The normal XX result by microarray was apparently inconsistent with that of the aneuploidy detection FISH (XXY in 83% of cells). Thus, additional cytogenetic investigations were performed. Interphase FISH was repeated in-house using the DXZ1/DYZ3/D18Z1 probe set (the same as the Abbott AneuVysion® kit) on the specimen used for microarray analysis, which showed an XXY result based on 200 interphase cells (Fig. 1A). Interestingly, metaphase FISH revealed that the DYZ3 probe

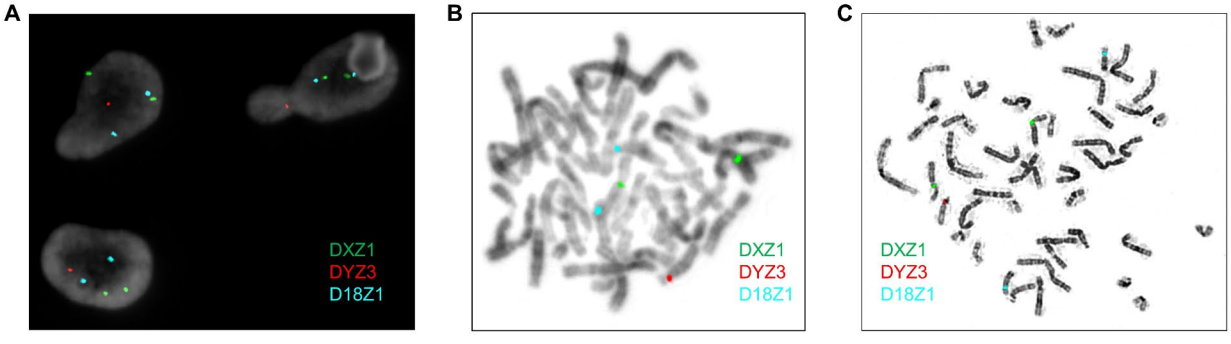

Fig. 1 Representative FISH images for case 1 using the DXZ1/DYZ3/D18Z1 probe set. (**A, B**) Interphase (**A**) and metaphase (**B**) FISH of the fetus (cultured amniocytes). There are two green signals and one red signal per cell, consistent with XXY. Metaphase FISH (panel B) shows that DYZ3 hybridized to the pericentromeric region of an acrocentric chromosome, most likely 15. (**C**) Metaphase FISH of the mother (PHA-stimulated peripheral blood) showing a DYZ3 signal, which indicates maternal inheritance

hybridized to the pericentromeric region of chromosome 15 (Fig. 1B). Interphase and metaphase FISH using a chromosome 15 centromere probe D15Z4 demonstrated a normal signal pattern (Fig. 2A and B). G-banded karyotype also demonstrated an apparently normal 46,XX karyotype in 20 metaphases from 3 primary amniotic fluid cultures (Fig. 2C).

Taken together, the XXY result by aneuploidy detection FISH was caused by hybridization of the Y chromosome centromeric probe DYZ3 to chromosome 15; otherwise, no Y chromosome material was detected by karyotype or microarray. Because the CytoScan™ HD array has adequate coverage for non-repetitive regions of the Y chromosome, we concluded that the DYZ3 hybridization to chromosome 15 in this fetus likely represents a heterochromatic variant of no clinical significance. Subsequent familial testing identified the same variant in the phenotypically unaffected mother of the fetus (Fig. 1C), further supporting the variant is likely a benign finding.

Case 2

An amniotic fluid specimen from a 26-year-old pregnant individual was referred to our laboratory for aneuploidy detection FISH and chromosomal microarray analysis due to intrauterine growth restriction. Aneuploidy detection FISH was performed on direct amniotic fluid using the DXZ1/DYZ3/D18Z1 probe set, which revealed an XXY result based on 200 interphase cells (Fig. 3A). Microarray was performed using DNA extracted from cultured amniocytes and showed a normal XX profile.

Similar to case 1, metaphase FISH showed that the DYZ3 probe hybridized to an acrocentric chromosome, likely chromosome 15 (Fig. 3B). For the same reasoning as in case 1, this finding was reported as likely a heterochromatic variant of no clinical significance.

Discussion and conclusions

We describe two cases of false-positive XXY results by interphase FISH in cytogenetically normal XX individuals, due to DYZ3 hybridization to the pericentromeric region of chromosome 15. In both cases, chromosomal microarray analysis clarified that there were no detectable gains of unique Y chromosome material; thus, the hybridization of the DYZ3 probe to chromosome 15 likely represents a heterochromatic variant of no clinical significance.

Our report marks the first documentation of DYZ3 hybridization to another chromosome in cytogenetically normal individuals. These cases are likely very rare. A retrospective review of more than 6,000 aneuploidy detection FISH cases tested at Mayo Clinic Cytogenetics Laboratory between 2015 and 2024 did not identify additional cases. Nonetheless, they, together with the previously reported cases involving D18Z1 and DXZ1, underscore the importance of comprehensive follow-up studies for abnormal aneuploidy detection FISH results. Had microarray analysis not been performed for the two cases in this report, an XXY result in a phenotypic female raises concern about differences in sex development (DSD), potentially leading to unnecessary anxiety, diagnostic tests, and/or pregnancy termination. The American College of Obstetricians and Gynecologists (ACOG) also recommends that clinical decision-making should not be based on FISH results alone [10].

In general, it is prudent to confirm abnormal aneuploidy detection FISH results by an orthogonal assay, e.g., chromosome analysis, chromosomal microarray, multiplex ligation-dependent probe amplification (MLPA) [11], or quantitative florescence PCR (QF-PCR) [12]. This is especially pertinent for abnormalities where clinical correlation could not be reliably made. For example, because features of Klinefelter syndrome could not be reliably observed prenatally or at birth, an XXY result by aneuploidy detection FISH should be orthogonally confirmed. On the other hand, a trisomy 21 result by aneuploidy detection FISH correlating with features of Down syndrome in a fetus or newborn might suffice as a genetic diagnosis without orthogonal confirmation.

When conflicting results are obtained from different assays, it is always important to rule out pre-analytical errors, such as specimen mix-up, prior to considering biological explanations. In case 1, we re-performed aneuploidy detection FISH on the same amniocyte culture used by chromosome microarray analysis, and observed similar results as reported by the external laboratory. In case 2, we performed short tandem repeat (STR)-based specimen source identification analysis and showed that the DNA aliquot used for chromosomal microarray analysis and the amniotic fluid specimen used for aneuploidy detection FISH belong to the same source.

DYZ3, along with D18Z1 and DXZ1, targets chromosome-specific alpha-satellite DNA. Found at the centromeres of all human chromosomes, alpha-satellite DNA consists of approximately 171-bp AT-rich monomers, which are organized into higher-order repeats that extend up to several million base pairs [13]. The structures of the higher-order repeats are largely distinct among chromosomes [14]; therefore, chromosome-specific alpha-satellite FISH probes are available for most chromosomes (except for 5, 13, 14, 19, 21, and 22) with high hybridization specificity.

Hybridization of D18Z1 [4–8], DXZ1 [9], and DYZ3 (this report) to other chromosomes are rare, but have been reported. The cause remains uncertain. These cases are unlikely due to random non-specific hybridization. For the two cases in this report, the DYZ3 hybridizations to chromosome 15 were confirmed by repeat FISH experiments. In addition, the authors of a previous case

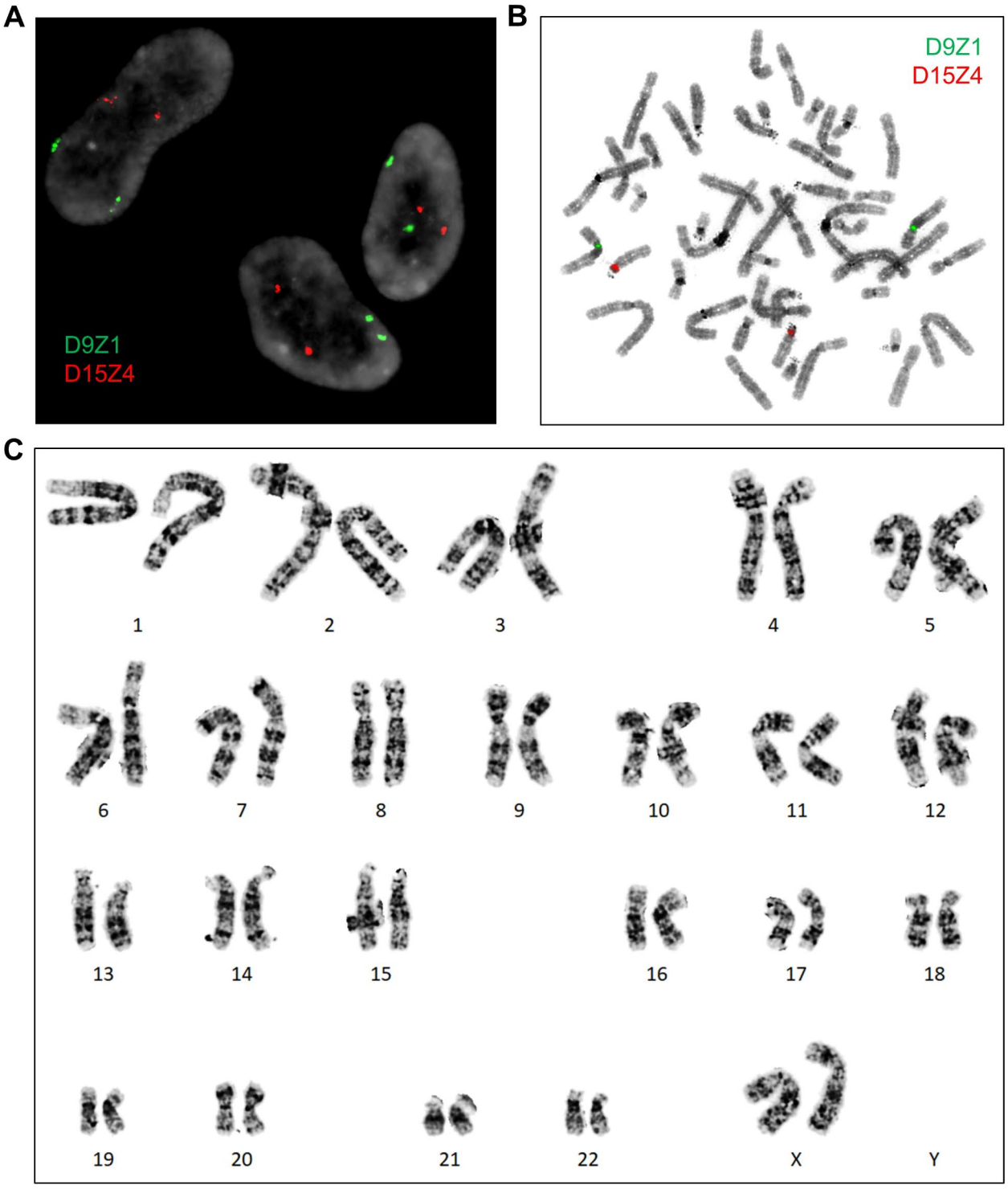

Fig. 2 Additional cytogenetic investigations for the fetus in case 1. (**A, B**) Normal interphase and metaphase FISH results of chromosome 15, using alpha-satellite probe D15Z4. (**C**) Karyotype consistent with a normal 46,XX individual

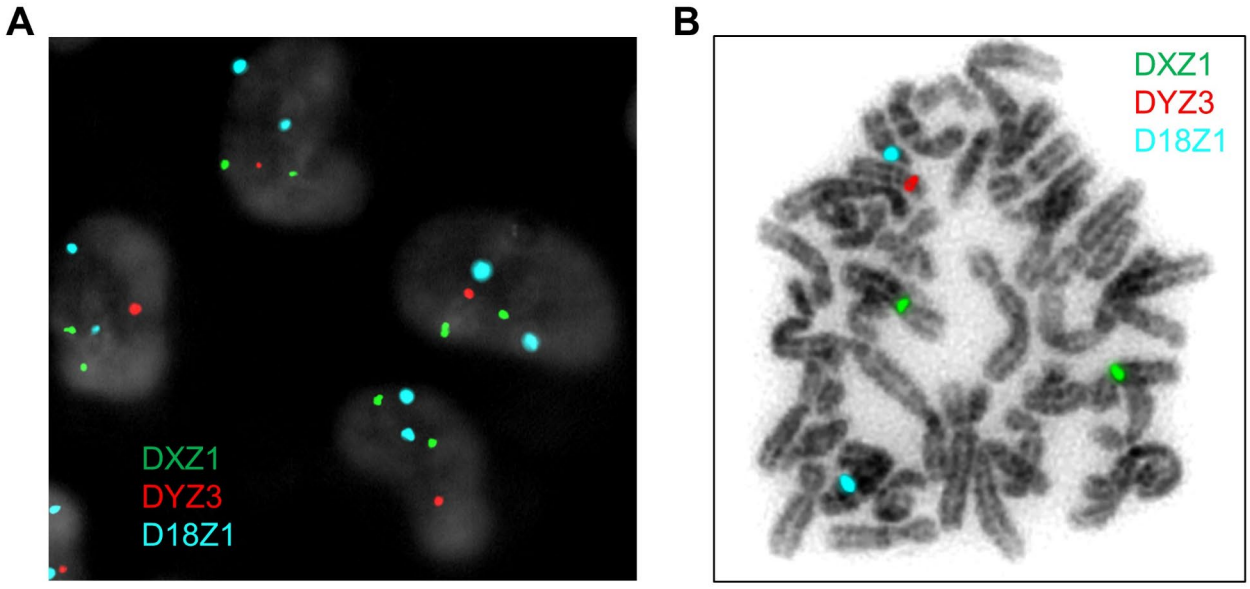

Fig. 3 Representative FISH images for the fetus in case 2 using the DXZ1/DYZ3/D18Z1 probe set. (**A, B**) Interphase (**A**) and metaphase (**B**) FISH of the fetus (cultured amniocytes). Same as in Fig. 1, FISH results are consistent with XXY, and the DYZ3 signal is at the pericentromeric region of an acrocentric chromosome most consistent with 15

reported that the three D18Z1 signals faded equally under stringent hybridization conditions, which is inconsistent with non-specific hybridization [4].

It is possible that these hybridization patterns are caused by the formation of a dicentric chromosome containing alpha-satellite DNA from two different chromosomes, possibly through a whole-arm translocation mechanism [15]. However, this scenario is unlikely to explain the two cases in this report, because no materials from either the p arm or the q arm of the Y chromosome were detected by microarray. Another possibility is that mutations in the centromeric alpha-satellite DNA of another chromosome could have altered its higher-order repeat structure to mimic the Y centromere, resulting in DYZ3 hybridization. Nonetheless, this is highly improbable as it would require hundreds of independent mutations.

The most likely explanation is insertional duplication. In this scenario, only the centromeric alpha-satellite DNA of the Y chromosome, but not the flanking euchromatic sequences, is duplicated and inserted into another chromosome. The pericentromeric region of chromosome 15 was previously found colocalizing with the sex vesicle during male meiosis [16], which possibly provides physical proximity between chromosomes 15 and Y for rearrangements. While we are unable to definitively conclude that insertional duplication is the cause of the observed DYZ3 hybridization patterns, this scenario could explain the observations without the need for complex rearrangements or extensive mutations. Supporting this scenario, a recent study, based on long-read sequencing, demonstrated a *de novo* insertion of about 50–300-kb of chromosome 18 alpha-satellite DNA into chromosome 15q26, leading to D18Z1 hybridization to chromosome 15 [8].

Regardless of the mechanism, the clinical significance of unexpected FISH signal patterns should be clarified by additional laboratory analyses. Cytogenetic methods such as karyotype or chromosomal microarray can reveal the presence of aneuploidy. Karyotype also provides information regarding the structural nature of copy number changes, which is important for recurrence risk counseling. Other molecular assays, e.g., MLPA and QF-PCR, may also be considered. In our cases, no copy-number changes involving the Y chromosome were detected by microarray. This indicates that the genomic alterations causing the hybridizations of the DYZ3 probe to chromosome 15 are limited to repetitive sequences that are not detectable by microarray. Therefore, we concluded that the observed patterns of DYZ3 hybridization likely represent heterochromatic variants of no clinical significance.

To conclude, our case report describes two cases of false-positive XXY results by interphase FISH in cytogenetically normal XX individuals, due to DYZ3 hybridization to chromosome 15. These findings highlight the importance of follow-up cytogenetic and/or molecular genetic studies to confirm and clarify abnormal aneuploidy detection FISH results.

Abbreviations

DSD	Differences in sex development
FISH	Fluorescence in situ hybridization
MLPA	Multiplex ligation-dependent probe amplification
QF-PCR	Quantitative fluorescence polymerase chain reaction
STR	Short tandem repeat

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Author contributions
Conceptualization: Q.D., R.A.R. and N.L.H.; Investigation: Q.D., A.L.B., K.A.B., A.A.E., E.B.M., C.K.R., R.A.R. and N.L.H.; Writing – Original Draft Preparation: Q.D.; Writing – Review & Editing, Q.D., C.K.R., R.A.R. and N.L.H. All authors reviewed the manuscript.

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Data availability
All data generated during this study are included in this published article.

Declarations

Ethics approval and consent to participate
This study was reviewed by the Mayo Clinic Institutional Review Board (IRB) and determined to be exempt from the requirement for IRB approval. The Mayo Clinic IRB has approved a waiver of informed consent (IRB Application #: 24-010046).

Consent for publication
The Mayo Clinic IRB has approved a waiver for consent for publication.

Competing interests
The authors declare no competing interests.

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