A Rare Combination of Functional Disomy Xp, Deletion Xq13.2-q28 Spanning the *XIST* Gene, and Duplication 3q25.33-q29 in a Female with der(X)t(X;3)(q13.2;q25.33)

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Abstract

Keywords

- ▶ 3q25.33-q29 duplication
- ▶ Xq13.2-q28 deletion
- ▶ translocation
- ▶ atypical Turner's syndrome
- ▶ functional disomy Xp
- ▶ duplication 3q syndrome
- ▶ *XIST*
- ▶ intellectual disability
- ▶ fluorescence in situ hybridization
- ▶ array comparative genomic hybridization

We report a 19-year-old female patient with a history of short stature, primary ovarian insufficiency, sensorineural hearing loss, sacral teratoma, neurogenic bladder, and intellectual disability with underlying mosaicism for der(X)t(X;3)(q13.2;q25.33), a ring X chromosome, and monosomy X. Derivative X chromosomes from unbalanced X-autosomal translocations are preferentially silenced by the *XIST* gene (Xq13.2) located within the X-inactivation center. The unbalanced X-autosomal translocation in our case resulted in loss of the *XIST* gene thus precluding the inactivation of the derivative X chromosome. As a result, clinical features of functional disomy Xp, Turner's syndrome, and duplication 3q syndrome were observed. Importantly, indications of the derivative X chromosome were revealed by microarray analysis following an initial diagnosis of Turner's syndrome made by conventional cytogenetic studies approximately 18 months earlier. This case demonstrates the importance of utilizing microarray analysis as a first-line test in patients with clinical features beyond the scope of a well-defined genetic syndrome.

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Introduction

Standard monosomy X accounts for approximately 46% of Turner's syndrome karyotypes and is associated with infertility and the absence of menstruation and secondary sexual characteristics.¹ Variant Turner's syndrome karyotypes (e.g., monosomy X mosaicism, isochromosome Xq, structural abnormalities of the Y chromosome, ring X chromosomes, and marker X chromosomes) account for the remaining 54% of Turner's syndrome diagnoses, and may vary clinically depending on the etiology.¹ Functional disomy Xp and partial 3q duplications are both rare with characteristic clinical features observed for each abnormality. Partial 3q duplications typically result from unbalanced rearrangements or de novo duplications, while functional disomy Xp in females can result from chromosomes containing Xp chromosomal material that lacks the *XIST* gene (i.e., X chromosomes with *XIST* deletion or autosomes harboring Xp chromosomal material).^{2,3} Located within the X-inactivation center on chromosome Xq13.2, the cis-acting *XIST* gene encodes RNA that "coats" the X chromosome thus preventing DNA from being transcribed with the exceptions of genes that escape inactivation, most of them located in the pseudoautosomal regions 1 and 2 (PAR1 and PAR2) but also seen dispersed throughout the X chromosome.^{1,4} While the normal chromosome X is preferentially silenced in balanced X-autosomal translocations, in unbalanced X-autosomal translocations the derivative chromosome X is preferentially silenced to best maintain a functionally balanced genome.¹ However, in any of these translocations loss of *XIST* prevents silencing genes located on the derivative chromosome.

We report a 19-year-old female patient with a history of short stature, primary ovarian insufficiency, bilateral sensorineural deafness, sacral teratoma, neurogenic bladder, and intellectual disability. Conventional cytogenetic studies were performed at the age of 18 years and revealed mosaicism for deletion Xq, a ring X chromosome, and monosomy X, and a diagnosis of Turner's syndrome was rendered. Approximately 1.5 years following conventional cytogenetic studies, microarray analysis was performed to better understand the etiology of a phenotype that surpasses the characteristic features of Turner's syndrome.

Case Report

We report a 19-year-old female patient that was referred to the genetics clinic with a diagnosis of Turner syndrome based on a history of short stature, primary ovarian insufficiency, and conventional cytogenetic results (mosaicism for deletion Xq, ring chromosome X, and monosomy X). Her birth weight was 2.61 kg, and birth length was 50.8 cm. Her medical history is also significant for bilateral sensorineural deafness, sacral teratoma, neurogenic bladder, and intellectual disability. Her communication was quite limited due to sensorineural hearing loss which required the use of hearing aids. She primarily expressed herself through sign language. She is currently enrolled in special education classes at the 12th-grade level. Her height is 149.5 cm (2% based on Centers for Disease Control

Fig. 1 (A and B) Proband at the age of 19 years; note the downslanting palpebral fissures, long eyelashes, and bushy eyebrows.

and Prevention [CDC] 2–20 years data), weight is 71 kg (85% based on CDC 2–20 years data), and body mass index is 31.77 (kg/m²). Notable facial features include downslanting palpebral fissures, long eyelashes, and bushy eyebrows (►Fig. 1A, B). Current treatment consists of hormone replacement therapy and prophylactic use of sulfamethoxazole and trimethoprim for neurogenic bladder. The family history of birth defects, deafness/hearing loss, developmental disorders, consanguinity, or other known genetic conditions were not reported.

Materials and Methods

Conventional cytogenetic and fluorescence in situ hybridization (FISH) studies were performed on a phytohemagglutinin-stimulated peripheral blood specimen collected from the proband. Metaphase and interphase cells were analyzed by American Society for Clinical Pathology–certified cytogenetic technologists and captured using Cytovision software (Leica Biosystems, Buffalo Grove, Illinois, United States). To confirm the location of the 3q25.33–q29 duplication, the FISH analysis was performed within the Xq deletion region using a *BCL6* (3q27) break-apart DNA probe (Abbott Molecular, Des Plaines, Illinois, United States). Quantitative polymerase chain reaction analysis (qPCR) was performed with the CFX Real-Time PCR Detection System (Bio-Rad, Hercules, California, United States) to determine the *XIST* deletion status using copy number assay Hs04096142_cn (chr X:73,828,912, NCBI Build 38, December 2013) (ThermoFisher Scientific, Waltham, Massachusetts, United States). Results of the qPCR analysis were compared with three males and four female controls.

Array comparative genomic hybridization (aCGH) analysis was performed on purified DNA from the proband using the CytoScan HD microarray platform (Affymetrix, Santa Clara, California, United States), and scanned with a GeneChip Scanner 3000 (Affymetrix). The reported nucleotide coordinates are based on UCSC hg19 (NCBI Build 37, February 2009). Nomenclature was reported using the International System for Human Cytogenomic Nomenclature (ISCN, 2013 and 2016). All aCGH and FISH procedures were performed according to the manufacturer's protocol.

Results

Conventional cytogenetic studies revealed what appeared to be a terminal Xq24 deletion in 37 cells, a ring X chromosome in six cells, and monosomy X in seven cells. At this time the karyotype was reported as mos 46,X,del(X)(q24)[37]/45,X[7]/46,X,r(X)(p22.1q24)[6] (►Fig. 2A–C).

Approximately 1.5 years following conventional cytogenetic studies, microarray analysis was requested for further genotype investigation and revealed a concomitant 38.5 Mb mosaic duplication of 3q25.33–q29 (►Fig. 2D), and an 82.9 Mb heterozygous Xq13.2–q28 deletion (►Fig. 2E). The 3q25.33–q29 mosaic duplication (predicted copy number: 2.79) contained 420 genes, 163 of these genes were described in Online

Fig. 2 Representative X chromosomes from the initial conventional cytogenetic study demonstrating mosaicism for (A) one normal chromosome X and one abnormal chromosome X with what appeared to be an Xq24 deletion (37 cells), (B) one normal chromosome X and one ring chromosome X (6 cells), and (C) monosomy X (7 cells). Array CGH profile showing (D) 82.9 Mb heterozygous Xq13.2–q28 deletion spanning the *XIST* gene, and (E) 38.5 Mb mosaic duplication of the 3q25.33–q29 chromosomal region. (F) Representative metaphase cell containing the derivative X chromosome. Arrows point to both normal copies of chromosome 3, the normal chromosome X, and the derivative chromosome X. (G) Sequential FISH analysis of the representative metaphase cell (see F) using a break-apart probe set (*BCL6*) targeting chromosomal region 3q27. Positive hybridization (arrows) was observed on both copies of chromosome 3 in addition to the long terminal arm of the derivative X chromosome. Metaphase cells containing the ring chromosome X and monosomy X were negative for *BCL6* hybridization. CGH, comparative genomic hybridization.

Mendelian Inheritance in Man (OMIM), while the Xq13.2-q28 deletion contained 962 genes, 356 of these genes were described in OMIM including *XIST*.

The discrepancy of the Xq deletion breakpoint between conventional cytogenetic studies (Xq24) and aCGH analysis (Xq13.2), along with a terminal 3q duplication that was not observed by conventional cytogenetics prompted an additional investigation by FISH. Positive hybridization for *BCL6* was observed on both copies of chromosome 3 and also on the long arm of the derivative X chromosome (►Fig. 2F, G). Metaphase cells with a ring X chromosome or monosomy X obtained positive hybridization for *BCL6* on both copies of chromosome 3 only. qPCR analysis confirmed a heterozygous *XIST* gene deletion when compared with three male and four female controls (►Fig. 3). The final karyotype was: mos 46, X,der(X)t(X;3)(q13.2;q25.33)[37]/45,X[7]/46,X,r(X)(p22.1q13.2)[6].arr[GRCh37]Xq13.2q28(72267404_155233731)x1,3q25.33q29(159339511_197851986)x3[0.79]. Parental chromosome studies could not be performed to determine balanced translocation carrier status.

Discussion

In healthy females silencing of a single X chromosome is random and begins in early fetal development.¹ The cis-acting *XIST* gene (Xq13.2) encodes RNA that essentially wraps itself around the X chromosome on which it is expressed, resulting in DNA silencing except for approximately 15% of genes that are currently known to escape X-inactivation.⁴ This process termed “lyonization” ensures that DNA is expressed from only one copy of chromosome X for healthy development.¹ Most of the human escape genes cluster in the PAR1 and PAR2 regions on the distal arms of both X and Y chromosomes.^{1,4,5} Balanced and unbalanced X-autosomal translocations alter the random inactivation of X to ensure the most functionally balanced genome. While balanced X-autosomal translocations in females result in preferential silencing of the normal X chromosome, unbalanced X-autosomal translocations lead to the preferential silencing of the derivative X chromosome.¹ Absence of the *XIST* gene on normal or abnormal X chromosomes precludes inactivation, thus enabling the expression of

Fig. 3 Quantitative PCR analysis (qPCR) results showing *XIST* gene copy number for our patient (arrow), three male controls (4, 5, and 7), and four female controls (1, 2, 3, and 6). Our patient had a predicted *XIST* gene copy number of 1.

DNA that would preferentially be silenced. This is the first case reporting an unbalanced translocation between the long arms of chromosomes X and chromosome 3 resulting in a concomitant Xq13.2-q28 deletion including the *XIST* gene and 3q25.33-q29 mosaic duplication. Also, deletion Xq13.2-q28 indicates a diagnosis of Turner's syndrome.⁶⁻⁸

The clinical features observed in our patient are consistent with functional disomy Xp, Turner's syndrome, and duplication 3q syndrome. Features of functional disomy Xp include early hypotonia, intellectual disability, hypertelorism, myopia, small hands and feet, and external ear malformations, while clinical features of duplication 3q syndrome include characteristic facial dysmorphism, hirsutism, microcephaly, intellectual disability, genitourinary anomalies, sacrococcygeal teratoma, hand and feet abnormalities, renal and congenital heart defects.^{2,3,9-16} Deletions of Xq result in Turner's syndrome with clinical features mainly including primary ovarian insufficiency.⁶⁻⁸ Consistent with duplication 3q syndrome our patient presents with intellectual disability, sacral teratoma, neurogenic bladder, and facial dysmorphisms including downslanting palpebral fissures, bushy eyebrows, and long eyelashes.² Primary ovarian insufficiency, short stature, and sensorineural hearing loss are consistent with features of Turner's syndrome.⁶⁻⁸ Importantly, genotype-phenotype correlation is limited when mosaicism has been established. While the origin of the der(X)t(X;3) in our patient is uncertain, the presence of additional cell lines (ring X chromosome in some cells, and monosomy X in other cells) strongly supports a de novo event. As the germline consists of the der(X)t(X;3), subsequent mitoses resulted in ring X chromosome formation by mechanisms not completely understood, and finally monosomy X due to the instability of ring X chromosomes.

Our case also illustrates the importance of communicating a comprehensive medical history to the genetic laboratory to guide appropriate testing. Based on the initial phenotypic description "premature ovarian failure" without any further symptoms, the interpretation of the conventional cytogenetic results was consistent with a diagnosis of mosaic Turner's syndrome. Subsequent microarray studies were initiated to investigate further the etiology of a much more complex phenotype that was not fully explained by the diagnosis of Turner's syndrome, such as intellectual disability, sacral teratoma, and a neurogenic bladder. The Xq breakpoint discrepancy between conventional cytogenetic (Xq24) and microarray (Xq13.2) studies is accounted for by the 3q25.33-q29 duplication located on the long terminal arm of the derivative chromosome X (—Fig. 2D–G). Moreover, similar banding patterns between Xq13.2-q24 and 3q25.33-q29 chromosomal regions precluded the identification of the unbalanced t(X;3) translocation. Although conventional cytogenetic analysis was initially performed due to features of Turner's syndrome, microarray is considered the first-line test in the initial evaluation of individuals with anomalies not specific to a well-delineated genetic syndrome as illustrated by our case.¹⁷

In summary, we present a 19-year-old female patient with the rare combination of functional disomy Xp, mosaic Turner's

syndrome, and duplication 3q syndrome. A multifaceted diagnostic approach including conventional cytogenetics, microarray analysis, and FISH identified the presence of a derivative X chromosome with a Xq13.2-q28 deletion spanning the *XIST* gene, and 3q25.33-q29 duplication. Also, this case highlights the importance of utilizing microarray as the first-line test in patients with clinical features that are beyond the scope of a well-defined genetic syndrome.

Conflict of Interest

None.

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