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Characterization of a prenatally diagnosed *de novo* der(X)t(X;Y)(q27;q11.23) of fetus

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A 31-year-old woman, who was pregnant with twins, underwent chorionic villus sampling because of increased nuchal translucency in one of the fetuses. Cytogenetic analysis showed a normal karyotype in the fetus with increased nuchal translucency. However, the other fetus, with normal nuchal translucency, had a derivative X chromosome (der(X)). For further analysis, fluorescence *in situ* hybridization (FISH) and additional molecular studies including fragile X analysis were performed. FISH analysis confirmed that the Y chromosome was the origin of extra segment of the der(X). The X-chromosome breakpoint was determined to be at Xq27 by *FMR1* CGG repeat analysis, and the Y-chromosome breakpoint was determined to be at Yq11.23 by the Y chromosome microdeletion study. To predict the fetal outcome, the X-inactivation pattern was examined, and it revealed non-random X inactivation of the der(X). To the best of our knowledge, the identification of an unbalanced Xq;Yq translocation at prenatal diagnosis has never been reported. This study was performed to identify precise breakpoints and the X-inactivation pattern as well as to provide the parents with appropriate genetic counseling.

Key words: Prenatal diagnosis, X chromosome, Xq;Yq translocation.

Introduction

X;Y translocations rarely occur in humans. Most of these aberrations, analyzed cytogenetically, have breakpoints at Xp22 and Yq11, probably because of their extensive sequence homology [1]. Males with Xp22;Yq11 translocations are usually nullisomic for a portion of Xpter, and their phenotypes depend on the X chromosome breakpoints. These phenotypes can include mental retardation, ichthyosis, chondrodysplasia punctata, and other phenotypic anomalies. Females carrying such translocations usually have a normal phenotype, except that they have short stature [1].

Xq and Yq interchanges are less frequent, and only a few cases of have been reported [2,3]. These reports described phenotypically normal females with secondary amenorrhea and translocation between Xq and Yq. To the best of our knowledge, the detection of Xq;Yq translocation at prenatal diagnosis has not been reported.

Herein, we report the prenatal diagnosis of a *de novo* unbalanced Xq;Yq translocation for the first time and discuss issues in genetic counseling to be given to patients with this particular abnormality.

Case

A 31-year-old nulliparous woman visited our fertility center and underwent ovulation induction. She became pregnant

with twins as a result of a successful intrauterine insemination procedure, and the fetal ultrasound examination at 11 weeks of gestation revealed an increased risk for Down syndrome in one fetus based on nuchal translucency (NT) measurement (first fetus NT = 3.1 mm, second fetus NT = 1.3 mm).
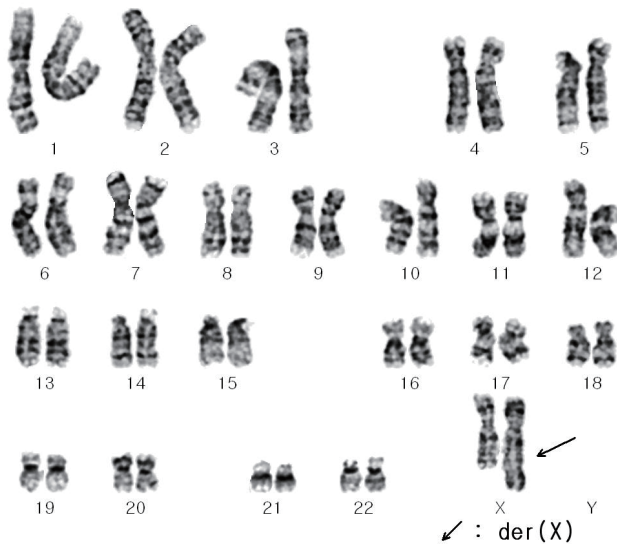

Fig. 1. Chromosome analysis by GTG-banding in the second fetus.

She opted for fetal karyotyping, and chorionic villus sampling (CVS) was performed. While a CVS chromosome analysis revealed a normal karyotype with 46,XY for the first fetus, the second fetus showed the presence of supplementary material at the telomeric end of one X chromosome in all 20 metaphases examined (46,X,der(X)) (Fig. 1). Both the parents' karyotypes were normal.

To confirm the origin of der(X), fluorescence *in situ* hybridization (FISH) analysis was performed with whole chromosome painting probes specific to the Y chromosome (WCP Y). The results demonstrated the presence of a Y-chromosome segment (red) on the X derivative (Fig. 2A). Based on this analysis, we represented the fetal karyotype as 46,X,der(X).ish der(X)t(X;Y) (q28;q11.2)(wcpY+).

For further study, amniocentesis was performed around 18 weeks of gestation. The additional FISH analysis using Y chromosome-specific probes demonstrated a hybridization signal for the CEP Y (DYZ1) probe and absence of a signal for the CEP Y (DYZ3) or SRY probe (Figs. 2B-D). These analyses revealed that the translocated Y segment largely consisted of heterochromatin and did not include the centromere.
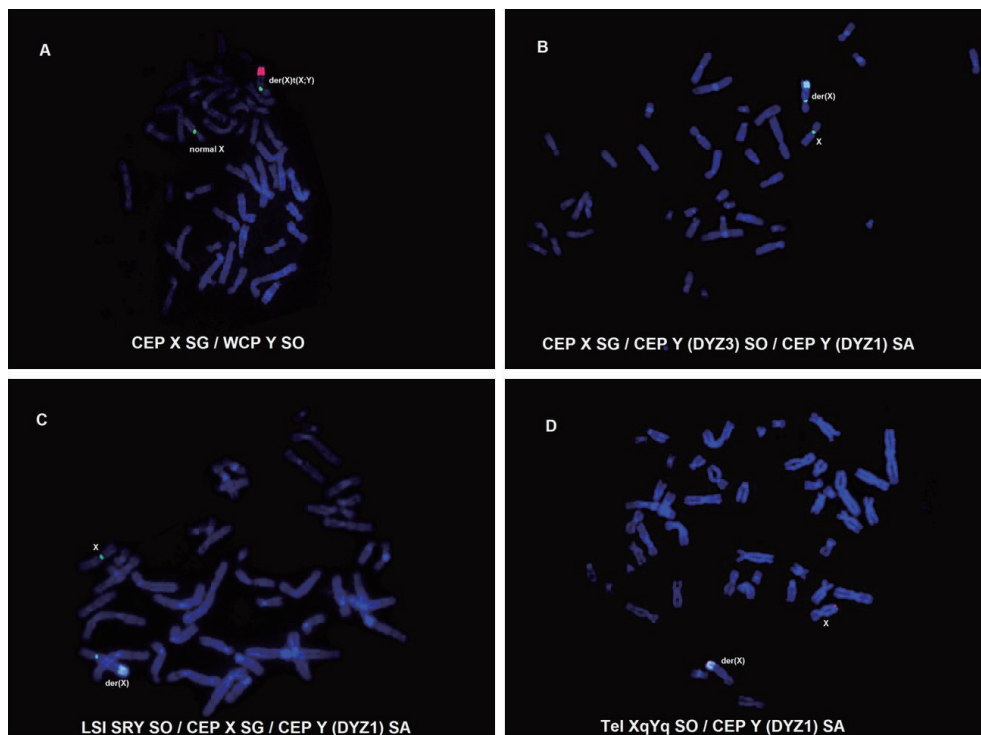

Fig. 2. (A) Fluorescence *in situ* hybridization (FISH) analysis with paint probe (WCP Y SO) demonstrating the presence of Y segment (red) on the X derivative. (B, C) FISH analysis showing no signal from the centromeric Y probe (CEP Y (DYZ3) SO) or the SRY probe. Aqua signal from the CEP Y (DYZ1) SA probe representing the presence of the Y heterochromatin region. (D) FISH analysis showing two signals from the sub-telomere Xq/Yq (red) probe. One red signal on the der(X) indicated the terminal deletion of the X chromosome and translocation of a Y chromosome segment.

Quantitative fluorescence polymerase chain reaction (QF-PCR) showed homozygous pattern at DXS8377 loci due to Xq28 deletion (data not shown). Multiplex ligation-dependent probe amplification (MLPA) was performed using two different MLPA kits (MLPA human telomere kit, SALSA P070 and MLPA microdeletion syndromes kit, SALSA P245; MRC-Holland b.v., Amsterdam, Netherlands), and the results showed a deletion of the Xq28 region, which included *MECP2*, and the presence of the

Xq/Yq terminal region, confirming the cytogenetic result (Fig. 3).

We conducted fragile X analysis to examine whether the affected X chromosome was inactivated. *FMR1* repeat determinations revealed only one allele of 33 CGG repeats (Fig. 4). Southern blot analysis of *Eco*RI/*Eag*I double digests with probe StB12.3 showed one unmethylated 2.8 kb fragment representing the normal, active X chromosome, but the normally methylated 5.2 kb fragment representing the inactive X chromosome
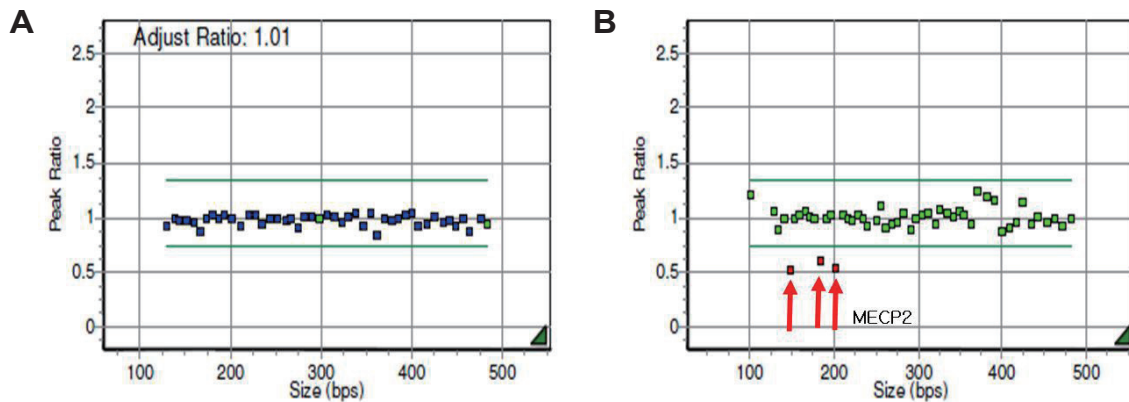

Fig. 3. (A) Multiplex ligation-dependent probe amplification (MLPA) (P070) results revealed a normal pattern, and (B) MLPA (P245) showed loss of the *MECP2* gene mapped on the Xq28 region in the second fetus.
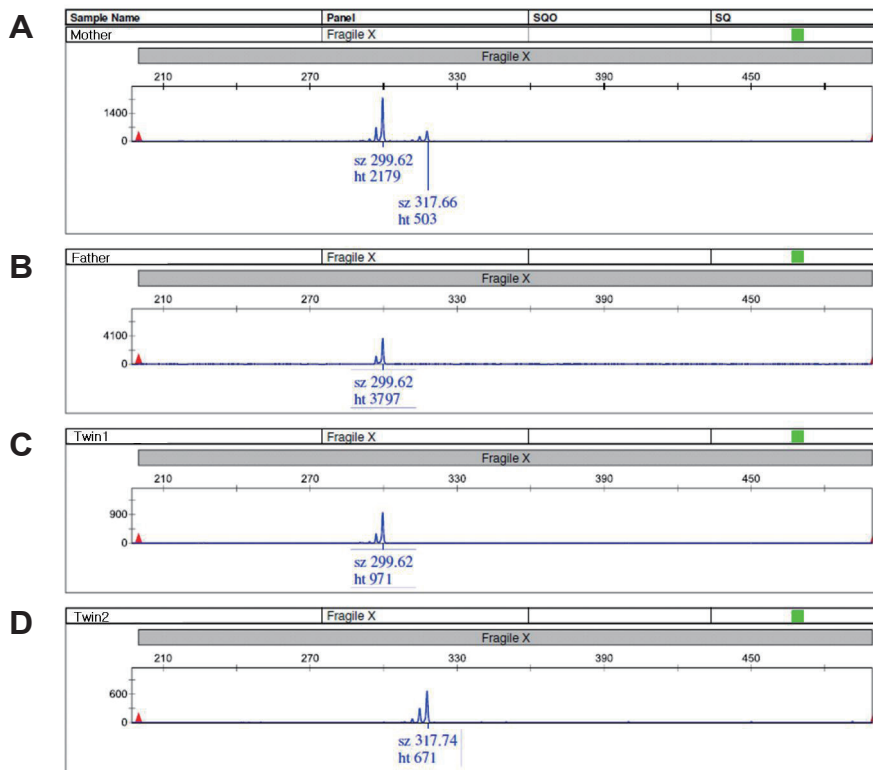

Fig. 4. *FMR1* gene CGG repeat determinations. (A) The mother showed two alleles of 27 and 33 repeats, and (B) the father showed one allele of 27 repeats. (C) The first fetus with a male karyotype had one allele of 27 repeats, and (D) the second fetus with der(X) had only one allele of 33 CGG repeats.

was absent (Fig. 5) [4]. These results revealed a deletion of the *FMR1* gene region on one X chromosome and non-random X inactivation in favor of the normal X chromosome. Based on these results, we modified the breakpoint of der(X) from Xq28 to Xq27.
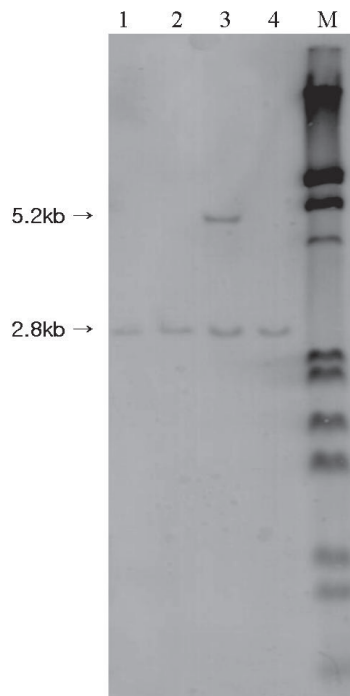

Fig. 5. *FMR1* Southern blot analysis of the fetus with a derivative X chromosome. DNA was digested with *Eco*RI/*Eag*I and hybridized with probe StB12.3.
Lane 1: The first fetus with 46,XY.
Lane 2: The second fetus with 46,X,der(X)t(X;Y) with the 2.8 kb fragment reflecting complete skewing of the X-inactivation patterns.
Lane 3: Normal female control with the 2.8 kb fragment representing the normal active X chromosomes and the 5.2 kb fragment representing the normal inactive X chromosomes.
Lane 4: Normal male control.
Lane N: Molecular weight marker.

Y chromosome microdeletion study showed one small peak in the sY1206 sequence, indicating the presence of part of the *AZFc* gene on the der(X). This study revealed the precise breakpoint of the Y chromosome as Yq11.23 within the *AZFc* gene (Fig. 6). The final karyotype was interpreted as 46,X,der(X).ish der(X) t(X;Y)(q27;q11.23)(wcpY+,SRY−,DYZ3−,DYZ1+,telXqYq+) by a combination of cytogenetic and molecular studies.

At 21 weeks of pregnancy, level II ultrasound and fetal echo-cardiogram assessment showed no structural abnormalities in either fetus. The parents decided to continue with the pregnancy. The twin babies were delivered by cesarean section at 35 weeks because of premature rupture of membranes. Both babies were admitted to the neonatal intensive care unit. The first baby was a boy with a birth weight of 2,060 g. The second baby was female with a birth weight of 2,300 g, and she was phenotypically normal. The babies were discharged after 10 days and are now seven months old. Both of them have been developing within the normal growth range.

Discussion

According to the University of California Santa Cruz (UCSC) human genome browser assembly hg19, Xq27-ter is a gene-rich region with over 100 genes, and many of these genes are associated with fatal X-linked syndromes [5]. Male infants who inherit the Xq deletions may be miscarried because of the deleted X chromosome in male infants who are nullisomic for the many essential genes contained within the deleted regions [5]. Yachelevich et al. [5] studied four female patients with terminal deletions of chromosome Xq that included the *FMR1* gene. The four female patients, and the two daughters of the second
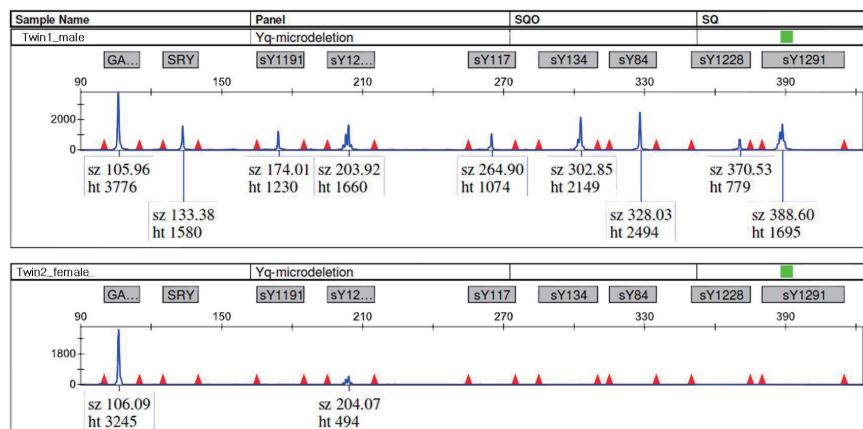

Fig. 6. Y chromosome microdeletion study showed a small peak of the sY1206 sequence in the second fetus, indicating the presence of part of the *AZFc* gene in the derivative X chromosome.

patient with the Xq deletion, had normal intelligence and stature, and no dysmorphic features [5]. Fragile X analysis revealed that each of the females had completely non-random X inactivation with only the normal X chromosome as the active one [5]. However, there are a few reports about girls with intellectual disabilities and microdeletion of Xq27.3 whose X inactivation pattern did not show complete skewing of X inactivation in favor of the normal X chromosome, probably because of the smaller-sized deletions in these patients [6,7].

We performed a Southern blot analysis to evaluate the methylation status of the unique *FMR1* allele present. The results of this analysis did not display any methylation pattern, which led us to conclude that the normal X-chromosome was active, whereas the derivative X-chromosome was preferentially inactivated. The non-random X-inactivation pattern indicated that the deletion is lethal at a cellular level during early stages of development, and that only the cells with the normal X chromosome as the active chromosome survive [5].

The parents were counseled that this result could not accurately predict fetal phenotype because the X-inactivation pattern in other tissues could be different [8]. We recommended a second X-inactivation study from postnatal blood samples, but the parents declined further investigation. Presently, the female infant is seven months old, developing within the normal growth range, and has a normal phenotype.

X;autosome translocations and X-chromosome deletions have been reported in premature ovarian failure (POF) patients, with two critical regions, *POF1* and *POF2*, most closely related to POF [9]. *POF1* (Xq23-q27 or Xq26-28) region deletions are much more commonly related to POF, and the haploinsufficiency of those deleted genes that normally escape X inactivation may be harmful to ovarian function [9]. Marozzi et al. [10] studied six POF patients with rearranged Xq chromosomes and revealed that the *POF1* region extends from Xq26.2 to Xq28.

However, some women with the same deletion exhibited POF at different ages and sometimes with different severities. Ferreira et al. [9] provided a possible mechanism explaining how the time during development can influence the onset of POF when non-random X-chromosome inactivation occurs. Taken together, it is thought that 5-10% of women with an Xq terminal deletion will be able to conceive but will need advice because of the high risks associated with fragile X syndrome or POF in their offspring [11].

The Xq27-q28 band is known to have high repeat density and recombination frequency, indicating that it is a hot spot for the generation of abnormal X chromosome in unbalanced translocations with different autosomal chromosomes [12-

14]. We believe that the generation of the X;Y rearrangement occurred in this region during paternal meiosis.

A number of studies have illustrated that females with Y-derived sequences in the der(X) seem to have a high risk of developing gonadoblastoma (10-30%) [15]. In fact, the presence of *GBY* in the Y chromosome, mapped in the pericentromeric region of Yp, seems to be related to an increased risk of gonadoblastoma [16]. In this study, the molecular analysis showed the absence of a Y-centromere and the presence of only a part of *AZFc* in the der(X). According to these results and previous literature reports, we suggest that the risk of developing gonadoblastoma in our patient might not be high later in life [17]. However, further studies are required to understand this abnormality, and our patient will be required to go through regular urogenital examinations to detect the development of gonadoblastoma [17].

In this communication, we report the first case of a *de novo* unbalanced Xq;Yq translocation identified at prenatal diagnosis. We performed a detailed assessment of the breakpoints and X inactivation study using fragile X analysis. Genetic counseling issues include a possibility of POF because of the X chromosome breakpoint and risks associated with random X-inactivation and gonadoblastoma.

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