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CASE REPORT

# X-AUTOSOME AND X-Y TRANSLOCATIONS IN FEMALE CARRIERS: X-CHROMOSOME INACTIVATION EASILY DETECTABLE BY 5-ethynyl-2-deoxyuridine (EdU)

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## ABSTRACT

Here we report one new case each of an X-autosome translocation (maternally derived), and an X-Y-chromosome translocation. Besides characterizing the involved breakpoints and/or imbalances in detail by molecular cyto-genetics, also skewed X-chromosome inactivation was determined on single cell level using 5-ethynyl-2-deoxyuridine (EdU). Thus, we confirmed that the recently suggested EdU approach can be simply adapted for routine diagnostic use. The latter is important, as only by knowing the real pattern of the skewed X-chromosome inactivation, correct interpretation of obtained results and subsequent reliable genetic counseling, can be done.

**Keywords:** 5-ethynyl-2-deoxyuridine (EdU); Genetic counseling; Molecular cytogenetics; Skewed X-chromosome inactivation; X-Autosome translocation; X-Y-Chromosome translocation.

## INTRODUCTION

The X-chromosome inactivation process (XCI) in mammalian female cells has been known for decades [1]. The goal of XCI is to balance the number of active X-chromosomes (Xa) with respect to a diploid set of autosomes. As we recently summarized: "Dosage compensation between genders in mammals is achieved by keeping only one Xa per diploid set of autosomes. Therefore, the majority of genes on one of the two X-chromosomes in female mammals is silenced and denoted as inactive X-chromosome (Xi) or Barr body" [2]. Interestingly, the molecular mechanism of XCI is not yet understood in detail, and especially, the choice of which of the present X-chromosomes have to be inactivated, remains overall enigmatic [2]. However, the X inactivation center as initial starting point of XCI has been identified, and the whole process was broken down to four stages including initiation, speeding, maintenance and reactivation [3]. In a normal female, the inactivation of one of the two X-chromosomes per cell is random; *i.e.,* in ~50.0% of the cells the paternal and in the other ~50.0% of the cells the maternal X-chromosome is active. In the case of balanced X-Y- or X-autsome-translocations, the X-inactivation becomes skewed. This can be due to the fact that only those cells are viable in which the whole autosomal content involved in the translocation remains active [4].

Identification of derivatives of X-chromosomes in females raises the question about the pattern of XCI. Previously, bromodeoxyuridine (5-bromo-2'-deoxyuridine; BUdR) was used to discriminate Xi from Xa in metaphase chromosomes [5]. However, recently a more reliable protocol was established based on 5-ethynyl-2'-deoxyuridine (EdU) [6]. The EdU is a labeled nucleoside analog of thymidine and it is incorporated during DNA synthesis of the cultivated blood cells. It is a marker that can highlight late replication regions and at the same time Xi. Here we report two female cases of X-autosome translocation (mother and daughter) and one female with an X-Y translocation leading to skewed X-inactivation. Molecular cytogenetics characterized the underlying chromosomal aberrations, and EdU-method according to Sisdelli *et al.* [6], could clearly identify the different patterns of X-inactivation.

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## MATERIALS AND METHODS

Overall, cytogenetically worked-up material from peripheral blood was used. The EdU incorporation and detection together with fluorescence *in situ* hybridization (FISH) using the probe DXZ1 were done as previously reported on this material [6]. A female (positive) and male (negative) were used as controls.

One female with an X-autosome translocation was originally studied due to infertility; during parental studies it turned out that the translocation was maternally derived. Another female was also studied for infertility and an X-Y-translocation was identified.

Banding cytogenetics was done according to standard procedures. Molecular cytogenetics was done as previously reported [7]. We applied a multicolor banding (MCB) probe set for the X-chromosome [8], a microdissection derived partial chromosome paint for chromosome 18p (pcp18p) [9], whole chromosome paints (WCP) for X- and Y-chromosomes [10] and commercially available probes for centromeres of X-chromosome (DXZ1) and chromosome 18 (D18Z1) (Abbott/Vysis, Wiesbaden, Germany). Also locus-specific probes for the Kallmann-syndrome region in Xp22.31 (KAL1; Abbott/Vysis), the subtelomeric region of the short arm of the X-chromosome (subtelX-pter; Abbott/Vysis) and a bacterial artificial chromosome derived probe for RP11-943F15 in Yq11.22 were used. 4,6-Diamidin-2-phenylindole (DAPI) was used as a counterstain and for computerbased inverted DAPI-banding.

## RESULTS

Two infertile females were studied by banding cytogenetics; in female 1, molecular cytogenetics could confirm the karyotype as 46,X,t(X;18)(q22.3;p11.2)mat (Figure 1A). In female 2, a complex karyotype was determined by banding cytogenetics and FISH as mos 46,X,der(X)t(X;Y)(p22.3;q11.22)[71]/45,X[8]/45,der(X)t(X;Y)(p22.3;q11.22)[3]/47,XX,+der(X)t(X;Y)(p22.3;q11.22)[2]/47,X,der(X)t(X;Y)(p22.3;q11.22),+der(X)t(X;Y)(p22.3;q11.22)[1]/46,der(X)t(X;Y)(p22.3;q11.22),+der(X)t(X;Y)(p22.3;q11.22)[1] (Figure 1B).

Results of a normal female and male after EdU-experiments are shown in Figures 2A and 2B. The EdU-test revealed that in female 1 (and her mother, results not shown) all 25 studied cells showed a skewed X-inactivation; *i.e.,*

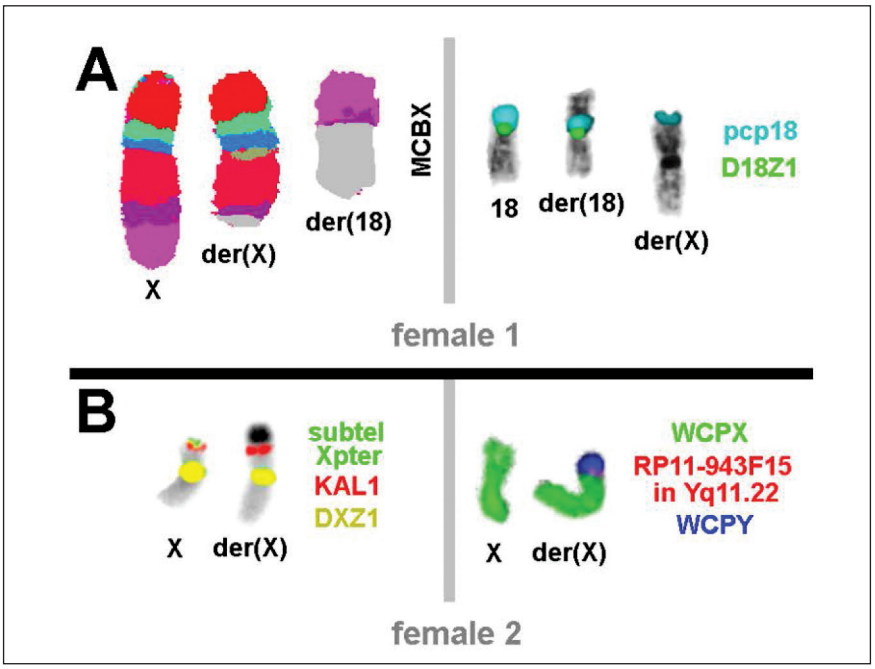


**Figure 1.** Results of molecular cytogenetic analyses for the case with X-autosome translocation (female 1) (A) and X-Y-translocation (female 2) (B) in females. Multicolor banding using a probe for the X-chromosome (MCBX) confirmed the break in Xq22.3; probes for the short arm of chromosome 18 (pcp18) together with a cen-tromeric probe for chromosome 18 (D18Z1) and inverted DAPI-banding mapped the breakpoint into subband 18p11.2. The break in the derivative X-chromosome could be mapped between the probe KAL1 and the subtelomeric probe, and for the Y-chromosome-part in Yq11.22 proximal to probes RP11-943F15.

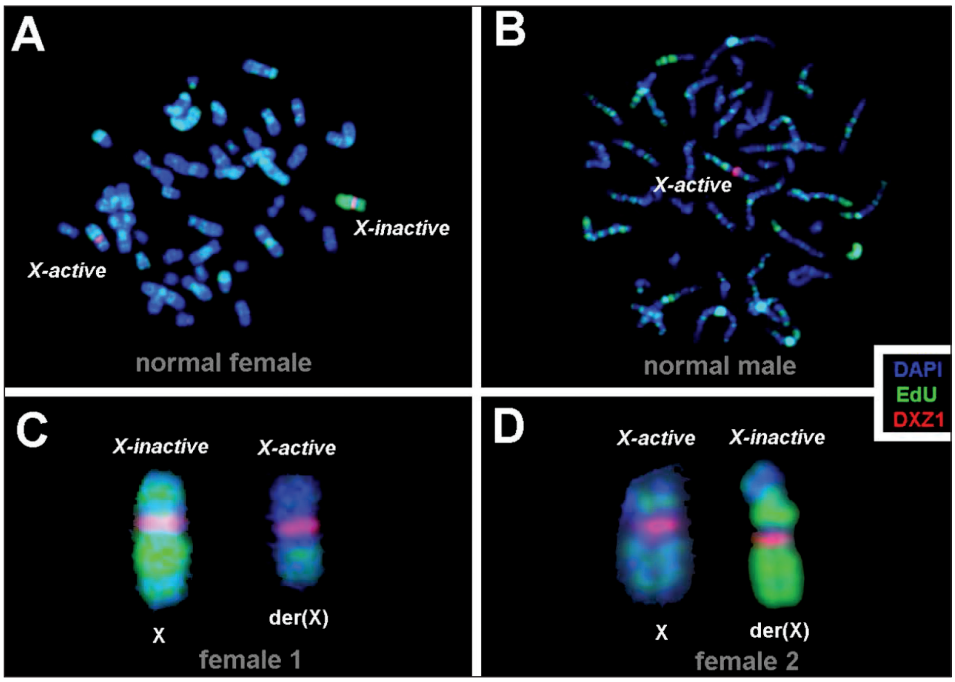


**Figure 2.** The EdU-test results are depicted for a normal female (A), a normal male (B), female 1 (C) and female 2 (D). X-Chromosomes are labeled in red by the centromeric probe for the X-chromosome (DXZ1). The inactive X-chromosomes are most intensely stained in green.

in all cells only the normal X-chromosome was inactivated (Figure 2C). In female 2, all studied cells had an inactive derivative X-chromosome (Figure 2D), here only cells with a karyotype 46,X,der(X)t(X;Y)(p22.3;q11.22) were taken into account.

## DISCUSSION

Up to now, female patients with derivative X-chromosomes due to X-autosome or X-Y translocation were not satisfactorily studied in routine molecular cytogenetic diagnostics (*e.g.,* [11]). As BUdR studies were not really reproducible, many laboratories stopped doing them; *e.g.,* in Germany no laboratory does them anymore. However, using the molecular genetic-based human androgen receptor (HUMAR)-test [12], XCI cannot be studied at the single cell level; *i.e.,* a skewed XCI can be detected but it can only be suggested which of the X-chromosomes is inactivated, the normal X-chromosome or the derivative one.

Here, we present two cases by which the XCI could be followed-up nicely on the single cell level based on a combination of banding cytogenetics, molecular cytogenetics and the EdU-test. The patterns were as to be expected in the two female patients showing no clinical symptoms (apart from infertility). Interestingly, female 1 suffers from infertility, even though for her mother this condition was not reported. Thus, the observed translocation can be the reason for the unfulfilled wish for children, but it was not completely clarified if this was the case. Still, for females 1 and 2, unbalanced products of conception may result from the maternal translocations, which may hamper their overall fertility.

Overall, the EdU-test was easy to establish just following the protocol of Sisdelli *et al.* [6] and can only be recommended to be used for complete clarification of similar cases. To the best of our knowledge, this is the first independent report confirming the excellent results of Sisdelli *et al.* [6]; the test was established in our laboratory within only 2 weeks.

**Declaration of Interest.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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