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# Identification of a Neocentromere in a Rearranged Y Chromosome With No Detectable DYZ3 Centromeric Sequence

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**An 18-year-old woman was evaluated because of primary amenorrhea and hypogonadism. Chromosome analysis from peripheral blood lymphocytes revealed a nonmosaic 46,X,+mar constitution. The marker was shown to be a rearranged Y chromosome consisting of an inverted duplication of the long arm: rea(Y)(qter-q11::q11-qter). Deletion mapping analysis with Y-specific STS showed that the marker lacked Yp and Y-centromeric (DYZ3) sequences, but it was positive for Yq sequences tested. Fluorescence in situ hybridization analysis with Y and X chromosome centromeric and pancentromeric probes showed no hybridization signals. The marker chromosome is present in 100% of the cells; therefore, it is mitotically stable despite the absence of DYZ3 centromeric sequence. Hybridization with CENP-A and CENP-C specific antibodies localized a neocentromere close to the breakpoint.**
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**KEY WORDS:** **chromosomal abnormalities; chromosomal structure/function; clinical cytogenetics; FISH; neocentromere**

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## INTRODUCTION

In eukaryotes, the centromere, cytogenetically defined as the primary constriction, is the site of sister chromatid attachment. It is essential for proper segregation of the chromosomes during mitosis and meiosis [Pluta et al., 1995]. The mammalian centromere contains large amounts of highly repeated satellite DNA, the best characterized of which is alpha-satellite DNA [Manuelidis, 1978].

Alpha-satellite DNA is believed to be important for centromeric function because it is the only type of sequence shown to be present at the primary constriction of all human chromosomes [Manuelidis, 1978]. The introduction of artificial chromosomes carrying alpha-satellite DNA into mammalian cells has demonstrated that this DNA sequence provides some, if not all, information required in cis for the formation of the centromere [Haff et al., 1992; Larin et al., 1994; Harrington et al., 1997, Ikeno et al., 1998].

Conflicting evidence for alphoid satellite as an essential DNA component of functional centromeres has emerged from the study of stable marker chromosomes, which fail to show labeling with specific alpha-satellite DNA probes. These analphoid chromosomes carry newly derived centromeres (called "neocentromeres") that are apparently formed within interstitial chromosomal sites that have not previously been known to express centromere function [Choo, 1997; du Sart et al., 1997; Warburton et al., 2000]. Moreover, in human dicentric chromosomes, the alpha-satellite DNA is present on both the active and inactive centromeres, suggesting that the presence of alpha satellite per se is insufficient to determine centromere function [Earnshaw et al., 1989; Warburton, 2001].

Five constitutive centromere-binding proteins have been implicated in centromere function: CENP-A, CENP-B, CENP-C, CENP-G and CENP-H. Three of them (CENP-A, -C and -H) associate specifically with active centromeres, that is, are present on normal

centromeres and neocentromeres and absent from inactive centromeres [reviewed by Warburton, 2001]. These CENPs may be responsible for the establishment of an epigenetic mark, which determines the propagation of human centromeres at particular chromosome locations.

One effective genetic approach to the learning about mammalian centromeres is to examine structurally rearranged chromosomes with abnormal centromeres. We report on an unusual rearranged Y chromosome with no detectable alphoid DNA bearing a functional neocentromere close to the breakpoint.

## PATIENTS AND METHODS

### Patient Description

An 18-year-old girl was referred to us due to primary amenorrhea and hypogonadism. She was born at term to nonconsanguineous and healthy parents. She had two healthy sibs (a 10-year-old sister and a 5-year-old brother). Family history was unremarkable. There was a history of a slight delay on motor and speech development, learning difficulties, and nocturnal enuresis. When she was 4 years old, a left inguinal hernia surgery was performed.

On physical examination, there was normal height (162 cm) and weight (64.4 kg), macrocephaly (head circumference = 59 cm), M-shaped nuchal hairline, posterior rotation of the ears, prominent forehead, a diffuse goiter, widely spaced nipples, cubitus valgus, short fourth and fifth metacarpals and fifth metatarsals, high frequency of whorl pattern on fingertips, a right simian crease, and clinodactyly of the fifth toes. There was no breast development, external genitalia were female, gonads were not palpable, and pubic hair was on Tanner stage IV with a female distribution.

Laboratory investigation showed high levels of follicle-stimulating hormone (68.7 mIU/mL) and luteinizing hormone (42.1 mIU/mL), low levels of estradiol (12 pg/mL) and total testosterone (1.5 ng/mL), normal levels of thyroid-stimulating hormone (0.61 μUI/mL) and free thyroxine (15.74 pmol/L), and negative thyroid peroxidase and thyroglobulin antibodies. Bone age was 14 years, and there was lumbar osteopenia. Echocardiogram showed mitral valve prolapse. Ultrasonography revealed hypoplastic uterus (4.95 $cm^3$) and the gonads were not detected. Bilateral gonadectomy was performed and histology revealed streak gonads.

### Cytogenetic and FISH Studies

Cytogenetic analysis was performed on metaphase chromosomes obtained from phytohemagglutinin-stimulated peripheral blood lymphocytes using GTG, CBG and QFQ-banding. One hundred cells were analyzed. Fluorescence in situ hybridization (FISH) to metaphase cells using alpha-DNA satellite probes for X and Y chromosomes (DXZ1 and DYZ3, Oncor, Gaithersburg, MD), X-painting probe (COATSOME$^r$ X, Oncor), and human alpha-satellite pancentromere probe (Oncor) were performed according to the manufacturer's instructions. Probe HY10 consists of a 3.4-kb Y-specific repeat, which is a major component of the Y heterochromatic long arm [Nakahori et al., 1986]. Immunofluorescence using antibodies to CENP-A or CENP-C and simultaneous FISH with probe HY10 were performed essentially as described [Warburton et al., 1997, 2000].

### Molecular Studies

DNA was extracted from peripheral blood [Sambrook et al., 1989]. The sequences TSPY and DYZ3 were investigated by polymerase chain reaction (PCR) using external primers as in Binder et al. [1995]. DYZ3 sequence was reamplified in a nested-PCR reaction with internal primers [Binder et al., 1995]. The following Y-specific STS—sy81, sy86, sy151, sy117, sy143, sy254, sy255—were analyzed by PCR as described by Vollrath et al. [1992] and Reijo et al. [1995]. The presence of the SRY gene was investigated with primers XES10 and XES11 [Hawkins et al., 1992]. Each PCR contained normal female and male controls. A female operator performed all reactions, including DNA extraction.

## RESULTS

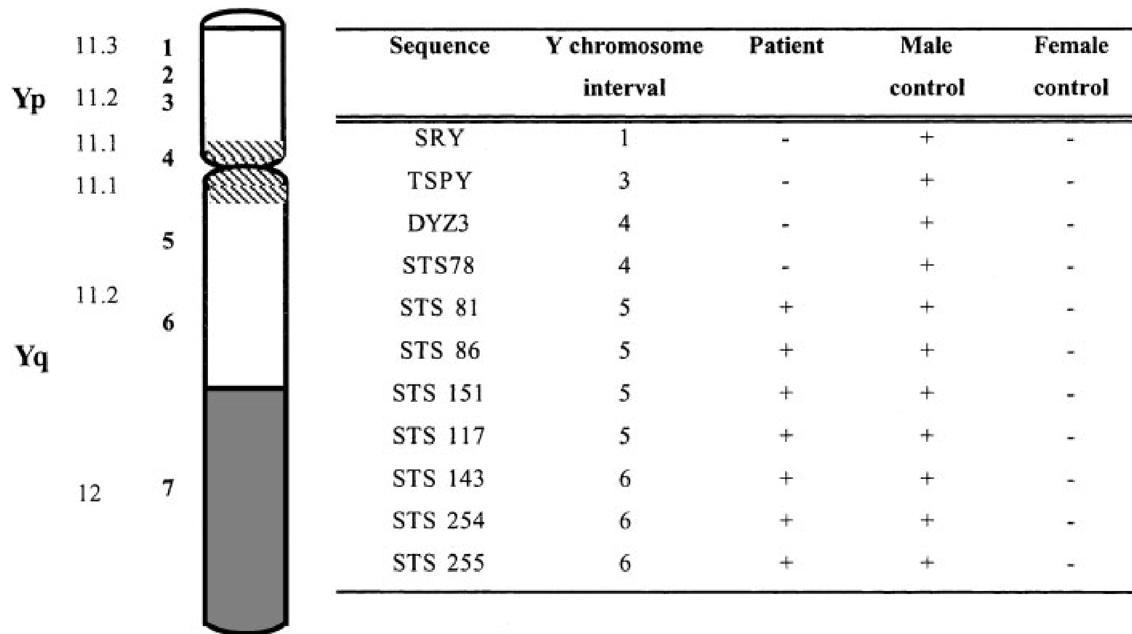
Chromosome analysis from peripheral blood lymphocytes with G-, Q-, and C-banding revealed a nonmosaic 46,X,+mar constitution. The marker consisted of two blocks of Q-positive heterochromatin separated by a region of Q-negative euchromatin. C-banding also revealed positive bands on both marker extremities. The banding patterns suggested that the marker might be an i(Yq). The karyotype of her brother was normal (46,XY), as well as that of her sister (46,XX). Although the father was not available for examination, the presence of a normal Y chromosome in the karyotype of the brother suggests that the formation of this Y rearranged chromosome occurred de novo, assuming same paternity of the sibs.

Molecular techniques were employed to confirm the Y origin of the marker, and a deletion map was established (Fig. 1). PCR studies showed positive results for all Yq sequences tested. However, Yp (SRY, TSPY) and Y centromeric (DYZ3) sequences tested negative. DYZ3, which corresponds to the chromosome Y centromeric alpha-satellite DNA, was assayed by nested PCR and the result was negative. The marker was therefore interpreted as a rea(Y)(qter-q11.2::q11.2-qter).

FISH experiments were carried out to search for possible centromere sequences within this marker. The marker failed to show labeling with X and Y centromeric probes and with X painting probe (data not shown). FISH with a human pancentromeric probe (Oncor) showed hybridization to all normal centromeres but not to the marker (Fig. 2a). CENP-C- and CENP-A-specific antibodies were shown to stain a region at or near the center of the chromosome, close to the breakpoint (Fig. 2b,c).

## DISCUSSION

We report in this article a rearranged Y chromosome found in a patient with a 46,X,rea(Y)(qter-q11.2::q11.2-qter) chromosomal constitution. This rearranged Y




| Sequence | Y chromosome interval | Patient | Male control | Female control |
|---|---|---|---|---|
| SRY | 1 | - | + | - |
| TSPY | 3 | - | + | - |
| DYZ3 | 4 | - | + | - |
| STS78 | 4 | - | + | - |
| STS 81 | 5 | + | + | - |
| STS 86 | 5 | + | + | - |
| STS 151 | 5 | + | + | - |
| STS 117 | 5 | + | + | - |
| STS 143 | 6 | + | + | - |
| STS 254 | 6 | + | + | - |
| STS 255 | 6 | + | + | - |

Fig. 1. Molecular map of the rearranged Y chromosome (+, sequence present; −, sequence absent).

chromosome is present in 100% of peripheral blood cells and therefore has been efficiently retained through cell divisions despite the absence of the endogenous centromere region. The presence of a neocentromere on this marker was confirmed by the absence of detectable alpha-satellite DNA and the presence of CENP-A and CENP-C (Fig. 2). This marker chromosome raises the question of the functional requirements for centromere formation.

Four neocentromeres have been reported on derivative Y chromosomes. One previously reported neocentric Y chromosome was similar to the one reported here, although apparently contained slightly less euchromatic material (Fig. 1) [Floridia et al., 2000]. This marker consisted of an inverted duplication of the long arm heterochromatin and a small amount of euchromatin, with deletion of the endogenous Y centromere and alpha-satellite DNA [Floridia et al., 2000]. The centromeric protein-binding domain in this marker was shown to be located within the DAZ gene cluster in Yq11.2. This chromosomal region is deleted in some infertile males [Reijo et al., 1995].

Thus, in the two reported cases of inverted duplication neocentric Y chromosome derivatives, the neocentromere appears to be found in the euchromatic DNA at or near the breakpoint [Floridia et al., 2000] (Fig. 2b,c). These chromosomes are consistent with the most common mechanism for the formation of analphoid marker

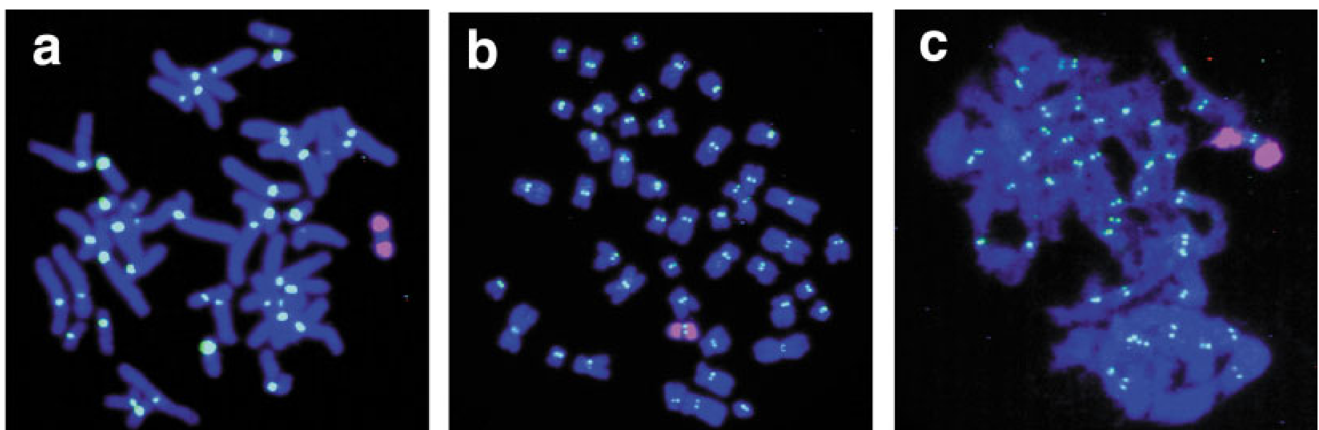



Fig. 2. Identification of alphoid DNA sequences and centromeric proteins. **a:** Fluorescence in situ hybridization (FISH) with a pancentromeric probe is shown in green and the derivative Y chromosome is identified by FISH using probe HY10 in pink. Immunofluorescence using antibodies to CENP-C **(b)** or CENP-A **(c)** is shown in green and the derivative Y chromosome is identified by FISH using probe HY10 in pink. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

chromosomes, the de novo inverted duplication of distal segments of chromosomes, resulting in mirror-image chromosomes [Choo, 1997; Warburton et al., 2000]. In the majority of these cases, the neocentromeres are found on one of the duplicated arms, although there are reports of metacentric chromosomes whose neocentromeres are at or near the breakpoints of the inverted duplications [Choo, 1997].

The other three cases of neocentromere-containing Y chromosomes are found on "neodicentric" Y chromosomes, which contain both an inactivated endogenous Y centromere and a neocentromere within or very close to the heterochromatin in the long arm [Bukvic et al., 1996; Rivera et al., 1996; Tyler-Smith et al., 1999]. It is of interest that to date such "neodicentric" chromosomes have only been observed on Y chromosomes. The endogenous Y centromeres may be less stable and more easily inactivated than other centromeres, due to the relatively small arrays of diverged alpha-satellite DNA and lack of binding sites for the heterochromatin protein CENP-B [Tyler-Smith et al., 1999]. Furthermore, the large amounts of constitutive heterochromatin in Y chromosome long arms may predispose formation of neocentromeres. Thus, the Y chromosome may be uniquely suited to form these unusual chromosomes.

The analysis of rearranged Y chromosomes allowed a mitotic centromeric interval to be defined consisting of approximately 150 kb of the alphoid array and about 300 kb of Yp adjacent short arm sequences [Tyler-Smith et al., 1993]. Yeast Artificial Chromosomes (YACs) containing human Y alphoid DNA were introduced into hamster and human cells and re-formed several of the properties of a centromere [Larin et al., 1994]. This suggests that Y alphoid DNA contains the information required to specify some of the centromeric functions. However, human markers with neocentromeres that contain no detectable alpha-satellite DNA indicate that this DNA is not mandatory for centromere function [Choo, 1997; Warburton et al., 2000]. The detailed analysis of a human chromosome 10-derived neocentromere revealed that the neocentromere sequence is not similar to known centromeric sequences. It is possible that the overall composition and distribution patterns of various unknown functional elements, or any "ordinary" DNA under appropriate epigenetic influences, determine centromere formation and function [Barry et al., 1999; Lo et al., 2001].

Hsu [1994] reviewed seven nonmosaic cases of monocentric isochromosomes for the long arm of Y. As expected, these individuals were all phenotypic females with sexual infantilism since they lack the *SRY* gene. Streak gonads were reported in all cases in which this information was available, whereas Turner syndrome (TS) features and short stature were described in at least half of them. Therefore, the female phenotype, the normal height, and the TS features found in our patient are in accordance with her karyotype. It is not clear yet whether the slight delay in motor and speech development observed in childhood has any relation with the chromosomal rearrangement observed. Further characterization of patients with rearranged Y chromosomes might help to clarify these observations.

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