

# Prenatal diagnosis of a karyotypically normal pregnancy in a mother with a supernumerary neocentric 13q21→13q22 chromosome and balancing reciprocal deletion

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An adult female patient with a history of miscarriages was found to be carrying a stable supernumerary chromosome. The patient also carried a reciprocal paracentric deletion in chromosome 13q21/22. Microdissection and reverse fluorescence *in situ* hybridization FISH revealed that this supernumerary chromosome was derived from region 13q21→13q22. The presence of a neocentromere on this supernumerary chromosome was confirmed by the absence of detectable alpha satellite DNA using FISH and the presence of centromere proteins CENP-C and CENP-A using immunofluorescence. The absence of telomere sequences suggests that the marker is a ring chromosome (r(13)). FISH using ordered BACs from the chromosome region 13q21→13q31 permitted the precise positioning of the r(13) chromosome and the corresponding deletion to chromosome bands 13q21.32→13q22.2. BAC 280J7 from within the r(13) was used as a FISH probe for the prenatal analysis of amniocytes at 16 weeks of gestation, which revealed a normal karyotype for the fetus. This r(13) chromosome represents the first description of chromosome 13 of the rarer class of neocentric chromosomes that are derived from interstitial deletions. It represents the first example of prenatal diagnosis in a phenotypically normal female that was ascertained to carry a neocentric marker. The presence of such a neocentric marker/deletion karyotype in a parent presents unique possible karyotypic outcomes for conceptions and unusual challenges for genetic counseling. Copyright © 2003 John Wiley & Sons, Ltd.

KEY WORDS: amniocentesis; marker chromosome; neocentromere; interstitial deletion

## INTRODUCTION

Endogenous human centromeres are characterized by large amounts of tandemly repeated alpha satellite DNA and heterochromatin. A class of mitotically stable human derivative chromosomes has been described that does not contain alpha satellite DNA or endogenous centromere regions, yet does contain fully functional centromeres, called neocentromeres, that have formed on low- or single-copy DNA in a previously noncentromeric location (Choo, 2001). Neocentromeres suggest that human centromere formation is not strictly dependent on alpha satellite DNA, but instead represents an epigenetic process that may be dependent on the formation of a distinct chromatin structure (Sullivan *et al.*, 2001).

Neocentromeres provide mitotic stability to rearranged chromosome fragments that do not contain an endogenous centromere and would otherwise be acentric and rapidly lost. Over 50 cases of neocentric chromosomes have been reported thus far. The majority of these are supernumerary inverted duplications of the distal region of an autosome, which generally present as partial tri- or tetrasomy of the duplicated region (Li *et al.*, 2002; Warburton *et al.*, 2000). A rarer class of neocentric chromosomes consists of

a supernumerary chromosome fragment derived from an interstitial deletion, which thus far has been found in karyotypes balanced by a corresponding reciprocal-deletion chromosome. The morphology of the phenotype and the severity of the abnormalities caused by neocentric chromosomes are dependent on the position and degree of aneuploidy.

Here we report a novel case of a morphologically normal female who carries an r(13)(q21.31→neo→q22.2) neocentric supernumerary chromosome and reciprocal del(13)(pter→q21.32::q22.2→qter) chromosome. Molecular cytogenetic analysis confirmed the origin of the supernumerary chromosome and the presence of a neocentromere. Prenatal diagnosis on cultured amniocytes from this patient revealed a karyotypically normal fetus. This is the first instance of an adult carrier of a neocentric chromosome ascertained prior to a pregnancy, permitting directed prenatal chromosomal analysis. This case represents the first example of a neocentric chromosome 13 derived from an interstitial deletion, and will permit the detailed examination of a 13q21 neocentromere.

## MATERIALS AND METHODS

### Patient history and prenatal diagnosis

A 32-year-old healthy female (with normal intelligence) was investigated because of three spontaneous

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Figure 1—TG banded karyotype of patient. Her karyotype was determined to be 47,XX, del(13)(pter→q21.32::q22.2→qter),+mar(13)(q21.32→neo→q22.2)

miscarriages. At 22 weeks gestation (GIV) of her fourth pregnancy, trypsin Giemsa (TG) banding chromosome analysis of cultured lymphocytes from the female revealed a supernumerary chromosome and a deletion in chromosome 13q21. Both parents of the female had normal karyotypes. Amniocentesis was performed with GTG-banding, which revealed a normal female karyotype (46,XX). A factor-V Leiden mutation combined with a reduced protein-C activity was found and was treated with low-molecular-weight heparin and aspirin until 36 weeks gestation. At 40 weeks, unexpected fetal death was found. A girl with no apparent dysmorphic features was born, weighing 2950 g. Post-mortem examination was denied. At 16 weeks gestation of her fifth pregnancy, amniocentesis was performed for prenatal diagnosis.

### Molecular cytogenetics

Chromosomes were prepared from peripheral blood lymphocyte cultures following standard procedures. For microdissection, a routinely fixed cell suspension stored at  $-20^{\circ}\text{C}$  was used to prepare metaphase spreads on coverslips. Dissection of five copies of the supernumerary chromosome and subsequent amplification using a degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) was performed as described previously (Engelen *et al.*, 1998). Reverse painting with the DOP-PCR product was performed following a published protocol (Guan *et al.*, 1995) with minor modifications.

FISH was performed with the PNA probe 'pan-telomere (Cy3)/pan-centromere (FITC)' (Boston Probes)

Figure 2—Molecular cytogenetic analysis of mar(13)(q21.32→neo→q22.2) (A) Reverse FISH to normal control metaphase using the probe (green) derived from microdissected marker chromosome 13, which hybridizes to chromosome region 13q21→13q22. (B) FISH using all centromere probe (green) and all telomere probe (red), neither of which hybridize to the marker chromosome 13 (white arrow). (C) Immunofluorescence with antibodies to CENP-A (green). (D) Immunofluorescence with antibodies to CENP-C (green). Inset: marker chromosome. Antibodies to CENP-A (C) and CENP-C (D) identify kinetochores on all chromosomes including the marker chromosome (white arrow). Normal chromosomes 13 and the marker chromosome hybridized with chromosome 13-specific painting probe (red). Chromosomes are counter stained with DAPI (blue)

Figure 3—FISH analysis of marker chromosome and prenatal diagnosis. (A) 850 band ideograms of the normal chromosome 13, pericentric deletion of chromosome 13 and corresponding marker chromosome are shown (marker is shown as linear for clarity). The positions of the breakpoints are indicated. The positions of the three BACs used for the combination FISH probe are shown. (B) FISH with a combination BAC probe. FISH with BACs 173N7 and 468L10 (red), both of which hybridized to both the normal (white arrow head) and the deleted chromosome 13 (filled arrow head). FISH with BAC 280J7 (green), which hybridized to the marker (white arrow) and the normal chromosome 13 (white arrow head). (C) Cultured amniocytes from 16 weeks of gestation in the fifth pregnancy were subjected to FISH analysis using probe 280J7, which identified two normal chromosomes 13 (white arrow heads)

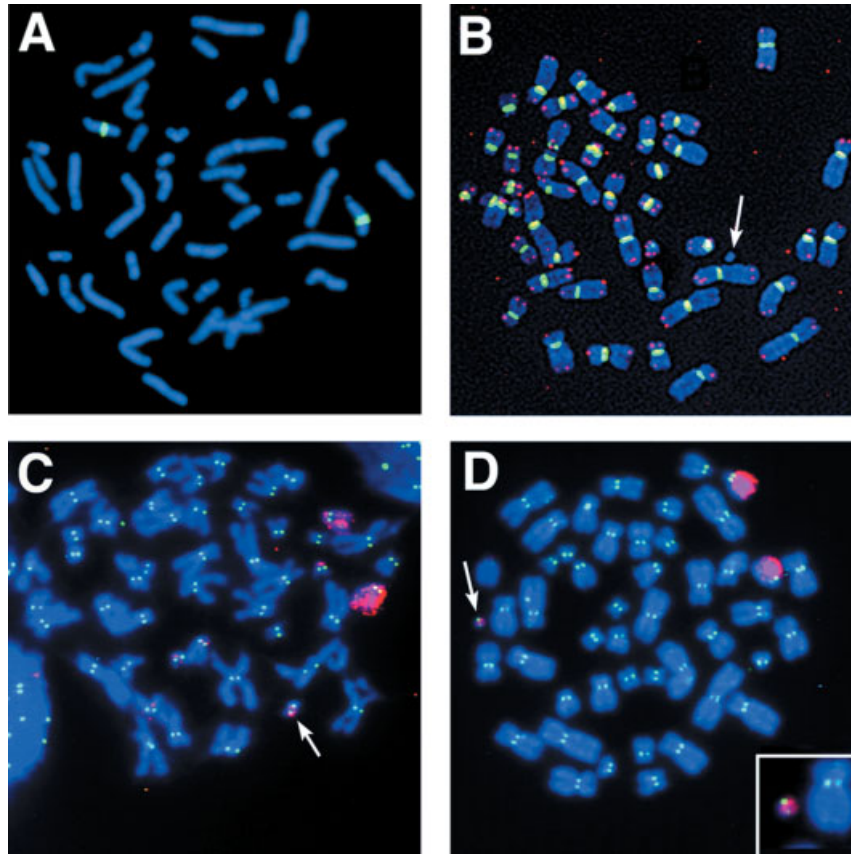


Figure 2

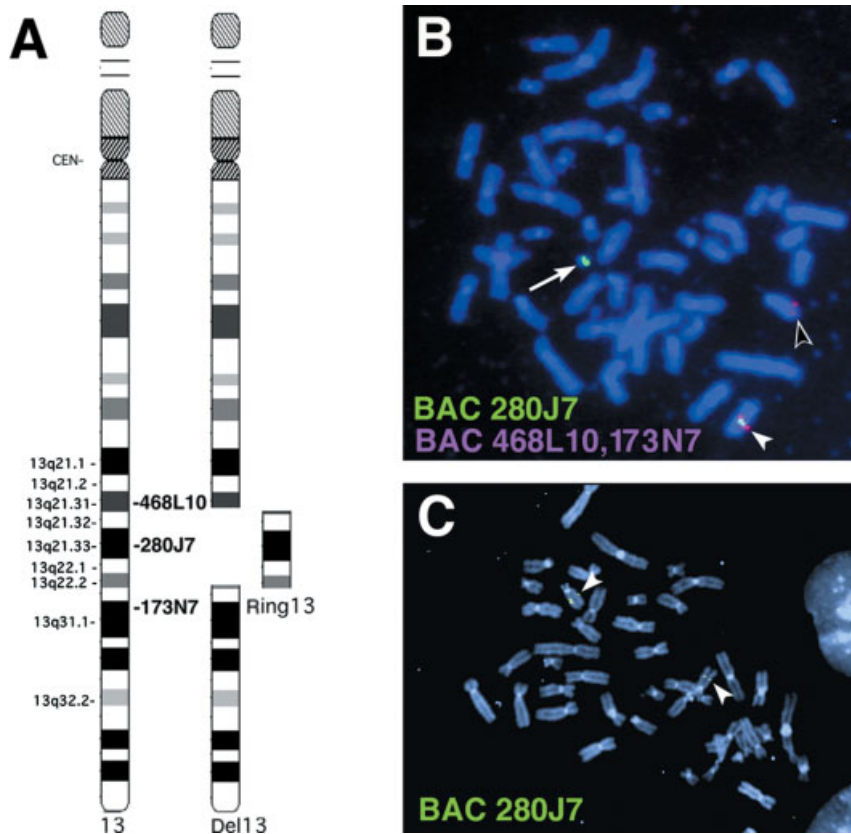


Figure 3

following manufacturers' protocols, a gift from Dr B. J. Eussen (University of Rotterdam). Polyclonal anti-CENP-C was a gift from William Earnshaw (University of Edinburgh). Monoclonal anti-CENP-A was a gift from Kinya Yoda (Nagoya University). WCP 13 was Alu PCR amplified from a somatic-cell hybrid containing only human chromosome 13, and was subsequently labeled with digoxigenin. Biotin-labeled BAC DNA was detected with streptavidin FITC and digoxigenin-labeled BAC DNA was detected with antidigoxigenin TRITC. Chromosomes were counterstained with DAPI (blue) or Propidium Iodide (red).

## RESULTS

Chromosome analysis of a phenotypically and mentally normal 32-year-old woman revealed the presence of a small supernumerary chromosome and a small deletion within one of the chromosomes 13 in 100% of her cells (Figure 1). Twelve of 50 metaphases examined showed an apparently double-sized supernumerary chromosome. Additional information regarding the origin and structure of this supernumerary chromosome and deletion was desired to aid in genetic counseling and future prenatal diagnosis. The constitution of the supernumerary chromosome was determined by microdissection and reverse FISH of DOP-PCR-amplified material, which hybridized to the chromosomes 13 (Figure 2A) from a normal control in the region 13q21→13q22.

This supernumerary chromosome did not appear to contain an endogenous centromeric region, yet was mitotically stable in 100% of the cells, suggesting the presence of a neocentromere. Therefore, FISH using a pan-centromere PNA probe (Boston probes) was performed, which hybridized to the centromere of all other chromosomes, yet proved negative on the supernumerary chromosome, suggesting that it did not contain detectable alpha satellite DNA (Figure 2B). The supernumerary chromosome was also negative for chromosome 13-specific alpha satellite DNA (data not shown). Furthermore, FISH using a pan-telomere PNA TTAGGG probe (Boston probes) that hybridizes to all normal telomeres was also negative on the supernumerary chromosome (Figure 2B). In order to confirm kinetochore formation at the neocentromere, centromere proteins CENP-A and CENP-C were detected on the supernumerary chromosome using immunofluorescence with specific antibodies (Figure 2C and 2D). Therefore, we conclude that the supernumerary chromosome is a neocentric ring chromosome derived from 13q21→13q22.

In order to determine more precisely the size and location of this neocentric ring chromosome 13 (r(13)), FISH analysis of cultured lymphocytes was performed using 15 ordered BACs from 13q21 to 13q31 spaced at 1- to 2-Mbp intervals. The assignment of BACs to chromosome 13 subbands was based on the current June 2002 freeze of the human genome sequence (<http://genome.cse.ucsc.edu>). The presence or absence of BAC FISH signals on the r(13) or deleted chromosome 13 permitted localization of the breakpoints.

This analysis showed that the r(13) chromosome and corresponding deletion encompass ~10 Mbp from distal 13q21.31 to proximal 13q22.2 (Figure 3A). FISH probes were chosen from these 15 ordered BACs that could distinguish between the normal, deleted and r(13) chromosomes in this case, for use in prenatal diagnosis. Figure 3B shows a metaphase of cultured lymphocytes from the patient hybridized with a combination FISH probe consisting of BAC 173N7 in 13q31.1, BAC 468L10 in 13q21.31, and BAC 280J7 in 13q21.33. All three of these probes hybridized to the normal chromosome 13. BACs 173N7 and 468L10 hybridized to the deleted 13 but not the r(13), while BAC 280J7 hybridized to the r(13) but not the deleted 13.

Use of both Q banding and FISH analysis with probe 280J7 (Figure 3C) on cultured amniocytes from 16 weeks of gestation in the fifth pregnancy of the patient revealed the presence of two normal chromosomes 13 and no r(13) chromosome.

## DISCUSSION

Here we report a phenotypically normal adult female who carries an r(13) chromosome derived from chromosome region 13q21.31→13q22.2 that is mitotically stable due to the presence of a neocentromere. She also carries a reciprocal deletion of chromosome 13, resulting in a balanced karyotype 47,XX,del(13)(pter→q21.32::q22.2→qter),+r(13)(q21.32→neo→q22.2). Prenatal diagnosis of cultured amniocytes at 16 weeks gestation using Q banding and FISH probes specific for the r(13) chromosome revealed a normal karyotype for this patient's conception. This case represents the first described example of a chromosome 13 paracentric deletion with a neocentromere, and the first case (for any chromosome) of the detection of a neocentric chromosome in an adult, which permitted directed prenatal screening for aneuploidy in the offspring.

The case reported here is an example of the rarer class of neocentric chromosomes that is derived from interstitial deletions. In such cases, both the centric fragment and the reciprocal acentric fragment are recovered because of the formation of a neocentromere. Pericentric deletions result in a centric fragment, and two fused distal acentric fragments that are stabilized by acquisition of a neocentromere (Maraschio *et al.*, 1996; Voullaire *et al.*, 1993; Wandall *et al.*, 1998). Paracentric deletions, such as the case described in this report, result in a centric fragment with a deletion, and stabilization of the acentric deleted fragment occurs by acquisition of a neocentromere, accompanied by ring formation in five of the six cases (Depinet *et al.*, 1997; Grimbacher *et al.*, 1999; Higgins *et al.*, 2000; Petit and Fryns, 1997; Slater *et al.*, 1999). The nine reported cases (including this report) of interstitial-deletion-derived chromosomes with neocentromeres have been found with a balanced karyotype, presumably owing to a bias for selection for recognized pregnancy or fetal survival. Six of these nine cases were observed *de novo* either prenatally or postnatally, and when examined were found to be associated with

mild dysmorphisms and/or developmental delay. The remaining three cases were observed in essentially phenotypically normal adults, an oligospermic male (Slater *et al.*, 1999), a 'borderline mentally retarded' male (Wandall *et al.*, 1998), and this case. The case reported by Wandall *et al.* (1998) involved a father who carried a neocentric chromosome del(3)(pter→p11::q11→qter) and a small centric fragment mar(3)(p11→q11). This case was ascertained after chromosomal analysis of a dysmorphic daughter who carried the large neocentric chromosome 3 but was missing the small centric marker. No examples of the more common distal inverted duplication neocentric chromosomes have been ascertained in normal parents, presumably because of a more severe phenotype of these tri- or tetrasomic karyotypes.

Supernumerary marker chromosomes occur *de novo* in about 1/2500 live births. The vast majority of these represents small centric heterochromatic fragments with little or no euchromatic coding sequences, and has an overall associated *de novo* risk of about 13% of birth defects/mental retardation (Warburton, 1991). However, supernumerary neocentric marker chromosomes could in theory have arisen from any part of the genome, and therefore may have a significant amount of euchromatic expressed sequences and a higher associated risk of birth defects (Levy *et al.*, 2000). The observed neocentric marker chromosomes derived from interstitial deletions are all found with a reciprocal-deletion chromosome, leading to a balanced karyotype. In contrast, centric marker chromosomes are not observed in cells with reciprocal centric deletions, which would lead to mitotic instability. When a cell that contains a centric marker chromosome undergoes meiosis, there is usually a 50% chance of transmission of the marker to the gamete. However, in balanced neocentric marker cases such as the one described here, several possible outcomes of meiosis can occur involving the neocentric marker and the deleted chromosome. The deleted and normal chromosome 13 will form a meiotic pair and disjoin at 50%. The neocentric chromosome will most likely randomly segregate at a 50% ratio independent of the two chromosomes 13. Thus, four karyotypic results of a prenatal chromosome analysis of this case were equally likely:

- (1) normal maternal and paternal chromosomes 13;
- (2) one normal paternal 13, and the maternal deleted 13 and r(13) (balanced);
- (3) one normal paternal 13 and one maternal deleted 13, (monosomy 13q21.32–13q22.2); or
- (4) two normal chromosomes 13, maternal and paternal, and the r(13) (trisomy 13q21.32–13q22.2).

Result 1 would be counseled as no risk, result 2 as minimal risk (as the mother has the same karyotype), while results 3 and 4 would be counseled as aneuploid and high risk. The risk associated with result 3 might be complicated by reports that monosomy for 13q21 is compatible with a normal phenotype (Couturier *et al.*, 1985). The fetus presented with a normal karyotype (result 1), and the patient was counseled appropriately.

In conclusion, the r(13)q21→22 chromosome reported here represents the first example of a neocentric chromosome resulting from an interstitial deletion of chromosome 13, confirmed by the absence of detectable alpha satellite DNA and the presence of kinetochore proteins CENP-A and -C (Figure 2). Eleven other cases of chromosome 13q neocentromeres have been described on symmetrical inverted duplication chromosomes, 3 in 13q21 (this case is the 4th example), 1 in 13q31, and 7 in 13q32 (Li *et al.*, 2002; Warburton *et al.*, 2000). The high-resolution localization and sequence identification of multiple independent examples of these common neocentromere-forming regions on chromosome 13 will provide information about the sequence and/or epigenetic requirements for human centromere formation. The lack of inverted duplicated material on the marker chromosome described here will make it highly valuable for further molecular analysis. Although the BAC mapping of this chromosome revealed it to be ~10 Mbp in size, this nevertheless provides a valuable starting point for the identification of the 13q21 neocentromere sequences.

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