Transmission of a Fully Functional Human Neocentromere through Three Generations

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Summary

An unusual Y chromosome with a primary constriction inside the long-arm heterochromatin was found in the amniocytes of a 38-year-old woman. The same Y chromosome was found in her husband and brother-in-law, thus proving that it was already present in the father. FISH with alphoid DNA showed hybridization signals at the usual position of the Y centromere but not at the primary constriction. Centromere proteins (CENP)-A, CENP-C, and CENP-E could not be detected at the site of the canonic centromere but were present at the new constriction, whereas CENP-B was not detected on this Y chromosome. Experiments with 82 Y-specific loci distributed throughout the chromosome confirmed that no gross deletion or rearrangement had taken place, and that the Y chromosome belonged to a haplogroup whose members have a mean alphoid array of 770 kb (range 430–1,600 kb), whereas that of this case was ~250 kb. Thus, this Y chromosome appeared to be deleted for part of the alphoid DNA. It seems likely that this deletion was responsible for the silencing of the normal centromere and that the activation of the neocentromere prevented the loss of this chromosome. Alternatively, neocentromere activation could have occurred first and stimulated inactivation of the normal centromere by partial deletion. Whatever the mechanism, the presence of this chromosome in three generations demonstrates that it functions sufficiently well in mitosis for male sex determination and fertility and that neocentromeres can be transmitted normally at meiosis.

Received December 1, 1998; accepted for publication February 25, 1999; electronically published March 26, 1999.

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0002-9297/99/6405-0025$02.00

Introduction

Under normal circumstances a chromosome must have one—and only one—centromere, in order to segregate properly. In the yeast *Saccharomyces cerevisiae*, short DNA sequences are sufficient to specify centromere function; if centromere DNA is present, the chromosome has a centromere, and if it is absent, the chromosome does not. In multicellular eukaryotes, including humans, no single DNA sequence has been shown to be essential for formation of a centromere, and it is thought that centromere activity is specified epigenetically (Karpen and Allshire 1997), perhaps by the binding of centromere protein (CENP)-A (Vafa and Sullivan 1997; Warburton et al. 1997). This hypothesis arose, in part, from studies of human neocentromeres, in which it was observed that, in rare patients with chromosomal rearrangements, sequences that are normally noncentromeric acquire centromeric function (duSart et al. 1997), and the hypothesis has been extended to *Drosophila* (Williams et al. 1998). Neocentromere segregation efficiency has not been measured in humans, but the mosaicism often seen in patients with neocentromeres suggests that it is lower than that of normal centromeres in mitosis, and it is not known whether neocentromeres function at all in human meiosis. We now describe a normal human family with a neocentromere on the Y chromosome. The presence of this chromosome in three generations demonstrates that it functions sufficiently well in mitosis for male sex determination and fertility, that it can be transmitted normally at meiosis, and that centromeric DNA evolution can be very rapid.

Methods

Chromosome analyses from amniocytes of the proposita and from lymphocytes of her husband (1106/93) and her brother-in-law were done as usual. A lymphoblastoid cell line was set up from the blood of 1106/93. FISH was performed as described elsewhere (Rossi et al.

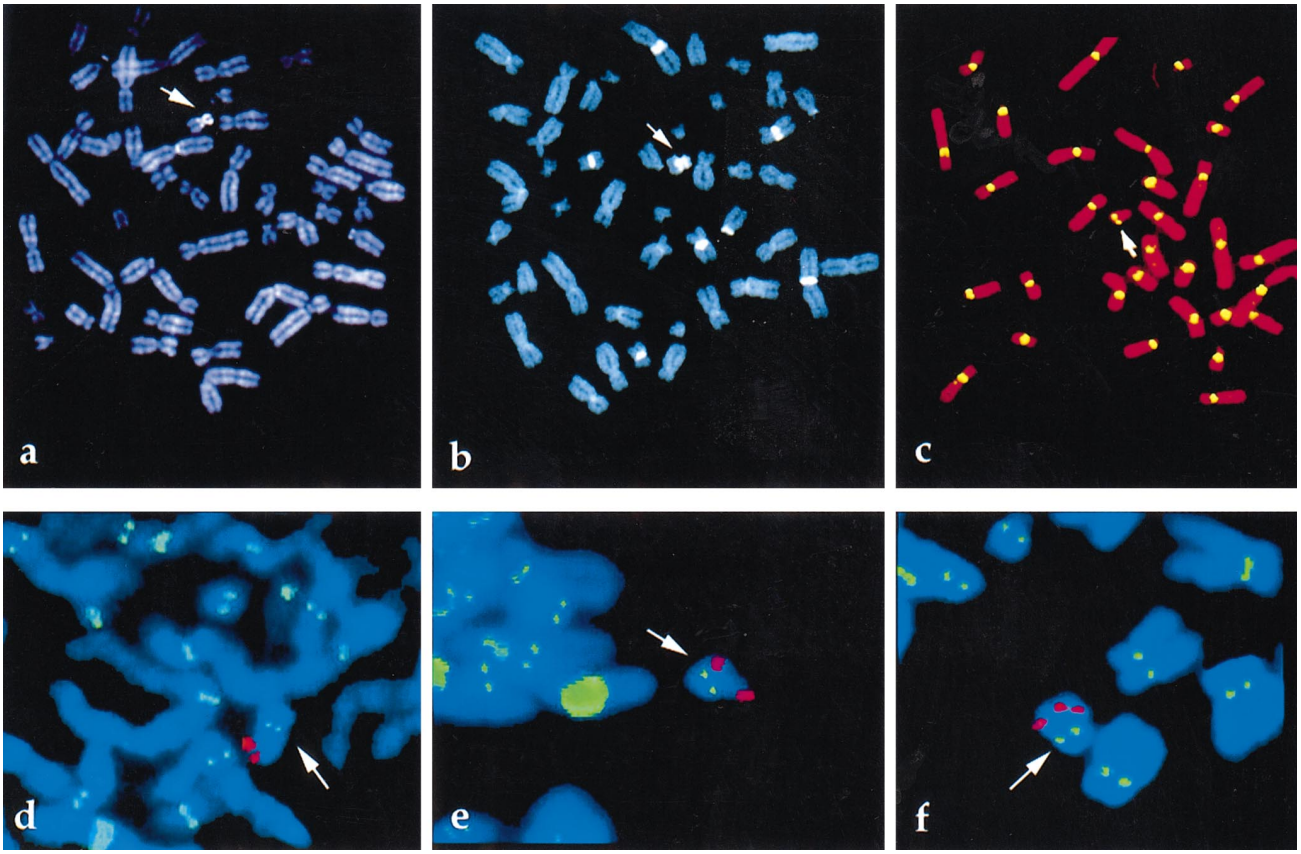

Figure 1 Metaphases from the proposita's amniocytes stained for Q-bands (*a*) and from the father's blood stained for DA-DAPI (*b*), showing the abnormal Y chromosome with the primary constriction in the Yq12 heterochromatin. FISH with D8Z2 *c* shows signals only at the inactive centromere of the paternal abnormal Y chromosome. After immunofluorescence with CENP-A (*d*), CENP-C (*e*), and CENP-E (*f*), signals (*green*) are localized at the neocentromere but not at the inactive centromere identified by the Y-alphoid DNA signals (*red*).

1994). Two alphoid probes, D8Z2 and DYZ3, were labeled by nick translation. D8Z2 was used under low-stringency conditions (hybridization mixture—30% formamide in 2 × SSC; posthybridization washes—50% formamide, 2 × SSC [37°C]). DYZ3 was used at high stringency (hybridization mixture: 50% formamide in 2 × SSC; posthybridization washes: 50% formamide, 2 × SSC [42°C]) and was detected with fluorescein isothiocyanate (FITC)–conjugated avidin. Chromosomes were stained with propidium iodide and counterstained with DAPI. No less than 50 metaphases were analyzed.

Antibodies to CENP-A (Warburton et al. 1997), CENP-B (Earnshaw et al. 1987), CENP-C (Saitoh et al. 1992), and CENP-E (Yen et al. 1992) were generated as described elsewhere. Slides with metaphase chromosomes from 1106/93 were prepared according to the method described by Haaf and Schmid (1989), with minor modifications. In brief, the cells were suspended in hypotonic solution (10 mM HEPES, pH 7.3), 2% FCS, 30 mM glycerol, 1.0 mM $CaCl_2$, and 0.8 mM $MgCl_2$, at a density of ~ 8×10^4 cells/ml, and incubated at +4°C for 15 min. Aliquots of 0.3–0.5 ml were cytocentrifuged (Cytospin 3; Shandon) onto clean glass slides at 1,000 *g* for 10 min with high acceleration. The slides were immediately removed and immersed in absolute

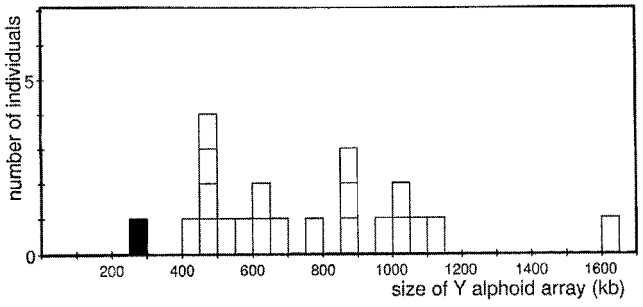

Figure 2 Distribution of Y alphoid DNA array sizes in haplogroup 9 individuals. 1106\93R is denoted by the blackened bar.

methanol for 30 min at −20°C and then in ice-cold acetone for 30 s. After air drying, the slides were used immediately or stored at −20°C. For the immunofluorescence staining, slides were treated essentially according to the method reported by Earnshaw et al. (1989). They were rehydrated in PBS-azide (10 mM $NaPO_4$ pH 7.4, 0.15 M NaCl, 1 mM EGTA (ethylene glycol-bis-[β-aminoethyl ether]-N_1,N,N^1,N^1-tetraacetic acid), 0.01% NaN_3) for 5 min, then washed three times (1 min each) with 1 × TEEN (1.0 mM triethanolamine: HCl pH 8.5, 0.2 mM Na EDTA, 25 mM NaCl), 0.1% Triton X-100, 0.1% BSA. The preparations were incubated for 30 min at 37°C with the specific antibody appropriately diluted in 1 × TEEN, 0.1% Triton X-100, 0.1% BSA. Antibodies were removed three washings with 1 × KB (10 mM Tris:HCl pH 7.7, 0.15 M NaCl, 0.1% BSA) for 2 min, 5 min, and 3 min and were detected by being incubated for 30 min at 37°C with FITC-conjugated antirabbit IgG (Sigma) diluted 1:20 in 1 × KB. Slides were washed for 2 min with 1 × KB. After the detection of CENP antibodies, we performed FISH with CEP-Y (Vysis), labeled with Spectrum orange, to obtain signals for both alphoid DNA and CENPs on the same preparation. Slides were fixed in a 10% formalin in 1 × KB solution for 10 min at room temperature, washed in distilled water for 10 min, fixed in 3:1 methanol/acetic acid for 15 min, air dried, and processed for FISH with Spectrum orange–labeled CEP-Y (DYZ1), as described by the Vysis protocol, slides being denaturated at 82°C for 8 min. For both FISH and immunofluorescence analyses of the centromeric proteins, slides were viewed through a Zeiss Axiophot fluorescence microscope. Metaphases were analyzed by means of DAPI, FITC, and triple-bandpass filter to visualize fluorescein, Spectrum orange, DAPI, and propidium iodide. Images were captured with a CCD camera (Perceptive Scientific Instruments) and were further processed with Adobe Photoshop software. Molecular methods were as described elsewhere (Tyler-Smith et al. 1993).

Results

Cytogenetic examination of amniocytes obtained from a 38-year-old woman revealed an unusual Y chromosome in all the cells (30 metaphases from 15 clones). The normal constriction was absent, and a novel constriction was seen within the long-arm heterochromatin (fig. 1*a*). Examination of the husband's chromosomes showed that this unusual chromosome was also present in 94 of 100 cells (fig. 1*b*), the remaining six metaphases being 45,X. No cytogenetic analysis could be performed on his first male child, but his older brother (56 years old) had the same Y chromosome in most cells, together with a minor 45,X cell line (16 of 110 metaphases). All further analyses were performed on the father's lymphoblastoid cell line (1106/93). FISH with alphoid DNA showed hybridization signals at the usual position but not at the new constriction (fig. 1*c*) of the Y chromosome. CENP-A, CENP-C, and CENP-E could not be detected at the normal centromere but were present at the new constriction (fig. 1*d–f*), whereas, as expected, CENP-B was not detected at all on this Y chromosome (results not shown). We therefore conclude that the normal centromere has been inactivated and a neocentromere has formed in the long-arm heterochromatin.

Initial PCR and filter hybridization experiments investigating 82 Y-specific loci distributed throughout the chromosome confirmed that no detectable deletion or rearrangement had taken place. The structure of the canonic centromere, as investigated by means of pulsed-field gel electrophoresis (Tyler-Smith et al. 1993), revealed that it contained an alphoid array ~250 kb in size, within the normal range of 220–1,600 kb (Mathias et al. 1994). However, more-detailed analysis showed that the Y chromosome belonged to the lineage "haplogroup 9," defined by the combination of two polymorphisms: an A→G transition at SRY-1532 (Kwok 1996) and a 2-kb deletion at the 12f2 (DYS11) locus (Casanova et al. 1985). Members of this lineage have all descended from a recent common ancestor who probably had an alphoid array similar in size to the mean for the haplogroup, 770 kb (range 430–1,600 kb, n = 41; fig. 2). The Y alphoid array in 1106/93 has therefore undergone a substantial deletion relative to others in this haplogroup.

Discussion

This neocentromere differs from those described elsewhere (Choo 1997), in that it lies on the same chromosome as does the normal centromere and is not associated with a cytologically detectable rearrangement. A similar Y chromosome, with a neocentromere inside the Yq heterochromatin, was reported by Bukvic et al. (1996) in an infertile woman. In that case, however, the abnormal Y chromosome was present as a supernumerary one in a minor cell line (5%), in mosaicism with both a normal 46,XY (40%) and a 45,X (55%) cell line. Thus the abnormal Y probably arose from a postzygotic event and was unstable.

What is the molecular basis for the inactivation of the normal centromere and for the activation of the stable neocentromere in the family studied? According to Warburton et al. (1997), the active centromere occupies only a portion of the alphoid array, as indicated by the small region of CENP-A staining, compared with CENP-B staining on other chromosomes. Thus, by using the in-

formation that the Y chromosome in this family is structurally normal except for a partial deletion of the alphoid array, we propose the following model. The deletion of part of the alphoid array, still leaving an array well within the size range observed for functional centromeres, removes part or all of the CENP-A–binding domain, thus leading to inactivation of the centromere. Activation of a neocentromere by a poorly understood mechanism, perhaps the binding of CENP-A to late-replicating DNA (Csink and Henikoff 1998), might stabilize the chromosome and prevent loss. CENP-A has been reported to be retained quantitatively in bull sperm (Palmer et al. 1990), so the modification might be transmitted to future generations. Alternatively, neocentromere activation could have occurred first and stimulated inactivation of the normal centromere by partial deletion, as seen in a dicentric Y;21 chromosome (Fisher et al. 1997).

Whatever the mechanism, the Y centromere in this family illustrates that one centromere sequence can replace another very rapidly. We do not know how many generations ago the centromere shift took place, but it must have been more than two, as demonstrated by the presence of the same Y chromosome in two brothers. Thus, the epigenetic change has been stable through at least three rounds of meiosis. Because it behaves as a neutral polymorphism (the minor 45,X cell line did not prevent normal fertility, and no abortions were reported in the entire family), it is possible (although unlikely) that in the future it could become fixed in the population. Perhaps this is how the centromeric DNA sequences of multicellular eukaryotes evolve so rapidly (Tyler-Smith et al. 1998). The finding that a similar Y chromosome has been reported (Bukvic et al. 1996) provides the first example of two independent neocentromeres arising in the same cytogenetic location and suggests that sequences along the Yq heterochromatin may be particularly likely to bind the centromeric proteins.

Acknowledgments

C.T.-S. was supported by the CRC, A.P. was supported by the Biotechnology and Biological Sciences Research Council, and O.Z. was supported by cofin98-MURST (Ministero Università Ricerca Scientifica Tecnologica). The financial support of Telethon to project C-18 cell bank and of Istituto di Ricerca e Cura a Carattere Scientifico Policlinico San Matteo, Pavia, is acknowledged.

References

Bukvic N, Susca F, Gentile M, Tangari E, Ianniruberto A, Guanti G (1996) An unusual dicentric Y chromosome with functional centromere with no detectable alpha satellite. Hum Genet 97:453–456

Casanova M, Leroy P, Boucekkine C, Weissenbach J, Bishop C, Fellous M, Purrello M, et al (1985) A human Y-linked DNA polymorphism and its potential for estimating genetic and evolutionary distance. Science 230:1403–1406

Choo KHA (1997) Centromere DNA dynamics: latent centromeres and neocentromere formation. Am J Hum Genet 61: 1225–1233

Csink AK, Henikoff S (1998) Something from nothing: the evolution and utility of satellite repeats. Trends Genet 14: 200–204

du Sart D, Cancilla MR, Earle E, Mao JI, Saffery R, Tainton KM, Kalitsis P, et al (1997) A functional neo-centromere formed through activation of a latent human centromere and consisting of non-alpha-satellite DNA. Nat Genet 16: 144–153

Earnshaw WC, Ratrie H, Stetten G (1989) Visualization of centromere proteins CENP-B and CENP-C on a stable dicentric chromosome in cytological spreads. Chromosoma 98:1–12

Earnshaw WC, Sullivan KF, Machlin PS, Cooke CA, Kaiser DA, Pollard TD, Rothfield NF, et al (1987) Molecular cloning of cDNA for CENP-B, the major human centromere autoantigen. J Cell Biol 104:817–829

Fisher AM, Al-Gazali L, Pramathan T, Quaife R, Cockwell AE, Barber JC, Earnshaw WC, et al (1997) Centromeric inactivation in a dicentric human Y;21 translocation chromosome. Chromosoma 106:188–206

Haaf T, Schmid M (1989) Centromeric association and nonrandom distribution of centromeres in human tumour cells. Hum Genet 81:137–143

Karpen GH, Allshire RC (1997) The case for epigenetic effects on centromere identity and function. Trends Genet 13: 489–496

Kwok C (1996) Mutation analysis of the 2 kb 5′ to SRY in XY females and XY intersex subjects. J Med Genet 33: 465–468

Mathias N, Bayes M, Tyler-Smith C (1994) Highly informative compound haplotypes for the human Y chromosome. Hum Mol Genet 3:115–123

Palmer DK, O'Day K, Margolis RL (1990) The centromere specific histone CENP-A is selectively retained in discrete foci in mammalian sperm nuclei. Chromosoma 100:32–36

Rossi E, Faiella A, Zeviani M, Labeit S, Floridia G, Brunelli S, Cammarata M, et al (1994) Order of six loci at 2q24-q31 and orientation of the HOX locus. Genomics 24:34–40

Saitoh H, Tomkiel J, Cooke CA, Ratrie HR III, Maurer M, Rothfield NF, Earnshaw WC (1992) CENP-C, an autoantigen in scleroderma, is a component of the human inner kinetochore plate. Cell 70:115–125

Tyler-Smith C, Corish P, Burns E (1998) Neocentromeres, the Y chromosome and centromere evolution. Chromosome Res 6:65–67

Tyler-Smith C, Oakey RJ, Larin Z, Fisher RB, Crocker M, Affara NA, Ferguson-Smith MA, et al (1993) Localization of DNA sequences required for human centromere function through an analysis of rearranged Y chromosomes. Nat Genet 5:368–375

Vafa O, Sullivan KF (1997) Chromatin containing CENP-A

and a-satellite DNA is a major component of the inner kinetochore plate. Curr Biol 7:897–900

Warburton PE, Cooke CA, Bourassa S, Vafa O, Sullivan BA, Stetten G, Gimelli G, et al (1997) Immunolocalization of CENP-A suggests a distinct nucleosome structure at the inner kinetochore plate of active centromeres. Curr Biol 7: 901–904

Williams BC, Murphy TD, Goldberg ML, Karpen GH (1998) Neocentromere activity of structurally acentric mini-chromosomes in Drosophila. Nat Genet 18:30–37

Yen TJ, Li G, Schaar B, Szilak I, Cleveland DW (1992) CENP-E is a putative kinetochore motor that accumulates just prior to mitosis. Nature 359:536–539