Letters to the Editor

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Sperm Chromosome Analysis in a Man Heterozygous for a Paracentric Inversion of Chromosome 14 (q24.1q32.1)

To the Editor:

Paracentric inversions are rarely reported, since they can only be detected by the use of banding procedures (Pettenati et al. 1995). The incidence is estimated to be .09–.49/1,000 (van Dyke et al. 1983; Ferguson-Smith and Yates 1984; Hook et al. 1984; Fryns et al. 1988). Some investigators have suggested that paracentric inversions in man are generally harmless (Madan 1995); however, recombinant chromosomes have been observed in 17 cases, and the risk of viable recombinants has been estimated to be 3.8% (Pettenati et al. 1995). Because the frequency of spontaneous abortions may be increased in carriers of paracentric inversions (Mules and Stamberg 1984), chromosomally unbalanced conceptions may be lost early in pregnancy. A direct analysis of chromosomes in gametes would overcome this potential loss of information. In 1986, I reported the first analysis of sperm chromosomes in a man heterozygous for a paracentric inversion of chromosome 7 (Martin 1986). The present report represents the second study of sperm karyotypes in a paracentric-inversion carrier.

A paracentric inversion of chromosome 14 (q24.1q32.1) was ascertained at amniocentesis performed because of advanced maternal age. Subsequent investigation of the family revealed that the inversion is present in the father. The couple had previously had two normal children and one spontaneous abortion at 11 wk gestation (chromosomes were not studied). The study was approved by the university ethics committee, and the sperm donor provided informed consent. The sperm donor had a normal sperm profile, with a volume of 3 ml, concentration of 138 × 10^6/ml, 80% motility, and forward progression of 8/10. A partial karyotype of the normal and inverted chromosomes 14 in the 41-year-old father is presented in figure 1. Sperm chromosome complements were obtained by fusion of golden hamster oocytes with human sperm and analysis of the Q-banded pronuclear chromosomes. This technique has been described in detail elsewhere (Martin et al. 1994*b*).

A total of 120 sperm chromosome complements were obtained. The results are summarized in the Appendix. Fifty complements (41.7%) contained a normal chromosome 14, whereas 70 (58.3%) had the inverted chromosome 14. This segregation was not significantly different from the expected 1:1 ratio ($\chi^2 = 3.3$, $P > .05$). An example of a sperm chromosome complement with an inverted chromosome 14 is shown in figure 2. The number of X- and Y-bearing sperm was 55 and 65, respectively, which was not significantly different from the expected (60 X- and 60 Y-bearing) number.

None of the spreads contained a recombinant chromosome 14. There were no dicentric, acentric, or duplicated/deficient chromosomes, as would be expected if a crossover had occurred within the inverted segment. Abnormal sperm chromosome complements were present in 10% of the spreads. Three complements had a numeric abnormality, six had a structural abnormality, and three had both. Details of these abnormalities are provided in the Appendix. This frequency of abnormalities is similar to my results from 84 chromosomally normal control donors, who had a mean frequency of 12.8% abnormal sperm (Martin 1995).

In a paracentric-inversion heterozygote, pairing of homologues during meiosis is maximized by the formation of an inversion loop. If an unequal number of crossovers occur within this loop, dicentric and acentric chromosomes are formed. The acentric chromosomes are generally lost in subsequent cell divisions. Dicentric chromosomes tend to break, since the two centromeres are pulled to opposite poles of the cell. Thus, the resulting gametes can have a variety of duplications or deficiencies.

Pettanati et al. (1995) have reviewed 446 cases of paracentric inversions, with 17 cases of recombinant chromosomes reported. The majority of the cases were monocentric chromosomes with duplications or deficiencies. Two maternally inherited cases had offspring with dicentric chromosomes: one case was an inversion of chromosome 9 (q22.1q34.3) (Worsham et al. 1989), and the other was an inversion of chromosome 14 (q24.2q32.3), with breakpoints very similar to those seen in the present case (Mules and Stamberg 1984).

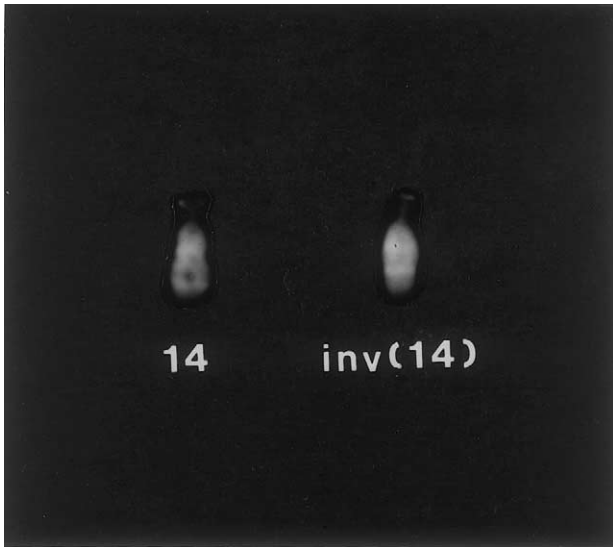

Figure 1 Q-banded human sperm chromosomes demonstrating normal and inverted chromosomes 14.

The paracentric inversion of chromosome 14 (q24q32) appears to be relatively common in humans (Pettenati et al. 1995), with six index cases reported, including this report. Two of the six cases were paternally inherited. The only case with recombinant chromosomes reported for this inversion is the maternally inherited dicentric case cited above. Because this inversion is ~30% of the length of long arm of chromosome 14, and because chromosome 14 has an average of 1.8 chiasmata (Chandley 1975), one would expect that a crossover within the inverted segment would occur ~50% of the time. Because one half of the chromatids are involved in the crossover, ~25% of the gametes should be chromosomally unbalanced. However, my results from sperm chromosome analysis did not detect a single recombinant chromosome in 120 spermatozoa. The lack of any recombinant chromosomes in sperm suggests either that the chromosomes 14 did not pair by an inversion loop or that crossing-over was suppressed within the loop.

Very few meiotic studies of inversion carriers have shed light on these possibilities. The only meiotic analysis of a paracentric inversion was performed in mice, by Poorman et al. (1981). They found that 100% of spermatocytes at early pachytene contained a fully synapsed loop. There have been no cytogenetic meiotic studies of paracentric inversions in humans, and the studies of pericentric inversions have produced conflicting results: some have shown homosynapsis whereas others have demonstrated heterosynapsis and asynapsis (Gabriel-Robez and Rumpler 1994). A recent study reported the analysis of meiotic recombination, by sperm typing in a man carrying a 9q32q34 inversion (Brown et al. 1998). The authors concluded that there was a reduced frequency of recombination within the inversion, suggesting that an inversion loop had not been formed.

Sperm karyotyping has been performed on a total of nine inversion heterozygotes: two with paracentric inversions (Martin 1986; present study) and seven with pericentric inversions. The other paracentric-inversion carrier (7q11q22) also did not have any recombinant sperm, despite expectation that the estimated frequency of recombinant sperm would be 25% (Martin 1986). Of the seven pericentric-inversion carriers, four had no recombinant sperm (Balkan et al. 1983; Jenderny et al. 1992; Martin et al. 1994*a;* Colls et al. 1997), whereas three demonstrated frequencies of 11%, 18%, and 31% recombinant sperm (Martin 1991, 1993; Navarro et al. 1993). The inversions that produced recombinant chromosomes in sperm were all large inversions encompassing more than half the chromosome length. The inversions that did not produce recombinant chromosomes were, in general, smaller, being less than one-third the length of the chromosome. The exception to this was

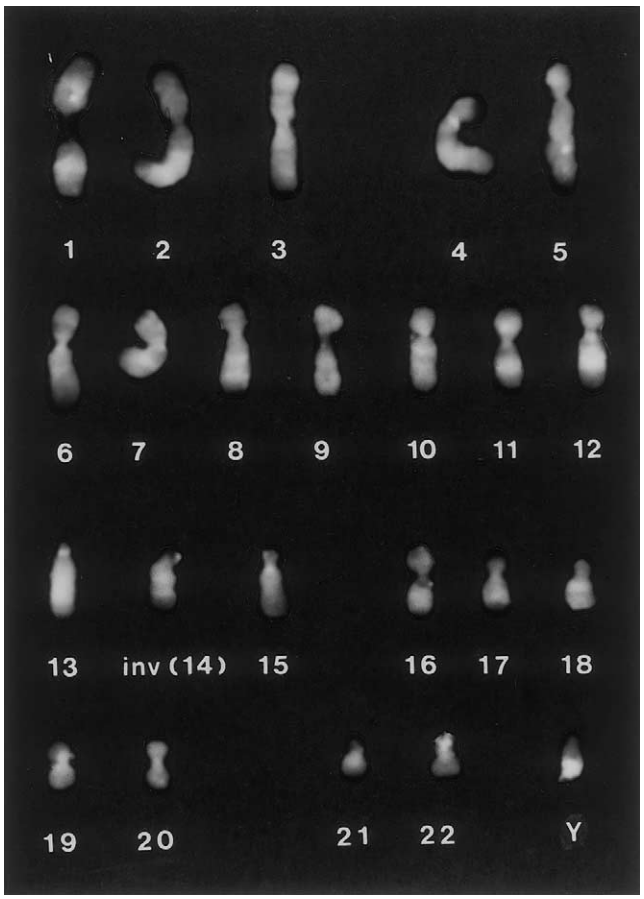

Figure 2 Q-banded karyotype of human sperm chromosomes, 23,Y,inv(14)(q24q32).

the pericentric inversion of chromosome 20 studied by Jenderny et al. (1992), which failed to produce recombinant sperm despite a relatively large size. However, this may simply be a matter of sample size, because only 26 sperm were analyzed. This dependence on size of the inversion to produce recombinant chromosomes is reminiscent of empirical studies that have suggested that, for a pericentric-inversion heterozygote to produce live-born children with a recombinant chromosome, the inverted segment must involve one-third of the chromosome length (Trunca and Opitz 1977). It is possible that, in small inversions, crossing-over is suppressed within the loop or that pairing is accomplished by heterosynapsis.

Ashley (1988) has formulated a hypothesis that predicts that, if G-light bands are aligned with G-light bands, lack of homology will be recognized and an inversion loop will be formed; in contrast, if the arrangement aligned two G-dark bands or a G-light band with a G-dark band, then lack of homology would not be recognized and heterosynapsis would proceed. de Perdigo et al. (1989) and Gabriel-Robez and Rumpler (1994) have reviewed synaptic data and localization of chromosomal breakpoints in human pericentric inversions and have determined that Ashley's hypothesis is consistent with the data: loops are formed when both breaks occur in G-light bands, whereas heterosynapsis without loop formation or asynapsis occurs when one of the breaks is in a G-dark band. The results of sperm chromosome–complement analysis of pericentric-inversion carriers are also generally consistent with this hypothesis. All three inversions with recombinant chromosomes in sperm had breakpoints in G-light areas (allowing homosynapsis, loop formation, chiasmata, and recombinant chromosomes). Of the four pericentric inversions with no recombinant chromosomes in sperm, all except the paracentric inversion of chromosome 20 (p13q11.2) studied by Jenderney et al. (1992) had at least one breakpoint in a G-dark region. However, as discussed above, 26 sperm complements may have been an insufficient sample size for detection of recombinant chromosomes. Of the two paracentric inversions studied by sperm karyotyping, both had breakpoints in G-light areas, yet neither had recombinant chromosomes in sperm. Thus, data from sperm karyotyping of the two paracentric inversions studied to date do not agree with Ashley's hypothesis. It may be that, for paracentric inversions, small size is a major detriment to homologous pairing and crossing-over.

The possibility of an interchromosomal effect for inversion heterozygotes has been raised, because children with unrelated chromosomal abnormalities have been born to inversion carriers. Canki and Dutrillaux (1979) described two cases of familial paracentric inversions associated with sex-chromosomal aneuploidy, and Fryns and van den Berghe (1980) reported a child with trisomy 21 who was born to a father heterozygous for a paracentric inversion. However, my data do not support such an effect, because the frequency of chromosomal abnormalities was not increased in either the case that was heterozygous for a paracentric inversion of chromosome 14 or the case that was heterozygous for a paracentric inversion of chromosome 7 (Martin 1986). Similarly, previous studies of sperm chromosomes in men with pericentric inversions have not demonstrated any increased frequency of abnormalities in other chromosomes (Balkan et al. 1983; Martin 1991, 1993; Navarro et al. 1993; Colls et al. 1997). Colls et al. (1997) specifically searched for an interchromosomal effect involving chromosome 21, by performing FISH analysis of >10,000 sperm nuclei from a case that was heterozygous for a paracentric inversion of chromosome 9, as well as by analyzing >300 sperm complements. Even these large sample sizes did not uncover an interchromosomal effect.

Further studies of sperm chromosomes in paracentric-inversion carriers are required, because only two have been reported. Information from these studies will provide estimates of the frequency of chromosomally unbalanced gametes. These studies will also help us to elucidate some of the factors that influence the production of recombinant chromosomes at meiosis.

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Appendix

Sperm Chromosome Complements in a Male Heterozygous for a Paracentric Inversion of Chromosome 14 (q24q32)

Normal sperm (n = 108):
  Normal chromosome 14, n = 44
  Inverted chromosome 14, n = 64
Abnormal sperm (n = 12):
  Normal chromosome 14, n = 6

Inverted chromosome 14, $n = 6$
Details of the 12 abnormal sperm complements:
Numerical:
22,Y,−6
22,X,−13
22,X,−18
Structural:
23,X,chrb(12)(q14or15)
23,X,chte(19;20)(q13;1or13.2;q13.3)(tr, incomplete)
23,X,chrb(12)(q12or13),[−14,+inv(14)]
21,Y,chte(1;3;17)(cx),[14,+inv(14)]
22,X,dic(7;18)(pter→cen::p11.3→qter), [−14,+inv(14)]
—,Y,MB+R,[−14,+inv(14)]
Both numerical and structural:
24,XiY,+ace
21,X,−22,dic(5;7)(p15;q35or36)+ace, [−14,+inv(14)]
22,Y,−17,chrg(7)(q21,chtg(9)(p21), [−14,+inv(14)]

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This letter is dedicated to R. Brian Lowry, clinic geneticist, on the occasion of his retirement.

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