Ectopic NORs on human chromosomes 4qter and 8q11: rare chromosomal variants detected in two families

M Guttenbach, T Haaf, C Steinlein, J Caesar, A Schinzel, M Schmid

Abstract

Two different NOR bearing non-acrocentric chromosomes were detected during prenatal diagnosis performed on two probands because of advanced maternal age. In the first case, a chromosome 4 carried a NOR in the telomeric region of the long arm (4qs), while in the second case a NOR was inserted into chromosome 8q11. Family analysis showed the variant chromosomes to be transmitted through at least three generations in each family. There were no reports of reproductive problems or phenotypic effects in the carriers of these chromosomes, indicating the benign character of the aberrant chromosomes. In order to characterise the chromosomal variants more precisely, various differential banding techniques were applied.

Keywords: nucleolus organiser region; non-acrocentric chromosome; 4qs; NOR insertion

In humans, the nucleolus organiser regions (NORs) are usually located in the satellite stalks (secondary constrictions) in the short arms of the acrocentric chromosomes 13, 14, 15, 21, and 22. In situ hybridisation studies showed these sites to contain the 18S and 28S rRNA genes. The transcriptional activity of these genes can be shown by specific silver staining of the NORs. Ectopic NORs, that is, NORs in positions other than the short arms of the acrocentric chromosomes, are very rare findings in humans. They are thought to arise from translocations between acrocentric and non-acrocentric chromosomes or by insertion of a nucleolus organiser region into a non-acrocentric chromosome. Depending on the particular rearrangement, unbalanced carriers can show phenotypic abnormalities or be phenotypically normal.

Here we present two more examples of non-acrocentric NOR bearing chromosomes. The unusual chromosomes were detected in two prenatal diagnoses for advanced maternal age. The segregation of the chromosomes was analysed in both families.

Material and methods

Proband

Prenatal diagnosis because of advanced maternal age led to the cytogenetic study of two families (A and B), which both included carriers of non-acrocentric NOR bearing chromosomes. In family A, an unusual chromosome 4 (4qs) was detected and in family B an interstitially located NOR was found in chromosome 8. The pedigrees of both families are shown in Fig 1.

Cytogenetic analyses

Silver staining and C and Q banding were done according to the methods of Goodpasture and Bloom, Sumner, and Caspersson et al, respectively.

Replication banding was performed on chromosomes of the carrier parent of the probands according to the method of Dutril-laux et al. In short, 24 hours before cell harvest, 300 µg/ml thymidine was added to the blood cultures. After 17 hours cells were washed twice in PBS, resuspended in BrdU substituted RPMI medium (100 µg/ml), and incubated for another five to seven hours at

Figure 1 Pedigrees of families A (A) and B (B). The probands are marked by arrows. (A) Transmission of chromosome 4qs. (B) Transmission of the NOR bearing chromosome 8.
37°C; 15 minutes before harvest colcemid was added to the cultures.

Differential chromosome staining was achieved with a modified fluorescence plus Giemsa (FPG) technique. The slides were kept for 30 minutes in buffered eosin Y solution (2 µg/ml). After rinsing in buffer, the slides were put into petri dishes, covered with buffer, and exposed to UV light at a distance of 10 cm for 7.5-20 minutes. Subsequently, the preparations were rinsed in buffer and incubated in 2 × SSC at 60°C for two hours. Finally, slides were stained in Giemsa solution.

Results

FAMILY A

In the prenatal diagnosis, a satellited chromosome 4 (4qs) was identified in all investigated metaphases of the fetus. Family analyses showed the father and the father’s sister to be carriers of the same chromosomal variant as well. Since the paternal grandmother had a normal karyotype, it could be concluded that the chromosome was transmitted by the paternal grandfather who had already died at the time of analysis (fig 1A). Detailed cytogenetic analysis was performed on lymphocyte chromosomes of the carrier father. Replication banding on prometaphase chromosomes (fig 2A) showed an additional bright band at the telomeric end of the long arm in one chromosome 4. Silver staining showed an active NOR to be located in this region (fig 2C-F). Apart from the satellited chromosome 4, eight acrocentric chromosomes (five D group chromosomes and three G group chromosomes) showed active NORs in their short arms (fig 2C-E). In C banded preparations, the terminal region of the 4qs appeared to be heterochromatic (fig 2G), indicating translocation of NOR associated heterochromatin to chromosome 4. In overexposed quinacrine stained chromosomes, the very small satellite region in 4qs becomes visible (fig 2B). The NOR bearing chromosome 4 was found to be involved in satellite associations with normal acrocentric chromosomes (fig 2H).

FAMILY B

Prenatal diagnosis identified an unusual chromosome 8 in all metaphases of the fetus. A faintly staining gap was visible in R banded chromosomes in the proximal long arm, close to the centromere. A silver positive nucleolus organis er was found to be inserted into this region (8q11). Family analyses detected the same chromosomal variant in the mother and in three of her brothers and sisters (fig 1B). One brother and one sister had a normal karyotype. Although the maternal grandparents were not available for chromosome analysis, it is evident that one of them must carry the variant chromosome 8 as well. Differential banding patterns on lymphocyte chromosomes of the carrier mother are shown in fig 3. After BrdU replication banding, an enlarged, very faintly staining region was visible beneath the centromere of one chromosome 8 (fig 3A). In Q banded preparations this region appears as a non-fluorescent segment (fig 3B). Silver staining showed an active NOR in this region as well as silver positive NORs in all acrocentric chromosomes (fig 3C, D, E), resulting in a total of...
11 actively transcribing nucleolus organiser regions. As indicated by a faint C band (fig 3F), NOR associated heterochromatin is present at the insertion site of the NOR. Similar to the satellited chromosome 4 in family A, the NOR inserted chromosome of family B also takes part in NOR associations with acrocentric chromosomes (fig 3G).

Discussion
Satellited non-acrocentric chromosomes are very rare events. The chromosome found to be most often involved appears to be the Y chromosome with 30, mostly familial, cases of Yqs (for review see Schmid et al17 and Couturier-Turpin et al18) and one case of Yps reported so far.19 It is generally accepted that these Yqs chromosomes are derived from reciprocal translocation events between a normal Y chromosome and an acrocentric chromosome. Thus, the structure of the Yqs chromosomes can show considerable interfamilial differences. In general, the presence of an additional NOR in the Y chromosomal heterochromatin region does not cause any phenotypic abnormalities.17

In contrast, unbalanced carriers of satellited autosomes may or may not display phenotypic abnormalities.6 7 20–22 In those cases showing no phenotypic effects, familial transmission of the satellited chromosome could usually be shown.

To our knowledge, apart from the present case, five cases of 4qs chromosomes have been published.8 9 22 27 Here, chromosome 4 represents the most often affected autosome and it has been suggested that the DNA sequence in terminal 4q is more likely to recombine with the short arms of acrocentric chromosomes than other chromosomes.21 In four of the cases, the father was found to have transmitted the 4qs, in one case the mother, and in one case the father was not available for analysis. Similarly, in all three cases of familial 4ps, the variant chromosome was inherited by the father.6 9 Thus, it can be speculated that the satellited chromosome 4 is preferentially transmitted through the male germ line. Moreover, a preferential transmission of the satellited chromosome 4 has been discussed22; in the family reported by Babu et al,6 the 4qs was detected in all six children of the carrier father and Miller et al22 found the 4qs chromosome to be preferentially inherited in their family as well. In the family investigated in the present study, the 4qs chromosome was transmitted to three family members. Thus, a distinction between random and preferential segregation of the satellited chromosome is not possible in this pedigree.

Interstitial insertions of nucleolus organiser regions into non-acrocentric chromosomes are observed even more rarely. Although such rearrangements have been described in some tumour cell lines,24 25 only four familial cases have been published so far. In a baby with mild Down syndrome features, the centromere and NOR of an acrocentric chromosome were inserted into the short arm of chromosome 12, resulting in a stable dicentric chromosome.26 Prenatal diagnosis led to the detection of a rearranged chromosome 11 in a fetus. Two active NORs joined by a centromere had been inserted into 11q21.27 Chromosome analysis of a malformed premature infant showed insertion of only the satellite stalks into 6q15.28 Finally, a NOR was observed in the long arm of chromosome 7 (7q21.3-q22.1) in a healthy male proband.29

The present investigation adds another case of a NOR insertion into a non-acrocentric chromosome (8q11). Comparable to the variants described by Prieto et al30 and Guttenbach et al31 only the satellite stalks and part of their adjacent constitutive heterochromatin seem to be inserted. The pedigree of the family shows that four out of six members of the second generation carry the variant chromosome.

Figure 3 NOR bearing chromosomes 8 (family B, proband II.1). (A, B) Selected chromosome 8 pairs from different metaphases after BrdU replication banding (A) and Q banding (B). The inserted NOR is indicated by arrowheads. (C, D) NOR bearing acrocentrics and NOR bearing chromosomes 8 after silver staining. (E) Selected chromosomes 8 after silver staining. (F, G) C banding of the NOR inserted chromosome 8. Selected chromosomes (F) and NOR association (G).
Although the ratio of 4:2 is not significantly different from 3:3, again the question about preferential transmission of NOR bearing chromosomes arises. However, in the investigation of the NOR bearing chromosome 7 we were not able to detect any preferential segregation of the variant chromosome in that large family\(^7\); 27 out of 51 family members exhibited the inserted chromosome 7. In this chromosome 7 variant, the inserted NOR seemed to represent a potential breakage region.

In neither of the two families reported here did the carriers of the satellited/inserted chromosome exhibit any phenotypic effects, confirming the benign/neural character of their chromosomal rearrangements. Furthermore, there was no evidence of reproductive problems in either family, suggesting normal meiotic behaviour of these chromosomes. In the other above mentioned familial cases of NOR insertions, phenotypic abnormalities detected in some carriers were related to factors other than the insertion. However, some satellited non-acrocentric chromosomes have been shown to result in cryptic terminal deletions that are associated with phenotypic abnormalities. In order to rule out such a possibility, high resolution banding or fluorescence in situ hybridisation with telomeric DNA probes or both seem to be indicated in the analysis of these chromosomes.

11 Sumer AT. A simple technique for demonstrating centro