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ORIGINAL INVESTIGATION

Ursula Friedrich · Monna Caprani · Erik Niebuhr
Aage Juhl Therkelsen · Arne Lund Jørgensen

Extreme variant of the short arm of chromosome 15

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Abstract Using fluorescence in situ hybridization, primed in situ labelling, and conventional cytogenetic staining we have characterized an excessively enlarged short arm of chromosome 15. The likely mechanism explaining this variant chromosome involves amplification of rDNA sequences followed by inverted insertional translocation between the enlarged sister chromatids of the short arm of chromosome 15.

Introduction

Nucleolar organizing regions (NORs) are present in the short arm stalks of all acrocentric chromosomes and contain genes for 18 S and 28 S ribosomal (r) RNA (Evans et al. 1974). Ribosomal RNA genes are GC-rich sequences and accordingly, the NOR regions stain weakly in Q-banding and strongly in R-banding. Also, silver staining, which normally reflects NOR activity, has been used to assess length variation in this region (Mikelsaar et al. 1977). Miller et al. (1978) were able to show two active NORs in an enlarged short arm of chromosome 14.

In recent years, it has become clear that the short arms of the acrocentrics consist of different DNA species. In a previous work we described the relative position of these different DNAs (Gravholt et al. 1992). Normally, the order to sequences is: centromeric α-satellite, satellite III, β-satellite, rDNA, β-satellite, cytological satellite, and telomere (Fig. 3). We were curious to see whether this order is maintained in the variant chromosome. Using molecular cytogenetic techniques we found that the variant differs from normal acrocentric chromosomes with respect to the order of the different satellite DNAs. Based on the enlargement and the changed DNA order of satellite DNAs we propose a two-step genetic mechanism explaining the origin of the extreme enlargement of the short arm.

U. Friedrich (✉) · A. J. Therkelsen · A. L. Jørgensen
Institute of Human Genetics, The Bartholin Building,
University of Aarhus, DK-8000 Aarhus C, Denmark
Tel. +45-8942-1684; fax +45-8612-3173;
e-mail zitta@humgen-aau.dk

M. Caprani
Cytogenetic Laboratory, Institute for Basic Research,
Psychiatric Hospital in Aarhus, DK-8240 Risskov, Denmark

E. Niebuhr
Department of Medical Genetics, The Panum Institute,
University of Copenhagen, DK-2200 Copenhagen N, Denmark

Patient and methods

The female proband was referred for cytogenetic evaluation because of two spontaneous abortions in the first trimester.

Peripheral lymphocytes were cultured for 72 h for preparation of metaphase chromosomes and for 96 h for prometaphase chromosomes, following standard protocols. The cell suspensions were stored at –20° C. The slides were freshly prepared for the different staining procedures which were: Q-, RFA-, C-banding, DA/DAPI, AgNOR. Fluorescence in situ hybridization (FISH) was performed as described by Gravholt et al. (1992). The primed in situ labelling method (PRINS) was used for in situ visualization of telomeres (Therkelsen et al. 1995). The probes and oligonucleotides employed are listed in Table 1.

Results

The variant chromosome 15 shows the same elongation of the short arm in all cells examined. The specific staining properties are visualized in Figs. 1 and 2. This region stains uniformly in Q-banding, the intensity being less than the one in chromosome 1qh (Fig. 1a). After incorporation of 5-bromo-2´-deoxyuridine (BrdU) late in S phase, the region shows two moderately dark-stained RFA bands interrupted by one light band (Fig. 1b). In C-banding, a dark-stained centromeric heterochromatin block is seen at the centromere, and a moderately dark block is seen in the elongated region (Fig. 1d). The variant chromosome does not stain with DA/DAPI, except for the juxta-centromeric heterochromatin normally found in chromosome 15 (Fig. 1c). Silver staining reveals the standard staining pattern of normal acrocentrics. In the variant chromosome, however,

Table 1 Characterization of probes and oligonucleotides

Name	Type of DNA	Chromosomal location	Reference
pTRA20	α-Repeat	15	Choo et al. (1990a)
pC_{BE}	Ribosomal	13, 14, 15, 21, 22	Sylvester et al. (1986)
P21β	β-Satellite	9, 13, 14, 15, 21, 22	Greig and Willard (1992)
pTRS-47	Satellite III	1, 9, 13, 14, 15, 21, 22, Y	Choo et al. (1990b)
telo 2$(CCCTAA)_7$	Telomere	All chromosome ends	Moyzis et al. (1988)

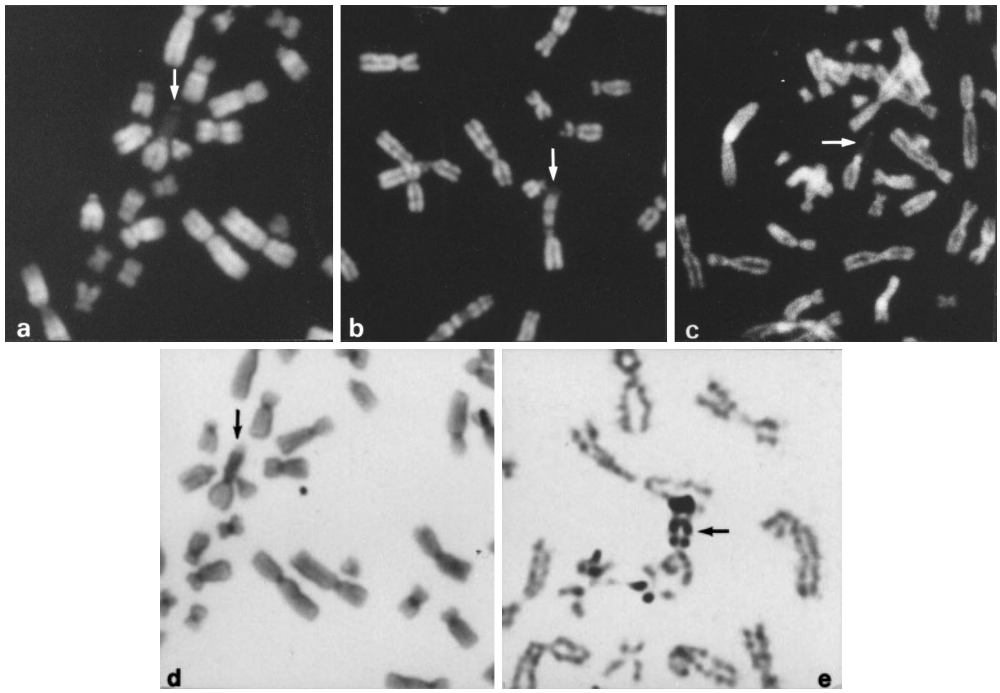

Fig. 1 **a** Q-banding; **b** RFA-banding; **c** DA/DAPI; **d** C-banding; **e** AgNOR

silver staining reveals three blocks, of which the distal block is more intensively stained and longer than the two proximal ones (Fig. 1e).

The presence of a huge amount of rDNA is confirmed by FISH. The rDNA probe hybridizes extensively to this region and lights up two blocks of chromatin of equal size that co-localize with the two GC-rich R-bands of this region (Fig. 2d). β-Satellite sequences that normally flank the rDNA region (Gravholt et al. 1992) have retained this location, as the β-satellite probe hybridizes distinctly to the proximal and also to the distal end of the huge rDNA region. No hybridization signal is detected within the rDNA region (Fig. 2c). The satellite III probe hybridizes distinctly to the proximal end of the rDNA region and no hybridization signal is detected within the rDNA region. However, a distinct hybridization signal is unexpectedly found at the distal end of the rDNA region (Fig. 2b). The identify of the variant chromosome is confirmed using as probe α-repeat DNA specific for the centromere of chromosome 15. No hybridization signal is detected outside the centromeric region (Fig. 2a).

The telomere probe detects telomeric sequences at the end of the long arm and at the end of the short arm of the variant chromosome (Fig. 2e).

Discussion

In Fig. 3, we propose a two-step mechanism for the creation of this extreme variant chromosome 15p+. The enlarged p arm exceeds the length of the q arm and is mainly made up of rDNA sequences. We estimate that chromosome 15 normally represents 3% of the human genome. If the size of the enlarged p arm equals or exceeds the size of the long arm, it will thus correspond to about 3% of the genome. If a haploid DNA content of 3×10^9 bp is assumed, the 3% will amount to some 10^8 bp. With an rDNA repeat unit of 44 kb (Worton et al. 1988), this DNA amount corresponds to 2200 rDNA copies (10^8 bp divided by 44×10^3 bp), which is a tenfold increase in the normal haploid number of rRNA genes. However, this figure may be an overestimate if DNA species other than rDNA, not detected by us, are present in the enlarged p arm. It is unlikely that the increase in rDNA is due to compensatory amplification, as silver staining also demonstrates the presence of active rDNA on the other acrocentrics.

In the first step we suppose that in one of the proband's ancestors a compensatory amplification of rRNA genes may have occurred on one chromosome 15 because of loss of rRNA genes on some of the other acrocentric chromosomes. We suggest that such an amplification results

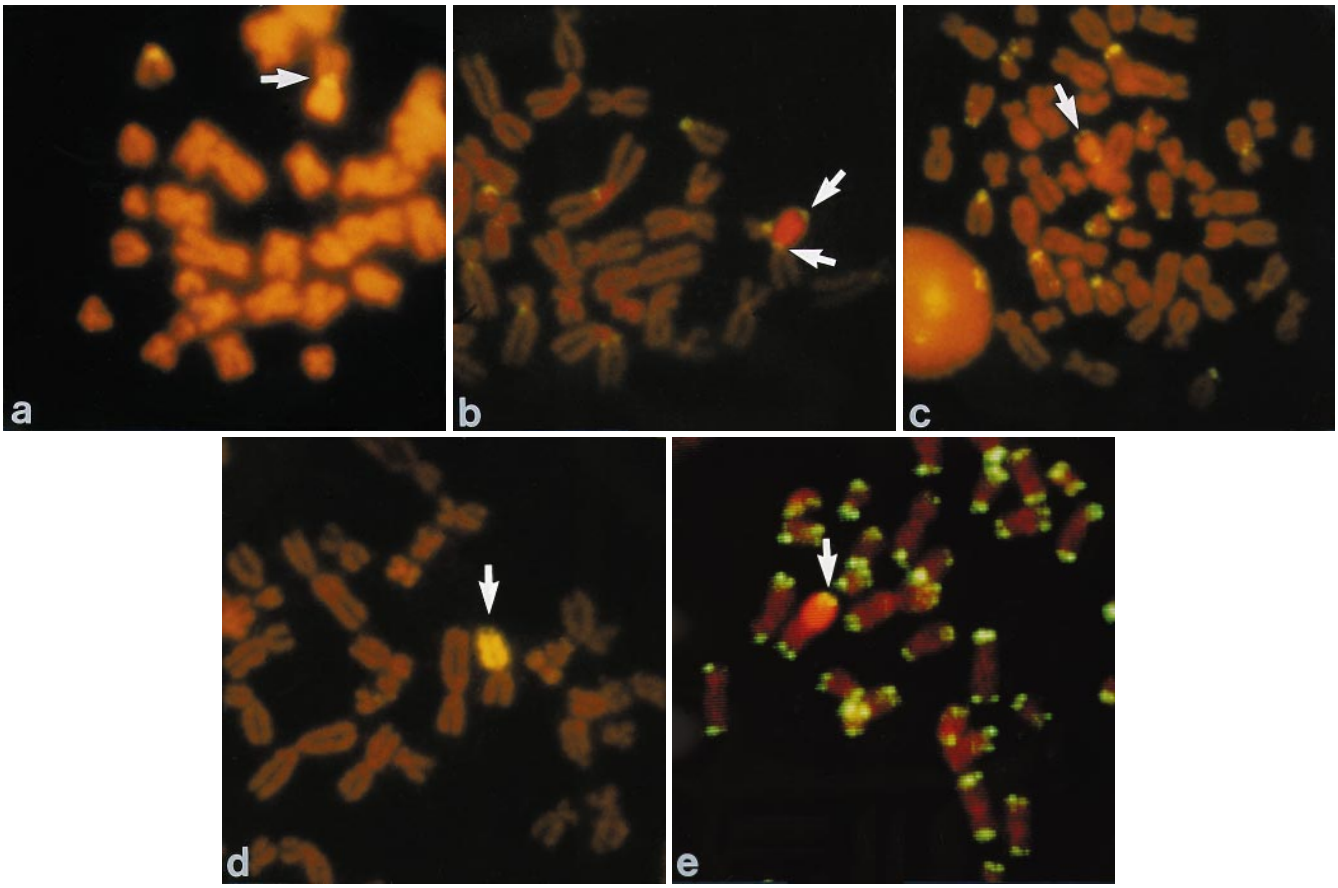

Fig. 2 **a** Fluorescence in situ hybridization (FISH) with α-repeat. **b** FISH with satellite III. *Arrows* indicate the normal location of the proximal satellite III in the centromere region and an additional block of satellite III at the distal short arm. **c** FISH with β-satellite. The β-satellite has retained its normal location and flanks the amplified ribosomal (r)DNA region. **d** FISH with rDNA. **e** Primed in situ labelling with a telomere probe showing normal telomeres at the end of the short and the long arms of chromosome 15

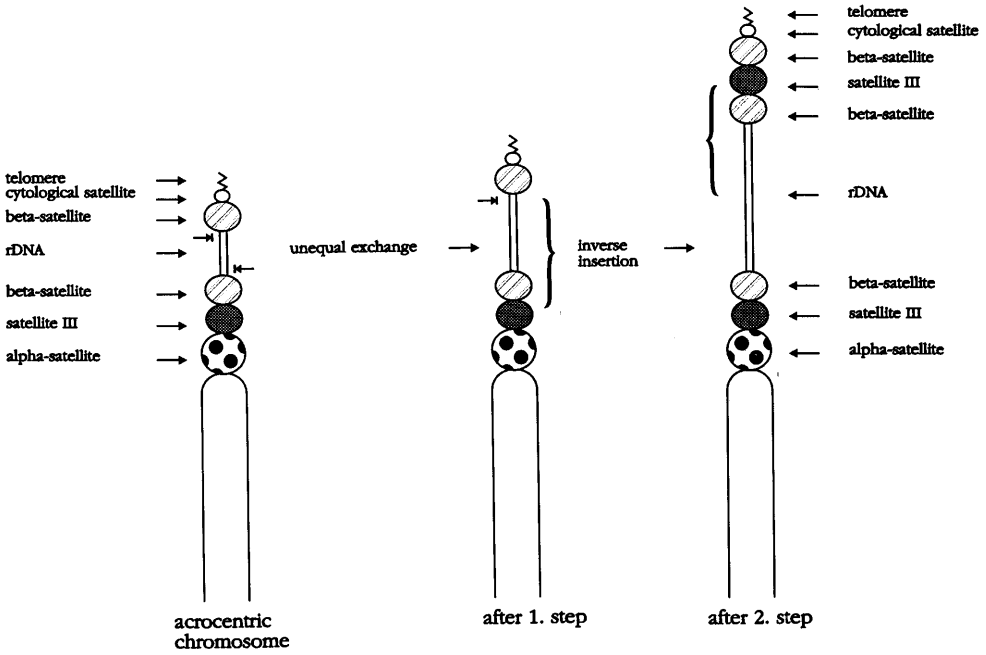

Fig. 3 Model of the short arm of an acrocentric chromosome. The locations of the different classes of tandemly repeated DNAs are given. The first step in the origin of the 15p+ chromosome includes unequal crossing-over between the two sister chromatids in the rDNA region. The region marked by a *bracket* is inserted in an inverted orientation into the sister chromatid at the location indicated by an *arrow*

from unequal crossing-over between sister chromatids of the short arm of chromosome 15 or between homologs. The duplication unit involves, at most, the whole rRNA gene array, excluding the flanking sequences, since we detect no β-satellite sequences interspersed in the enlarged cDNA region. In the next step, a further amplification has taken place as a result of an inverted tandem duplication. Normally, the short arm region of chromosome 15 is built up by DNA repeats in the following order: α-centromeric satellite – satellite III – β-satellite – rDNA – β-satellite – cytological satellite – telomere (see Fig. 3). In the variant chromosome 15, however, satellite III is also seen at the distal end of the enlarged rDNA region, which suggests that an exchange between two sister chromatids has taken place. In the donor strand there are two breakpoints, which most probably are localized in the satellite III and in the rDNA region (Fig. 3). In the recipient strand there is one breakpoint placed distally between the rDNA and β-satellite region. Following the strand breaks, a duplication of the rDNA – β-satellite – satellite III region has occurred as an inverse insertion with attachment of corresponding DNA strands. This mechanism would create the location of the two β-satellite blocks side-by-side, interrupted only by satellite III sequences, as seen in Fig. 2c. Since the two duplicated blocks of enlarged rDNA are of nearly the same size, it is most likely that the exchange has taken place between the two sister chromatids and not between homologs. A similar mechanism has been described for non-acrocentric chromosomes (Taylor et al. 1977).

Telomere sequences are present in the elongated chromosome 15 on both arms. The mechanism described for the origin of this variant chromosome does not interfere with the structure of the telomere.

As already mentioned, the easiest explanation for this extreme variant chromosome would have been unequal crossing-over in the rDNA region. By the hybridization technique employed, however, a much more complicated and complex mechanism could be disclosed.

Enlarged NORs have been suggested as etiologic causes of nondisjunction. Many discrepant results have been published. So far, no convincing clinically associated implications have been observed (for review, see Schwartz et al. 1989). Therefore, it is not possible to conclude that the extreme variant chromosome is causally related to the two spontaneous abortions in the proband.

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