Breakpoints in α, β, and satellite III DNA sequences of chromosome 9 result in a variety of pericentric inversions

K H Ramesh, R S Verma

Abstract

Human chromosome 9 with a pericentric inversion involving the qh region is considered normal. It has probably evolved through breakage and reunion and is retained through mendelian inheritance without any apparent phenotypic consequences. Fluorescent in situ hybridisation (FISH) technique using α, β, and satellite III DNA probes showed that the breakpoints are variable and can be localised in the α or in the satellite III and β DNA regions or both. Three types of inversions are proposed which appear similar by CBG banding: pericentric inversions with two alphoid, one β, and one satellite III hybridisation signals were classified as type A. Type B were those with two β, one α, and one satellite III hybridisation signals, while type C was complex, and most likely involved two inversions, since two separate hybridisation signals were detected in each of the alphoid, β satellite, and satellite III DNA regions. Based on eight cases, type A is likely to be the most frequent, but the frequencies, which at present appear non-random for these different types of inversions in the population, can only be estimated by studying a larger sample size. Inversion heteromorphisms may promote reshuffling of tandem arrays of DNA repeat sequences, thereby giving rise to new heteromorphic domains. Alternatively, the repetitive nature of the sequences lends to the structural variations observed within the inv(9) chromosomes (or any other abnormal chromosome that is the result of recombination between, or breakage within, repetitive DNA).

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Key words: chromosome 9; heterochromatin; secondary constriction region; heteromorphisms.

Division of Genetics, The Long Island College Hospital-SUNY Health Science Center at Brooklyn, Hicks Street at Atlantic Ave, Brooklyn, NY 11201, USA
K H Ramesh
R S Verma

Correspondence to:
Dr Verma.

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Human chromosome 9 displays the highest degree of morphological variation owing to the heterochromatin confined mainly to the secondary constriction (qh) region. It is considered a normal heteromorphism whose inheritance is familial.[1-3] Although approximately 10–15% of the general population exhibits this pericentric inversion,[4] its cause and clinical consequences remain the subject of controversy and debate.[5]

Selective conventional techniques (especially CBG banding and *Alu*I/Giemsa) depict qh regions as homogeneously stained heterochromatic regions,[6 7] while molecular techniques have recently shown that the pericentromeric heterochromatin is composed of different fractions of satellite DNA.[8 9] The heterogeneity is presumed to be the result of accumulation of different fractions of repeated satellite DNA sequences, termed α, β, and satellite III DNA.[10-13] The centromeric region contains alphoid DNA, while classical satellite III DNA and β satellite DNA are at the pericentromeric region and can be directly visualised by the fluorescence in situ hybridisation (FISH) technique.[14-16] Earlier, we had hypothesised that the occurrence of pericentric inversion of chromosome 9 is the result of the breakage prone region involving α satellite repeat DNA sequences.[14] The study of additional cases of pericentric inversion of chromosome 9 in our laboratory showed that breakage can also occur in the β satellite DNA. This enabled us to localise the breakpoints in these inversions and further establish the causal relationship between heterochromatin and the frequent occurrence of pericentric inversions involving secondary constriction regions.

Materials and methods

Chromosome preparations from six peripheral blood and two amniotic fluid samples, which had previously been confirmed as pericentric inversion of chromosome 9, were used for this investigation. Culture and chromosome banding by the GTG technique were according to standard protocols.[17] The inversions were further characterised by the FISH technique, using biotin labelled α satellite all human centromeres, chromosome 9 specific satellite III, and β satellite DNA probes according to the manufacturer's protocol (Oncor, MD). Briefly, slides aged for four to six days were treated in 2 × SSC (pH 7·0) at 37°C for 10 minutes and then dehydrated in 70%, 85%, and 100% ethanol and air dried. Chromosomal DNA was denatured in 70% formamide/2 × SSC (pH 7·0) at 70°C followed by ethanol dehydration. All probes except those used in dual colour FISH were denatured at 70°C for five minutes and chilled on ice immediately. For dual colour FISH, biotin labelled α satellite all human centromeres, and digoxigenin labelled 9 specific β satellite probes were mixed in the appropriate buffer, denatured together, and allowed to hybridise to the target chromosomal DNA without preannealing. Hybridisation of probes to target DNA was at 37°C for 16 hours.

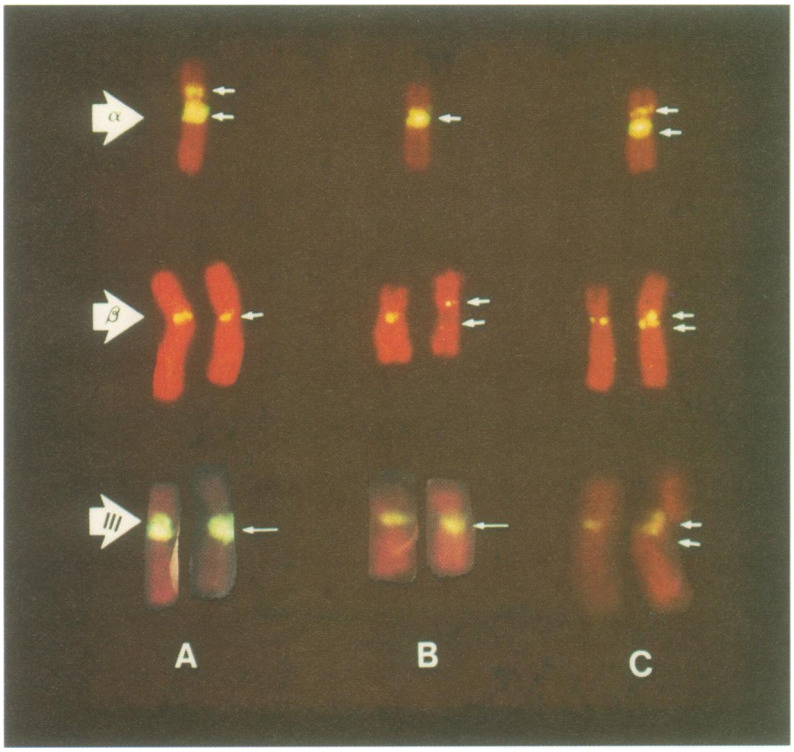

Figure 1 Characterisation of pericentric inversion of chromosome 9 by FISH. (A) Arrows indicate the two alphoid (top), one β (middle), and one satellite III (bottom) hybridisation signal(s); inversion type A (five cases). (B) Arrows indicate one alphoid (top), two β (middle), and one satellite III (bottom) hybridisation signal(s); inversion type B (two cases). (C) Arrows indicate the two alphoid (top), two β (middle), and two satellite III (bottom) hybridisation signals; inversion type C (one case). The normal non-rearranged chromosome 9 is shown on the left for the chromosomes hybridised with β and satellite III DNA probes.

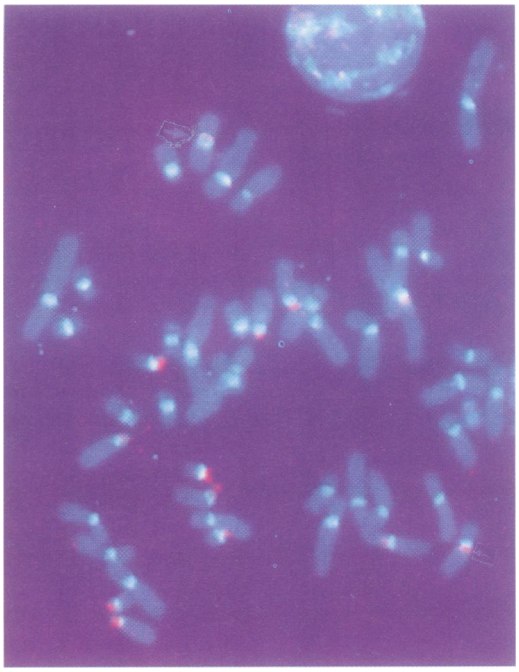

Figure 2 Analysis by dual colour FISH showing the position of the β satellite DNA region (red signal) in relation to the α satellite DNA region (green signal) in the normal non-rearranged (small arrow) and the inverted chromosomes 9 (big arrow) respectively (see text for details).

Posthybridisation washes were as specified by the manufacturer: for all human centromeres α satellite probe, the first wash was in 50% formamide/2 × SSC (pH 7·0) at 37°C for 15 minutes, and for chromosome 9 specific β satellite probe it was in 65% formamide/2 × SSC (pH 7·0) at 43°C for 15 minutes followed by a second eight minute wash in 2 × SSC (pH 7·0) at 37°C for both. Satellite III DNA probe slides were washed in 0·5 × SSPE (pH 7·4) at 72°C for five minutes. For detection and amplification, FITC-avidin and anti-avidin antibody were used. Slides were counterstained with propidium iodide/antifade. The posthybridisation wash for the dual colour FISH slides was in 1 × SSPE buffer (pH 7·4) at 72°C for five minutes followed by detection using a mixture of rhodamine-antidigoxigenin and FITC-avidin. No amplification was required and the counterstain used was DAPI/antifade. At least 20 metaphases were analysed in each experiment and photomicrographs were taken using 35 mm EPH-1600 Kodak Ektachrome film and also the Oncor 3 CCD cooling integrating camera.

Results

We characterised the human chromosome 9 inversion involving the qh region by FISH technique in eight subjects and found that breaks could occur within the α or in the satellite III and β satellite (DNA) regions or both. Based on the location of breaks, at least three types of inversion are proposed. In five cases, two independent hybridisation signals in the alphoid sequence and only one hybridisation site each in the β and satellite III regions could be detected, which we called type A (fig 1A). In two other cases, we noted two hybridisation signals in the β and only one in each of the alphoid and satellite III DNA regions which we called type B (fig 1B). The last case had two separate hybridisation signals in each of the α, β, and satellite III DNA regions and this class has been called type C (fig 1C). Dual colour FISH was performed using a case with chromosome 9 just to show the relative position of the α and β DNA regions in the normal and inverted qh region without rearrangement (fig 2). As seen in fig 2, the β DNA region is in the proximal q arm below the α DNA region in the non-rearranged normal chromosome 9, whereas in the inverted chromosome 9 it is present in the proximal p arm above the α DNA region.

The breakpoints and the position of α, β, and satellite III DNA regions in the three different types of inversions is shown schematically in fig 3. The visualisation of two separate alphoid hybridisation signals and one signal each for the satellite III and β DNA regions indicated that in type A inversions, a break occurred in the α satellite DNA region (figs 1A and 3A). The one break was localised in the β satellite DNA region in type B inversions; this was identified by the presence of two distinctly separate β hybridisation signals and only one hybridisation signal each in the α and satellite III DNA regions (figs 1B and 3B). In both type A and type B inversions, an additional break appears to have occurred in band 9q13. Type C inversion is complex and possibly resulted

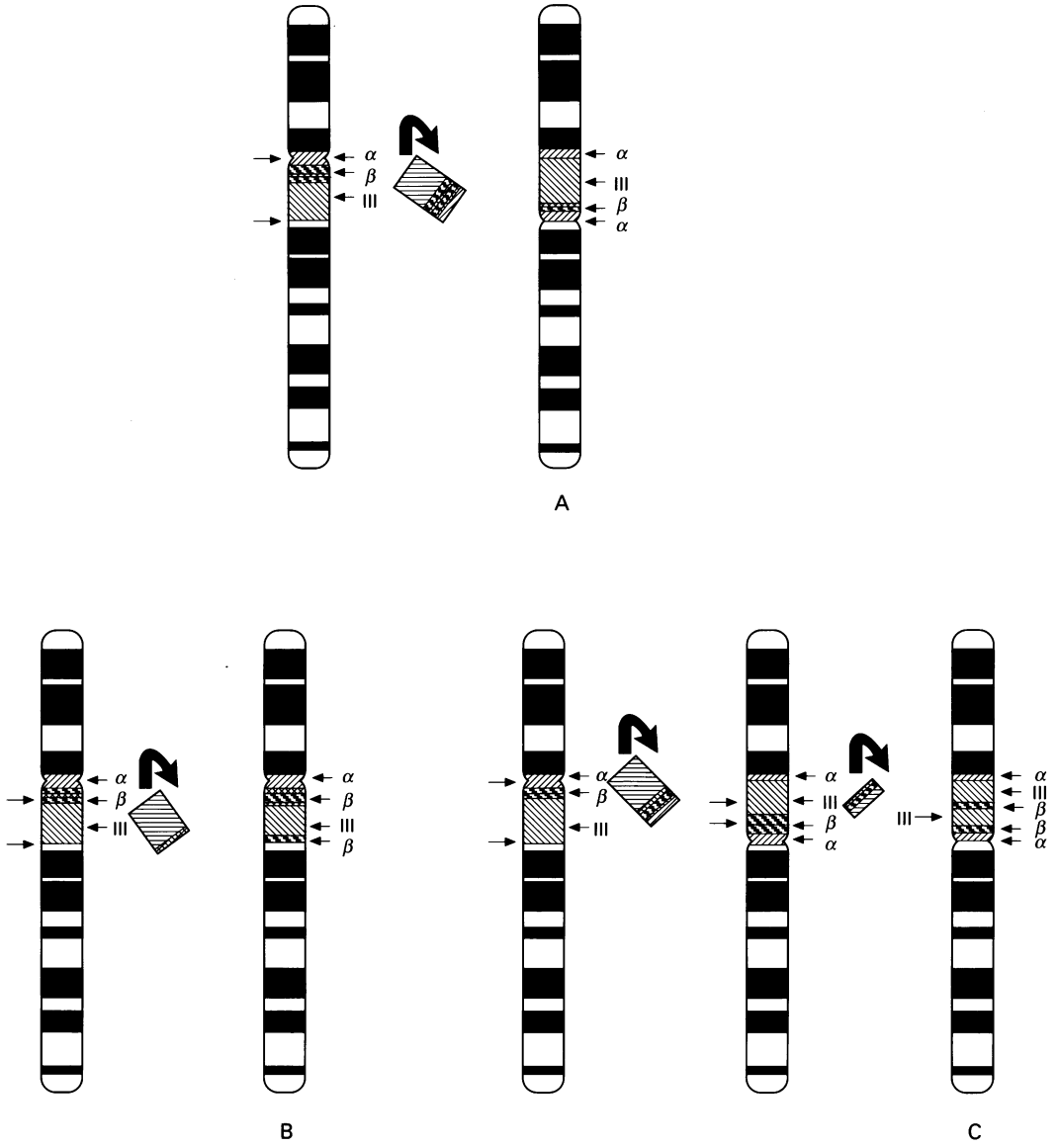

Figure 3 Schematic representation of the three different types of pericentric inversions involving the qh region of chromosome 9. (A) Inversion type A showing the two separated alphoid regions; the normal chromosome 9 on the left shows the position of α, β, and satellite III DNA regions, with long arrows (on the left) indicating the breakpoints. (B) Inversion type B showing the two separated β satellite DNA regions; on the left is the normal chromosome 9 with long arrows indicating the breakpoints. (C) Inversion type C showing two alphoid, β, and satellite III DNA regions; breakpoints involved in the first and second inversion are shown by the long arrows.

from two separate events (figs 1C and 3C). In the initial inversion, one break must have occurred in the alphoid region and the other break in band 9q13, producing two separate alphoid hybridisation signals. A subsequent inversion occurred with one break in the β satellite DNA region and a second break in the satellite III DNA region, which is documented by the presence of two separate β and satellite III DNA hybridisation signals. The proximity of the two β and satellite III DNA hybridisation signals indicates that the second inversion probably involved a very small area within and close to the β and satellite III DNA regions of the inverted chromosome 9.

Discussion

The repetitive nature of the α, β, and satellite III DNA sequences makes them useful markers for the in situ detection of chromosomes and chromosome fragments, as well as in determining the nature of translocations, inversions, and the breakpoints involved.[18-20] For example, exchange among short arms of different acrocentric chromosomes is the model that has been put forth to explain the homogeneity of β satellite sequences.[18 21] Previous studies have shown that in acrocentric chromosomes, a common location for breakpoints is the region between rRNA genes, α satellite DNA regions, and satellite III DNA.[13 22-27] Our study shows that the β satellite region of chromosome 9 is also involved in breakage, thereby bringing about rearrangement within the repeat sequences.

Molecular studies have shown the Sau3A repetitive alphoid DNA family to be unstable and susceptible to breakage in chromosomes.[28 29] Perhaps intrachromosomal or even interchromosomal recombinations involving α and β satellite DNA repeat sequences through in-

versions could be one explanation by which these repeat sequences undergo divergence. During the evolutionary process of alphoid DNA sequences, divergence in base pair accumulation probably produced chromosome specific alphoid subsets.[10 30] However, the homogeneity of α satellite DNA in individual chromosomes suggests the possible occurrence of unequal crossing over between sister chromatids over a period of time which has been called the "horizontal process of homogenization".[31 32]

The pericentric inversion involving the qh region of chromosome 9 is regarded to be a chromosomal mutation, without any phenotypic consequences. Although it involves rearrangement of repetitive DNA sequences, it has so far not been evolutionarily unfavourable. It is tempting to hypothesise that α and β satellite DNA sequences are prone to frequent breakage in chromosomes and can undergo rearrangement within the repeat units, thus giving rise to a variety of inversions.[33] This and the fact that there appears to be no reproductive or selective disadvantage or founder effect could also explain the high incidence of pericentric inversion of human chromosome 9, which has been termed an inversion heteromorphism.[34] Using molecular methods, further analysis may provide insight into not only the concerted evolutionary process itself, but also the mechanisms that bring about homogeneity or divergence in α and β satellite DNA sequences of human and other primate chromosomes as well. No doubt, it is an arduous task to reach a meaningful conclusion concerning these so-called "rare familial variants or heteromorphisms" whose origin could result from the aforementioned mechanisms. Heterochromatin, which has been regarded as a meaningless filler in the genome, may very well carry some genetic function that could alter certain genes surrounding it.[35]

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