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Homologous sequences at human chromosome 9 bands p12 and q13-21.1 are involved in different patterns of pericentric rearrangements

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A thorough study of the heterochromatin organisation in the pericentromeric region and the proximal long (q) and short (p) arms of human chromosome 9 (HSA 9) revealed homology between 9p12 and 9q13-21.1, two regions that are usually not distinguishable by molecular cytogenetic techniques. Furthermore, the chromosomal regions 9p12 and 9q13-21.1 showed some level of homology with the short arms of the human acrocentric chromosomes. We studied five normal controls and 51 clinical cases: 48 with chromosome 9 heteromorphisms, one with an exceptionally large inversion and two with an additional derivative chromosome 9. Using fluorescence *in situ* hybridisation (FISH) with three differentially labelled chromosome 9-specific probes we were able to distinguish 12 heteromorphic patterns in addition to the most frequent pattern (defined as normal). In addition, we studied one inversion 9 case with the recently described multicolour banding (MCB) technique. Our results, and previously published findings, suggest several hotspots for recombination in the pericentromeric heterochromatin of HSA 9. They also demonstrate that constitutional inversions affecting the pericentromeric region of chromosome 9 carry breakpoints located preferentially in 9p12 or 9q13-21.1 and less frequently in 9q12.

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Introduction

Among the non-acrocentric human chromosomes, chromosome 9 presents with the highest degree of morphological variations.¹ Variants, such as 9qh+, 9qh– or inv(9)(p11q13) are very common findings in routine cytogenetics, with frequencies of approximately 8%, <1% and approximately

1.5%, respectively.² These variants, termed ‘heteromorphisms’, have been studied intensely and are usually ascertained by GTG- and CGB-banding in routine cytogenetics.² The ISCN 1995³ describes the following variants for chromosome 9: (i) increase or decrease in the length of the heterochromatin of the long arm (9qh+ and 9qh– respectively), and (ii) heterochromatin in the short arm only (9ph), or in both the long and the short arm (9phqh). Surprisingly, the most common inversion, inv(9)(p11q13), cytogenetically classified as a variable but normal chromosome feature⁴ is not mentioned in the ISCN 1995.³

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Since the introduction of fluorescence *in situ* hybridisation (FISH) techniques only a few systematic studies have been performed to classify the chromosome 9 heteromorphisms in more detail. In one study, four different types of inversion and duplication (ie 9qh+) were reported,⁵ and overall, seven different types of rearrangement leading to inversions on chromosome 9 have been described.^{1,6,7} However, neither the origin nor the clinical significance of these heteromorphisms is well understood. This is mainly due to the fact that the centromeric regions of the human chromosomes are poorly assembled by current mapping and sequencing efforts, as they are poor in euchromatin but repeat-rich and organised in a highly complex fashion.⁸

The present study used a specific probe set of three chromosome 9-specific FISH probes (chromosome 9 α -satellite, satellite III DNA and a microdissection-derived probe for 9q12/9q13-21.1) to identify the full range of heteromorphic patterns and any breakpoint cluster regions related to the pericentric inversions of chromosome 9. We also wanted to assess whether the multicolour banding (MCB) technique would detect any heteromorphic patterns of clinical significance.

Patients and methods

Patients

The study group was chosen based on previous findings of heteromorphic patterns in the pericentric region of chromosome 9. Informed consent for the study was obtained from all patients. Metaphase spreads from short-term cultures of peripheral blood or aminocytes from a total of 56 patients were analysed by standard GTG- and CGB-banding in eight German Centres (Cottbus, Düsseldorf, Essen, Halle/Saale, Homburg/Saar, Jena, Leipzig and Magdeburg; Table 1). With the exception of case 52, who was the mother of case 56, patients were not related. A familial 9ph+ heteromorphism (defined by GTG-banding) was found in metaphases from cases 52 and 56, but the child also carried a supernumerary marker chromosome (SMC) of chromosome 9 origin. Case 12 was the partner of case 16.

Twenty-five healthy patients were analysed cytogenetically for reasons not relevant in the context of our study. Another 17 patients were analysed due to a history of reproductive problems. One patient was healthy but cytogenetic analysis revealed an SMC in 75% of the metaphases (case 55). In 12 cases, the chromosome 9 variants were detected in children or young adults in connection with minor physical abnormalities or psychomotor retardation (defined as morbid slowing of action or movement presumed to result from psychic rather than physical disturbance). Although it could not be verified, it is probable that the heteromorphisms detected in these cases were familial variants.

Molecular cytogenetics

The chromosomes of all 56 patients were studied using a FISH-probe set comprised of: (a) a chromosome 9-specific α -satellite probe (SpectrumGreen, green; Vysis, Downers Grove, IL, USA); (b) a satellite III probe (digoxigenin, detected with anti-digoxigenin-rhodamine, red; Q-BIOgene, Heidelberg, Germany); (c) a microdissection-derived⁹ probe from the SMC of case 56 ('midi36'), specific for 9p12 and 9q13-21.1; diethylaminocoumarin, blue (NEN-Dupont, Boston, MA, USA). Additionally, a microdissection derived whole chromosome and chromosome arm-specific painting probes,¹⁰ microdissection probe for all human acrocentric chromosomes ('midi'54) (derived from the SMC of an ICSI patient and representing heterochromatin from the short arms of chromosome 13 or 21),¹¹ and a probe specific for all human centromeres (Q-BIOgene) have been applied in some of the cases. Labelling of the microdissection derived probes and hybridisation were carried out as described.^{10,12} We used a chromosome 9-specific MCB probe set¹¹ to further characterise the heteromorphic chromosome 9 in case 13 following the published MCB FISH procedure.^{11,13} All images were captured on a Zeiss Axioplan microscope (Zeiss, Jena, Germany) with the IKAROS and ISIS digital imaging system (MetaSystems, Altlussheim, Germany) using a CCD camera with on-chip integration (XC77, Sony, Vienna, Austria).

Results

A chromosome 9-derived small marker chromosome

The DNA-probe midi36 (derived from the SMC of case 56) stained the chromosomal regions 9p12 and 9q13-21.1 on both normal chromosomes 9 in addition to the SMC (Figures 1A1 and 2C). Both the mother (case 52) and child (case 56) carried one chromosome defined cytogenetically at 9ph+ (Figures 1A1 and 2B), caused by a pericentric inversion with breakpoints in 9p12 and 9q12-13 (Figures 1A2). In addition to the SMC, the child carried one normal chromosome 9 and one chromosome with a '9ph+' heteromorphism. It is possible that the SMC originated from the region composed of 9p12 and 9q13-21.1 (Figure 1A2). However, no further studies of the origin of the SMC could be performed due to the unavailability of a parental sample. Hybridisation of probe midi36 and post-hybridisation slide processing under less stringent conditions results in signals in the pericentromeric regions of most human chromosomes including 1qh and 16qh, and the short arms of the acrocentric chromosomes (Figure 1E). Cohybridisation of midi36 with a microdissection-derived probe for the p-arms of all acrocentric chromosomes (midi54)¹¹ highlighted identical regions on chromosome 9 (Figure 1F). The probe midi54 gave the signals only on the pericentromeric region of chromosome 9 regardless of stringency levels (Figure 1F).

The probe midi36 completely stained the CBG-negative/GTG-positive extra band in 9q12 in cases 13-17 (Figure

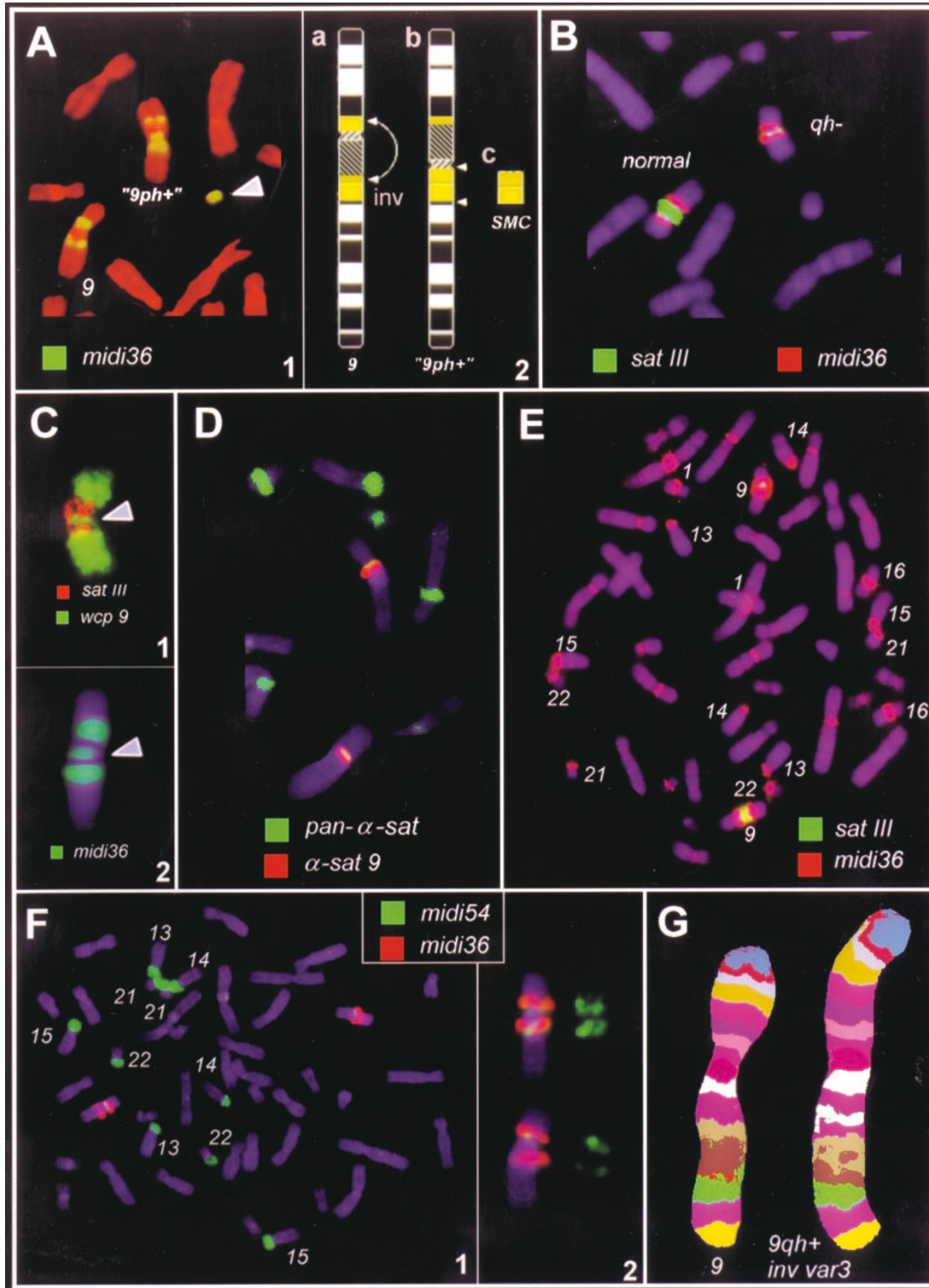


Figure 1 Representative examples of the FISH patterns observed. (A) (1) Representative partial metaphase from the foetus (case 56) carrying the SMC from which the microdissection probe midi36 was derived. In addition to painting the SMC itself (arrowhead), this probe allowed the identification of the heteromorphic pattern (var2) (9qh+ and inversion) for the chromosome 9 cytogenetically defined as '9ph+'. (2) Schematic depiction of the postulated origin of the SMC of case 56. A pericentric inversion with breaks in distal 9p12 and proximal 9q13 led to the '9ph+' variant detected in case 52 (mother of case 56). The SMC is a derivative of that variant of chromosome 9

1C). Combining midi36 together with a satellite III DNA probe showed that the size variations 9qh+ and 9qh- are due to variations in copy number of the satellite III DNA (Figures 1B and 2A).

Characterisation of heteromorphic patterns

In cases characterised as 9qh+, 9ph+, or inv(9)(p11q13), 12 different heteromorphic patterns (besides the normal) could be distinguished using our three probe set. The results are summarised in Figures 2A–C, 3 and Table 1.

Previous studies^{6,7} of the 9qh region used a satellite III DNA probe combined with a β -satellite probe and an α -satellite probe specific for all human centromeres (pan- α satellite probe; Figure 2C,D). In the present study, we compared the patterns obtained with the chromosome 9 specific α satellite probe with the pan- α satellite probe by cohybridisation. As shown in Figure 3, the chromosome 9 specific α satellite probe covers a similar but larger region to the pan- α satellite probe (Figure 1D).

The normal pattern

All 10 homologues of chromosomes 9 in the control group (cases 1–5) and one chromosome each of the 51 patients studied showed the following hybridisation pattern: two blue signals (corresponding to the midi36 probe) in 9p12 and 9q13–21.1 respectively and flanking (from p to q) (i) a small green α satellite and (ii) a red satellite III signal. If the latter was of about the same size as the sum of both midi36 signals (for the normal pattern see Figures 2A and 3A–C, upper row), the region 9q12 was considered normal. The region 9q12 was defined as 9qh- if the red band was smaller than either blue midi36 signals, and as 9qh+ if the red band was larger than the sum of midi36 signals.

Abnormal patterns: inv(9)(p11q13) and 9qh-

Thirty-two cases (cases 18–49) classified as inv(9)(p11q13) by GTC- and CBG-banding could be sub-classified based on three different FISH patterns (Figure 2A). All 32 cases showed an inverted pattern of FISH signals. Twelve cases (cases 18–29) presented with blue signals of equal size at 9p12 and

9q13-21.1 (variation type 1=var1). Twenty cases showed a weaker signal at 9p12 and a large signal at 9q13-21.1 (variation type 2=var2, cases 30–49). These two groups could be divided further into: (a) cases with a satellite III region of normal size and (b) cases with a red fluorescent signal of reduced size (less than half the size of the midi36 signals). This was defined as 9qh- with inversion. Only one case of this variant was found in the patients studied. While var1 was present in only six of the 26 cases with inversion and a satellite III region of normal size, var1 was found in all six 9qh- cases with inversion (Table 1).

The 9ph+ pattern

Five cases (cases 50–53, 56) were described as 9ph+ by GTG banding. Cases 50 and 51 showed a duplication of either the region 9p12 or 9q13-21.1, which are indistinguishable in our model. In case 53, a similar enlargement was followed by an inversion with breaks in 9q13-21.1 and in 9q12 (inversion type var3). In case 52 and her unborn child (case 56) FISH revealed an enlargement of satellite III DNA in connection with a pericentric inversion of var2 type as cause of the 9ph+ (see Figures 1A and 2).

The 9qh+ pattern

We included eleven cases described as 9qh+ after cytogenetic analysis (cases 7–17). Five of these had an enlarged satellite III (red) signal but no aberrant pattern for the other two probes. In all 9qh+ cases, the enlarged heterochromatin in the pericentric region of chromosome 9 was larger than the sum of blue midi36 signals. In five cases an extra band was observed on GTG-banding (cases 13–17; Table 1). This additional band was delineated by the probe midi36 (see Figures 1C and 2A). Three of these (cases 13–15) showed a normally sized blue signal in 9p12, and a proximal green signal, but two blue signals in the long arm of chromosome 9 (one in 9q12 and 9q13-21.1, respectively). The signal in 9q13-21.1 was less intense than that in 9p12, but both signals in 9q together were as intense as that in 9p12. This pattern could be explained by a paracentric inversion with breaks in 9q12 and 9q13 (inversion variation 3=var3).

with breaks in 9p10 and 9q13-21.1 (B) Partial metaphase from case 6 of the probe midi36 (red) co-hybridised with a classical satellite III DNA probe (green). The reduced green signal on the 'qh-' chromosome indicates that this is due to a diminished copy number of the classical satellite III DNA. (C) Representative chromosomes from case 14 hybridised with: (1) a whole chromosome 9 paint (wcp 9) (green) and a classical satellite III DNA probe (red). The CBG negative GTG-positive extra-band in 9q12 is highlighted by wcp 9, and (2) the midi36 (green) probe. (D) Cohybridisation of the chromosome 9 specific α -satellite DNA probe used in the present study (red) with an alpha satellite probe (pan α -sat, green) specific for all human centromeres. The chromosome 9 specific α -satellite DNA probe covers a similar but larger region to the pan α -satellite probe. (E) Under low stringency conditions the midi36 probe hybridized to homologous sequences in the pericentric region of most human chromosomes including 1qh and 16qh, and the p-arms of the acrocentric chromosomes. Under the same low stringency hybridisation conditions, the satellite III DNA probe (green) highlighted only the two chromosomes 9. (F) (1) Metaphase showing cohybridisation of the midi36 probe (red) and a microdissection probe for the acrocentric p-arms (midi54, green). The enlarged chromosomes in (2) show identical regions of chromosome 9 labelled by both probes. (G) The var3 (9qh+ and inversion) type inversion of case 14 analysed using the multicolor banding (MCB) probe set for chromosome 9. Using the MCB probe set, the α -satellite region is pseudo-colored in pink, the satellite III DNA region in white and the region 9p12/9q13-21.1 in violet. These results confirm those obtained with a set of three centromeric chromosome 9 probes (Figure 2).

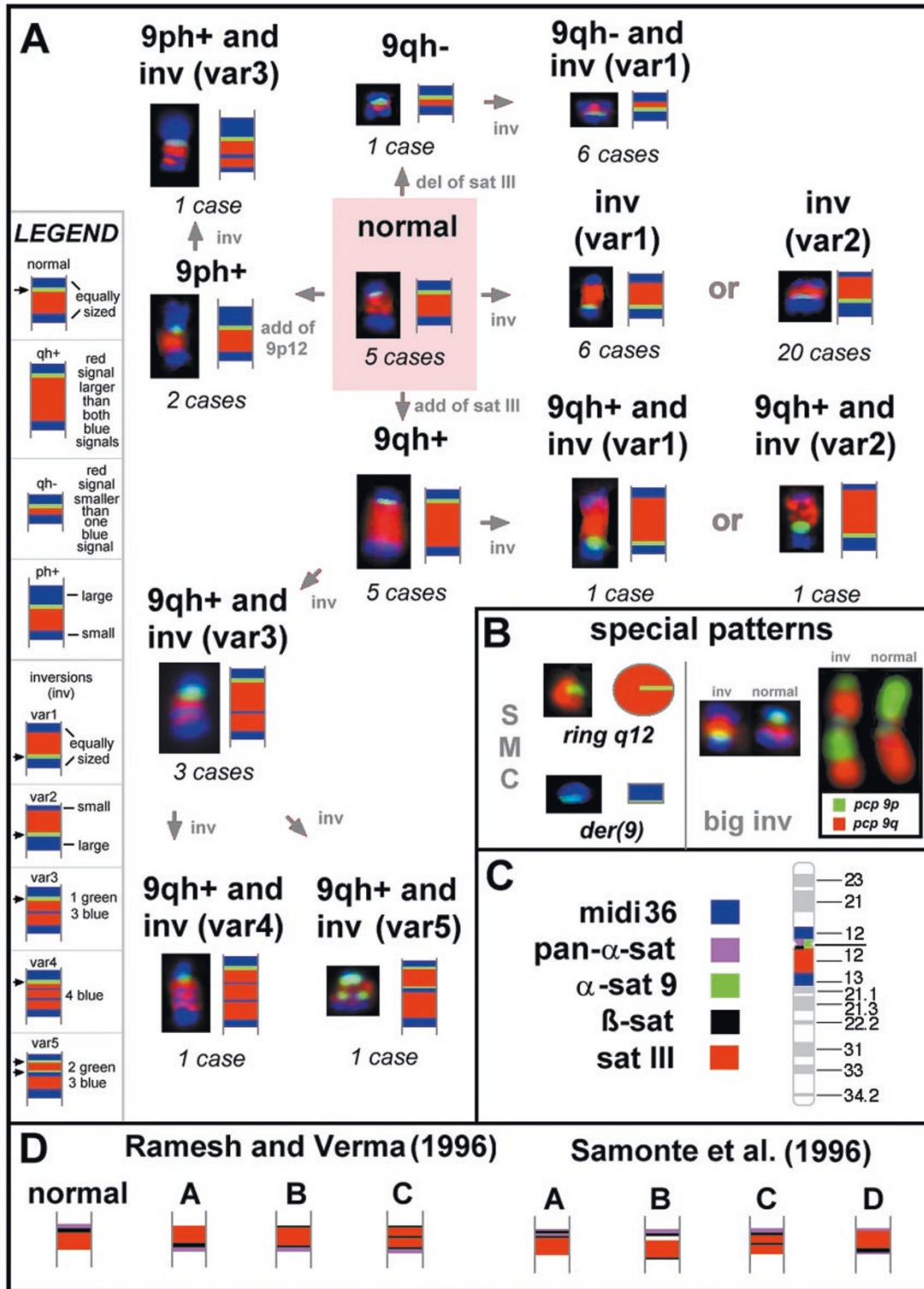


Figure 2 Representative FISH patterns and schematic representative of heteromorphous patterns observed in the study. (A) The 12 observed heteromorphous patterns and a 'normal' variant detected by a probe set of chromosome 9 specific alpha satellite DNA (green), classical satellite III DNA (red) and midi36 (blue) are shown. On the left hand side, the Legend shows a schematic version of the normal, qh+, qh-, ph+, and five variant types of inversion (inv var1 – 5). The number of cases detected with the corresponding heteromorphous pattern is given below each group. The suggested type of rearrangement leading to the 12 heteromorphous pattern is given in grey near

In case 16 two additional signals for the probe midi36 were present in the long arm of chromosome 9. This pattern can be explained by the occurrence of a second inversion (after the var3 inversion), with breakpoints in proximal 9q12 and the midi36 positive extra band (inversion type 4=var4) (see Figure 2A). The hybridisation patterns found in case 17 are also most likely due to a second inversion following a var3 inversion. In this case the breakpoints are likely to be in 9q11 and the midi36 positive extra band on the var3, leading to split signals within the α satellite region (inversion type 5=inv5) (see Figure 2A).

Additional aberrant pattern

There were three cases of abnormalities detected in apparently healthy individuals. The first (case 54), was discovered to have an exceptionally large pericentric inversion with breakpoints in 9p24.1 and 9q31.2 (Figure 2B). Although the true constitution of this rearrangement could not be elucidated using our probe set, the observed pattern was similar to an inversion type var1 (Figure 2B). The second, an SMC detected in a healthy women (case 55), was characterised as a ring chromosome comprised exclusively of α -satellite and satellite III DNA (ring q12) (see Figure 2B). The third was the SMC of case 56, described above.

A recently developed multicolour banding approach^{11,13} using a chromosome 9 specific probe set¹¹ was applied to analyse the inversion type 3 in case 14 (Figure 1G). The MCB results confirmed results obtained with the set of three chromosome 9 DNA repeat probes (Figure 2A).

Mechanism of formation of 9q heteromorphisms

Six potential breakpoints are postulated within the two regions stained by the probe midi36: a proximal, a centric and a distal breakpoint in 9p12 and analogous regions in 9q13-21.1. Therefore, nine different breakpoint combinations are possible, three of which could result in the 'var1' and two in the 'var2' heteromorphic pattern (Figure 3A,B respectively). Only one breakpoint combination could result in a pattern similar to that observed in the 9ph+ cases (Figure 3C). It is possible that the enlarged signal in 9q13-21.1 corresponding to the midi36 probe in the 9ph+ cases 50, 51 and 53 is an artifact due to flaring effects of the microdissection-derived probe, previously described by Speicher *et al.* for whole chromosome painting probes.¹⁴ However, due to the consistency of the signal size we

believe that this reflects amplification of 9p12 in 9ph+ karyotypes. Three of the possible breakpoint combinations lead to signal patterns not observed in the present study (Figure 3D,E). It remains to be determined if these variants exist or if there is a specific mechanism that prohibits their formation. Additionally, a combination of the postulated centric breakpoint in 9q13-21.1 and a breakpoint in 9q12 would lead to the inversion type 'var3'. This was present in four cases: three of which were 9qh+ (cases 13-15) and one a 9ph+ (case 53). The inversion types 9qh+/var4 and 9qh+/var5 can easily be traced from 9qh+/var3 by postulating breaks in the proximal region of 9q13-21.1 and 9q12 or in 9q12 and 9q10, respectively (Figure 3F-H).

Discussion

Due to their paralogous organization, multiple gaps are still present in the primary sequence of the pericentromeric regions of chromosome 9.⁸ However, even without specific knowledge of the DNA sequence, molecular cytogenetic studies such as the one presented here can provide important information. The present study has confirmed the homology between 9p12 and 9q13-21.1 reported by other investigators^{15,16} (Figure 2B). The identification of 9qh+ individuals with an additional GTG-positive and CBG-negative band in the satellite III region at 9q12 (Figure 1C1) has also been reported previously.^{17,18} However, the observation that the midi36 (9p12) and midi54 (9q13-21.1) probes cross-hybridise with the short arms of the acrocentric chromosomes is a new finding, and suggests that these regions might be evolutionarily related. These observations confirm other evolutionary data concerning *Homo sapiens* (HSA) chromosome 9 homologues in primates.¹¹ HSA 9 developed from an acrocentric ancestor by a pericentric inversion with breaks in regions homologous to 9p24.3 and 9q13.¹¹ During further evolution a satellite III DNA block may have been inserted *de novo* into the DNA block stained by the probe midi36, and in some cases, it was subsequently amplified (9qh+) or diminished (9qh-) (Figure 4). The finding of homology between the 9p12/9q13-q21.1 regions and acrocentric short arms suggests that these regions might be more prone to chromosomal rearrangements. However, a search of the literature has shown that this is not the case, with only a single report of translocations involving these regions.¹⁹⁻²²

This study has identified 12 heteromorphic patterns of the pericentromeric region of chromosome 9, in addition

the arrows. (B) Special hybridization patterns observed in the ring chromosome of case 55 and the derivative chromosome 9(p10q12) of case 56. The hybridisation pattern of the large inv(9)(p24.1q31.2) of case 54 (big inv) using the probe set of (A) (left) and partial chromosome painting probes specific for 9p (green) and 9q (red) is also shown. (C) Localisation of the probes used in the present study and in former studies. (D) Summary of the heteromorphic pattern detected in the studies of Ramesh and Verma⁶ and Samonte *et al.*⁷ Abbreviations: inv=inversion; add of sat III=addition of material; del=deletion of material; sat III=classical satellite III DNA; pan- α -sat=pan-alpha-satellite DNA probe; α -sat 9=chromosome 9 specific alpha-satellite DNA probe; β -sat=chromosome 9 specific beta satellite DNA probe; sat III=satellite III DNA probe.

Table 1 Clinical details of patients included in the study and heteromorphic patterns identified after cytogenetics and FISH

Case	Sex	Clinical status	GTG/CGB pattern	FISH pattern
1	M	healthy	normal	normal
2	M	healthy	normal	normal
3	F	healthy	normal	normal
4	M	healthy	normal	normal
5	F	healthy	normal	normal
6	M	healthy	9qh-	9qh-
7	M	healthy-ICSI	9qh+	9qh+
8	F	healthy-ICSI	9qh+	9qh+
9	F	healthy-ICSI	9qh+	9qh+
10	M	healthy-ICSI	9qh+	9qh+
11	F	healthy	9qh+	9qh+
12	M	healthy	9qh+	9qh+ and inv (var1)
13	M	healthy-ICSI	9qh+/extra band	9qh+ and inv (var3)
14	F	healthy	9qh+/extra band	9qh+ and inv (var3)
15	F	healthy-ICSI	9qh+/extra band	9qh+ and inv (var3)
16	F	healthy-ICSI	9qh+/extra band	9qh+ and inv (var4)
17	F	healthy-ICSI	9qh+/extra band	9qh+ and inv (var5)
18	F	healthy	inv	9qh- and inv (var1)
19	M	hyposomia	inv	9qh- and inv (var1)
20	M	healthy	inv	9qh- and inv (var1)
21	F	healthy	inv	9qh- and inv (var1)
22	M	ICSI due to andrological sterility	inv	9qh- and inv (var1)
23	M	psychomotor retardation	inv	9qh- and inv (var1)
24	M	intersexual genitals	inv	inv (var1)
25	M	adipositas	inv	inv (var1)
26	M	psychomotor retardation	inv	inv (var1)
27	F	healthy-habitual abortions	inv	inv (var1)
28	F	healthy	inv	inv (var1)
29	F	healthy	inv	inv (var1)
30	F	healthy-habitual abortions	inv	inv (var2)
31	M	healthy	inv	inv (var2)
32	M	psychomotor retardation	inv	inv (var2)
33	F	healthy-ICSI	inv	inv (var2)
34	M	fra X (?)	inv	inv (var2)
35	F	healthy-ICSI	inv	inv (var2)
36	M	healthy	inv	inv (var2)
37	F	healthy	inv	inv (var2)
38	M	psychomotor retardation	inv	inv (var2)
39	F	healthy-ICSI	inv	inv (var2)
40	F	healthy-ICSI	inv	inv (var2)
41	F	healthy	inv	inv (var2)
42	M	psychomotor retardation	inv	inv (var2)
43	M	healthy	inv	inv (var2)
44	M	hyposomia	inv	inv (var2)
45	F	healthy-habitual abortions	inv	inv (var2)
46	F	healthy	inv	inv (var2)
47	F	healthy	inv	inv (var2)
48	F	healthy	inv	inv (var2)
49	M	psychomotor retardation	inv	inv (var2)
50	F	healthy	9ph+	9ph+
51	F	healthy-ICSI	9ph+	9ph+
52	F	healthy	9ph+	9qh+ and inv (var2)
53	M	psychomotor retardation	9ph+	9ph+ and inv (var3)
54	M	healthy	inv	inv(9)(p24.1q31.2)
55	F	healthy-marker in 75% of metaphases	mar	r(9)(q12)
56	M	prenatal-marker in 25% of metaphases	mar (=midi36) 9qh+	der(9)(q10p12;q13q21.1) 9ph+ and inv (var2)

Abbreviations: extra band=CBG-negative/GTG-positive extra-band; ICSI=intra-cytoplasmic sperm injection; the other abbreviations are according to ISCN 95.³

to the 'normal' variant (Figures 2A and 3). It is difficult to compare the heteromorphic patterns detected in the present study with other studies from the literature, due

to the variety of techniques used, many of which lacked the resolution presented in this investigation. The inv(9)(p11q13) types var1 and var2 have not been described

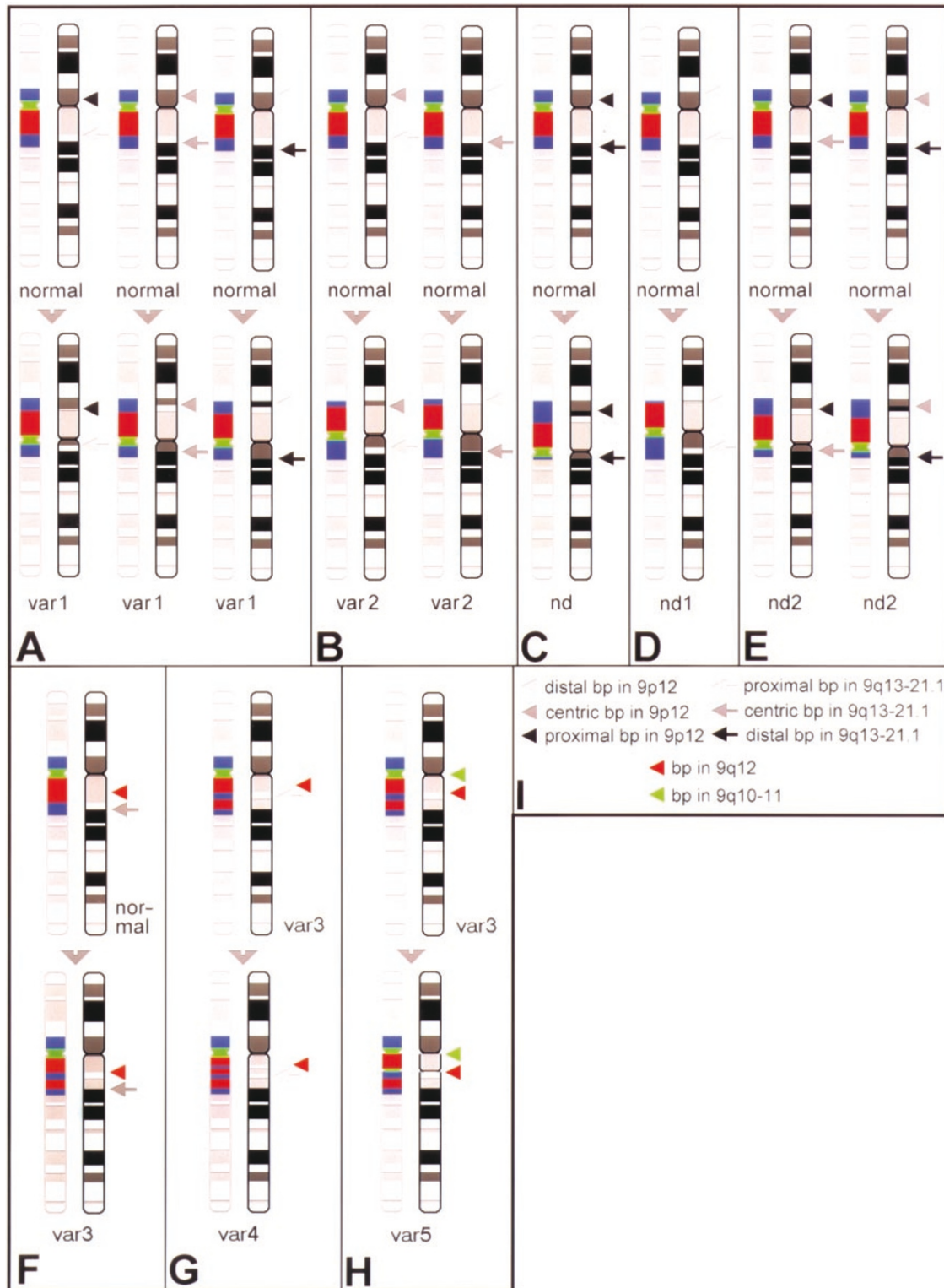


Figure 3 Mechanisms of formation of heteromorphisms observed. Six theoretically possible breakpoints in the two regions stained by the probe midi36 are postulated: a proximal (black arrowhead), centric (dark-grey arrowhead) and distal breakpoint (light-grey arrowhead) in 9p12, and a proximal (light-grey arrow), centric (dark-grey arrow) and distal breakpoint (black arrow) in 9q13-21.1. Therefore, nine different breakpoint-combinations are possible (A–E). Additionally, breakpoints in 9q12 (green arrowhead) and in the alpha-satellite region (blue arrowhead) are postulated (see I). In F–H the postulated breakpoints for the development of the inversion types var3, var4 and var5 are given (for further details refer to the text). (Abbreviations: bp=breakpoints; nd, nd1, nd2=different possible variants which have not been detected in the present study).

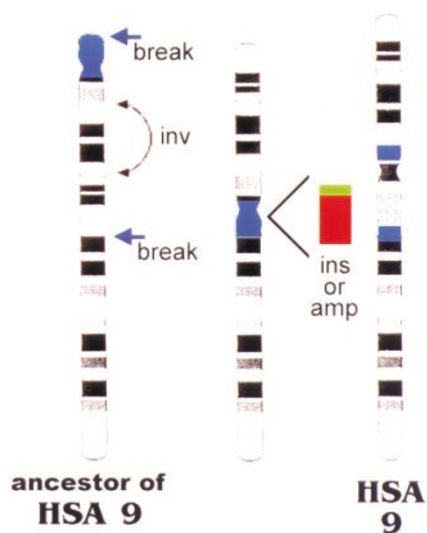


Figure 4 Development of human chromosome 9 (HSA 9) from its presumed ancestor. HSA=*homo sapiens*. Schematic representation of the origin of HSA 9, based on hybridization of the midi54 probe to chromosomes of the *Gorilla gorilla* (GGO).¹¹ The p-arm of acrocentric GGO 13 (homologue to HSA 9) has homologous sequences to the short arm of all human acrocentric chromosomes,¹¹ the region stained by midi36 (blue). A pericentric inversion (inv) of GGO 13, with breaks in regions homologous to 9q24.3 and 9q13 leads to a human chromosome 9 banding pattern. During evolution human specific α -satellite DNA (red) has developed and inserted (ins) together with the satellite III DNA block (green).

before. However, the unusual chromosome 9 polymorphism described by Fryns *et al.*²³ in a healthy individual may be similar to the one found in our case 12 (9qh+ and inversion type var1). The heteromorphic patterns identified by Ramesh and Verma⁶ and Samonte *et al.*⁷ were detected with FISH using a pan- α -satellite and a chromosome 9 specific β -satellite DNA probe (see Figure 2D). However, as the β -satellite probe used in these studies is no longer available, we can only speculate that our inversions type var1 and var2 could be subdivided into the same subtypes. An acquired inv(9)(p11q13) has been described in essential thrombocythemia,²⁴ but the type of inversion is not evident from the literature.

Healthy individuals with a 9ph+ variant have been reported. In some of these (eg Family D in the study of Wang and Miller²⁵), the additional material in 9p originates from the satellite III DNA, similar to case 52 in the present study. The cases described by Webb *et al.*²⁶ show more similarity to cases 50, 51 and 53 of the present study. Interestingly, Webb *et al.*²⁶ detected an amplification of the 9ph+ region transmitted through all generations. Reddy,²⁷ Knight *et al.*²⁸ and Jalal *et al.*²⁹ all report healthy carriers with a CBG negative 9qh+ variant. This variant was not detected in the present study. The five cases of 9qh+ with simple amplification of satellite III DNA are simi-

lar to previously reported cases.³⁰ Published cases of individuals with a GTG-positive/CBG-negative extra band in 9qh+^{27,31–37} probably correspond to 9qh+ and inversion of type (i) var3, (ii) var4 or (iii) var5. This study therefore indicates that the incidence of pericentric inversions involving the heterochromatin may be higher than the suggested 1.5%.³⁸ Moreover, the results presented here indicate that the conclusions of Wojiski *et al.*³⁶ and Park *et al.*¹⁵ that the additional material in the extra band in 9q12 is derived from 9p12 (based on the hybridisation pattern of YAC 878e3) are incorrect – it also could be derived from 9q13-21.1. Several variants have been described that do not fit any of the heteromorphic patterns described here. Verma *et al.*³⁹ described an inverted duplication 9qh in a prenatal case. Inverted duplications of 9p10-q12 were also reported by Wang and Miller²⁵ and Macera *et al.* (Type II)⁵ using FISH with a pan- α -satellite probe and a chromosome 9-specific β -satellite DNA probe. The inversion type C described by Samonte *et al.*⁷ is similar to our 9qh+ with inversion type var3, while their types A, B and D were not detected in our study.

A possible connection between the 9ph polymorphisms and reproductive problems has been postulated from the beginning of the banding era^{2,3,4} up to the present day.^{40,41} In our study, one couple (case 13 and 17) that underwent intra-cytoplasmic sperm injection (ICSI) for fertility problems carried a 9qh+ combined with inversion(s) (var3 and var5) in both partners. Furthermore, four of the five cases with 9qh+ and an extra band in 9q12 (including the couple mentioned before) reported fertility problems. Additionally, 17 out of the 47 cases studied with heteromorphism of chromosome 9 (36%) were referred for chromosome analyses because of reproductive failure or reduced fertility (ie repeated foetal miscarriages and/or ICSI patients). In a control group of 100 patients referred for cytogenetic analysis because of reproductive failure in Jena between December 2000 and January 2001, the corresponding percentage of cases without heteromorphism of chromosome 9 was 24%, providing further evidence for a possible correlation. Even more strikingly, 13 out of the 17 cases with heteromorphism of chromosome 9 referred because of reproductive failure were female (see Table 1). In contrast, the risk to male inv(9)(p11q13) carriers of producing chromosomally abnormal offspring or spontaneous abortions was not increased in the study by Colls *et al.*⁴⁰ There are many and varied clinical conditions reported to have an association with pericentric inversion 9 including mental retardation,⁴² schizophrenia,⁴³ the Walker-Warburg syndrome,⁴⁴ the oculo-auriculo-vertebral (Goldenhar) spectrum⁴⁵ and cancer predisposition.⁴⁶ To date therefore, the clinical significance of the chromosome 9 heteromorphism still remains to be clarified.

The majority of the heteromorphic patterns revealed in this study (apart from 9qh+ or 9qh– copy number variations of classical satellite III DNA) can be explained by

simple inversions with breakpoints in the repetitive elements of the regions 9p12 and 9q13-21.1 or in the satellite III region (Figure 3). The finding that the majority of these breakpoints are within repetitive sequence regions is important, and may explain why such inversions do not have any clinical consequences. There is also evidence to suggest hotspots for recombination in these chromosomal sections (see Figure 3). Large DNA sequence duplications flanking the centromere of human chromosome 10 and promoting pericentric inversions have been described recently.⁴⁷ It is possible that sequence similarities of this type could form the molecular basis for inversions involving chromosome 9 as well. However, the large amount of repetitive DNA at the centromere of chromosome 9 has so far hampered mapping and sequencing efforts.⁸

In summary, 12 heteromorphic patterns and the normal variant of the pericentric region of chromosome 9 are described. Previous studies attributed the extensive degree of variability in this region to unequal meiotic exchanges involving the various classes of repetitive sequences in the centromeric region^{5,7,25} and to unequal exchanges involving stretches of homologous DNA sequences in the proximal short and long arms of chromosome 9.¹⁵ The results of the present study would support such an assumption. The microdissection-derived probes described here and elsewhere¹⁶ do not permit the discrimination between the 9p12 and 9q13-21.1 regions, and the origin and exact composition of the region 9p12/9q13-21.1 remains unclear. However, the enigma of the region 9p12/9q13-21.1 has to some extent been clarified by the observation that these regions are homologous to the short arms of the acrocentric chromosomes. Significantly, this study has also indicated the presence of breakpoint clustering in the regions stained by the microdissection-derived probe midi36.

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References

- 1 Verma RS. A reply: pericentric inversion of chromosome 9qh are 'real' but the mechanisms of their origin are highly complex. *Hum Genet* 1999; **105**: 183–184.
- 2 Verma RS *Heterochromatin: molecular and structural aspects*. New York: Cambridge University Press, 1988, pp 276–299.
- 3 Mitelman F. *ISCN 1995: An international system for human cytogenetic nomenclature*. Basel: S. Karger, 1995, pp 1–115.
- 4 Gardner RJM, Sutherland GR: Chromosome abnormalities and genetic counseling. In: *Oxford monographs on medical genetics No. 29*. New York and Oxford: Oxford University Press, 1996, pp 139–158.

- 5 Macera MJ, Verma RS, Conte RA, Bialer MG, Klein VR: Mechanisms of the origin of a G-positive band within the secondary constriction region of human chromosome 9. *Cytogenet Cell Genet* 1995; **69**: 235–239.
- 6 Ramesh KH, Verma RS: Breakpoints in alpha, beta, and satellite III DNA sequences of chromosome 9 result in a variety of pericentric inversions. *J Med Genet* 1996; **33**: 395–398.
- 7 Samonte RV, Conte RA, Ramesh KH, Verma RS: Molecular cytogenetic characterization of breakpoints involving pericentric inversions of human chromosome 9. *Hum Genet* 1996; **98**: 576–580.
- 8 Horvath JE, Bailey JA, Locke DP, Eichler EE: Lessons from the human genome: transitions between euchromatin and heterochromatin. *Hum Mol Genet* 2001; **10**: 2215–2223.
- 9 Senger G, Weimer J, Claussen U, Chuboda I: Microdissection and reverse chromosome painting; In: Wegner RD (ed): *Diagnostic Cytogenetics; Springer Lab Manual*. Heidelberg: Springer Verlag, 1999, pp 356–375.
- 10 Senger G, Chudoba I, Plesch A: Multicolor-FISH—the identification of chromosome aberrations by 24 colors. *BIOforum* 1998; **9**: 499–503.
- 11 Mrasek K, Heller A, Rubtsov N *et al*: Reconstruction of the female Gorilla gorilla karyotype by Zoo-FISH using 25-color FISH and multicolor banding (MCB). *Cytogenet Cell Genet*; **93**: 242–248.
- 12 Liehr T, Thoma T, Kammler K *et al*: Direct preparation of uncultured EDTA-treated or heparinized blood for interphase FISH analysis. *Appl Cytogenet* 1995; **21**: 185–188.
- 13 Liehr T, Heller A, Starke H, Rubtsov N, Trifonov V, Mrasek K, Weise A, Kuechler A, Claussen U: Microdissection based high resolution multicolor banding for all 24 human chromosomes. *Int J Mol Med* 2002; **9**: 335–339.
- 14 Speicher MR, Gwyn Ballard S, Ward DC: Karyotyping human chromosomes by combinatorial multi-fluor FISH. *Nat Genet* 1996; **12**: 368–375.
- 15 Park JP, Wojcicki SA, Spellman RA, Rhodes CH, Mohandes TK: Human chromosome 9 pericentric homologies: implications for chromosome 9 heteromorphisms. *Cytogenet Cell Genet* 1998; **82**: 192–194.
- 16 Müller-Navia J, Nebel A, Schleiermacher E: Complete and precise characterization of marker chromosomes by application of microdissection in prenatal diagnosis. *Hum Genet* 1995; **96**: 661–667.
- 17 Luke S, Verma RS: Genetic consequences of 'euchromatic' band within 9qh region. *Am J Med Genet* 1993; **45**: 107.
- 18 Hoo JJ, Szego K, Wong P, Roland B: Evidence of chromosome 9 origin of the euchromatic variant band within 9qh. *Clin Genet* 1993; **43**: 309–311.
- 19 Levy B, Dunn TM, Hirschhorn K, Kardon N: Jumping translocations in spontaneous abortions. *Cytogenet Cell Genet* 2000; **88**: 25–29.
- 20 Gebhart E, Liehr T: Patterns of genomic imbalances in human solid tumors (Review). *Int J Onc* 2000; **16**: 383–399.
- 21 Bleichert A, Fiedler W, Claussen U *et al*: A long distance-PCR derived FISH probe detects a deletion between p15 and p16 in CML and T-ALL patients. *Int J Mol Med* 2001; **7**: 591–595.
- 22 Schinzel A: *Catalogue of unbalanced chromosome aberrations in man*. Berlin, New York: W. de Gruyter, 2001, pp. 1–966.
- 23 Fryns JP, Kleczkowska A, Londers L, van den Berghe H: Unusual chromosome 9 polymorphism and reproductive failure. *Ann Genet* 1985; **28**: 49–51.
- 24 Wan TS, Ma SK, Chan LC: Acquired pericentric inversion of chromosome 9 in essential thrombocythemia. *Hum Genet* 2000; **106**: 669–670.
- 25 Wang JC, Miller WA: Molecular cytogenetic characterization of two types of chromosome 9 variants. *Cytogenet Cell Genet* 1994; **67**: 190–192.
- 26 Webb GC, Krumin EJ, Eichenbaum SZ, Voullaire LE, Earle E, Choo KH: Non C-banding variants in some normal families might be homogeneously staining regions. *Hum Genet* 1989; **82**: 59–62.

- 27 Reddy KS: Variants of chromosome 9 with additional euchromatic bands: two case reports. *Am J Med Genet* 1996; **64**: 536–538.
- 28 Knight LA, Soon GM, Tan M: Extra G positive band on the long arm of chromosome 9. *J Med Genet* 1993; **30**: 613.
- 29 Jalal SM, Kukolich MK, Garcia M, Day DW: Euchromatic 9q+ heteromorphism in a family. *Am J Med Genet* 1990; **37**: 155–156.
- 30 Hansmann I: Structural variability of human chromosome 9 in relation to its evolution. *Hum Genet* 1976; **31**: 247–262.
- 31 Madan K: An extra band in human 9qh+ chromosomes. *Hum Genet* 1978; **43**: 259–264.
- 32 Berg JM, Gardner HA, Gardner RJ *et al*: Dic(21;21) in a Down's syndrome child with an unusual chromosome 9 variant in the mother. *J Med Genet* 1980; **17**: 144–148.
- 33 Donlon TA, Magenis RE: Structural organization of the heterochromatic region of human chromosome 9. *Chromosoma* 1981; **84**: 353–363.
- 34 Silengo MC, Davi GF, Franceschini P: Extra band in the 9qh+ chromosome in a normal father and in his child with multiple congenital anomalies. *Hum Genet* 1982; **60**: 294.
- 35 Docherty Z, Hulten MA: Extra euchromatic band in the qh region of chromosome 9. *J Med Genet* 1985; **22**: 156–157.
- 36 Wojiski SA, Rhodes CH, Brodhurst CA, Mohandas TK, Park JP: The G positive band of the rare euchromatic 9qh variant is derived from 9p12. *Appl Cytogenet* 1997; **23**: 125–129.
- 37 Luke S, Verma RS, PeBenito R, Macera MJ: Inversion-duplication of bands q13–q21 of human chromosome 9. *Am J Med Genet* 1991; **40**: 57–60.
- 38 Madan K, Bobrow M: Structural variation in chromosome no. 9. *Ann Génét* 1974; **17**: 81–86.
- 39 Verma RS, Luke S, Brennan JP, Mathews T, Conte RA, Macera MJ: Molecular topography of the secondary constriction region (qh) of human chromosome 9 with an unusual euchromatic band. *Am J Hum Genet* 1993; **52**: 981–986.
- 40 Colls P, Blanco J, Martinez-Pasarell O *et al*: Chromosome segregation in a man heterozygous for a pericentric inversion, inv(9)(p11q13), analyzed by using sperm karyotyping and two-color fluorescence in situ hybridization on sperm nuclei. *Hum Genet* 1997; **99**: 761–765.
- 41 Sasagawa I, Ishigooka M, Kubota Y, Tomaru M, Hashimoto T, Nakada T: Pericentric inversion of chromosome 9 in infertile men. *Int Urol Nephrol* 1998; **30**: 203–207.
- 42 Cora T, Demirel S, Acar A: Chromosomal abnormalities in mentally retarded children in the Konya region–Turkey. *Genet Couns* 2000; **11**: 53–55.
- 43 Miyaoka T, Seno H, Itoga M, Ishino H: A case of small cerebral cyst and pericentric inversion of chromosome 9 that developed schizophrenia-like psychosis. *Psychiatry Clin Neurosci* 1999; **53**: 599–602.
- 44 Zenker M, Dorr HG: A case associated with Walker Warburg syndrome phenotype and homozygous pericentric inversion 9: coincidental finding or aetiological factor? *Acta Paediatr* 2000; **89**: 750–751.
- 45 Stanojevic M, Stipoljev F, Koprčina B, Kurjak A: Oculo-auriculo-vertebral (Goldenhar) spectrum associated with pericentric inversion 9: coincidental findings or etiologic factor? *J Craniofac Genet Dev Biol* 2000; **20**: 150–154.
- 46 Milan C, Lamberti L: Study of C-polymorphisms of chromosomes 1, 9 and 16 in lymphocytes of patients with laryngeal carcinoma. *Boll Soc Ital Biol Sper* 1996; **72**: 187–194.
- 47 Jackson MS, Rocchi M, Thompson G *et al*: Sequences flanking the centromere of human chromosome 10 are a complex patchwork of arm-specific sequences, stable duplications and unstable sequences with homologies to telomeric and other centromeric locations. *Hum Mol Genet* 1999; **8**: 205–215.