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D. F. Callen · H. Eyre · S. McDonnell
S. Schuffenhauer · K. Bhalla

A complex rearrangement involving simultaneous translocation and inversion is associated with a change in chromatin compaction

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Abstract Detailed fluorescence in situ hybridisation analysis of a previously described translocation revealed it to be a more complex rearrangement consisting of both a translocation and a paracentric inversion with an apparent coincident breakpoint at 16p13.3, t(14;16)(p32;p13.3) inv16(p13.3p12.1). This unusual three-breakpoint rearrangement was not obvious from examination of G-banding. Such rearrangements may be undiagnosed in cytogenetic studies. The presence of an interstitial deletion of 16p was unlikely as the rearranged chromosome contained probes distributed along the short arm of chromosome 16. Fluorescence in situ hybridisation studies suggested that the inverted segment was smaller in size than that on the normal chromosome. Measurements of distances between probes on metaphase chromosomes confirmed that there was differential compaction of the inverted portion on 16p. The inverted region was significantly reduced in size by 21% compared with the same region on the normal chromosome 16. The size reduction across the region was non-uniform, with one region showing a 55% increase in compaction. The change in compaction was also associated with a change in the lateral position of a probe on the chromatids. The finding that a single chromosome breakpoint can change the compaction of chromatin over an extensive region has implications for models of the structure of metaphase chromosomes. Possible explanations are either a localized severe disruption of DNA packaging over relatively short distances (hundreds of kilobases) or a more generalized change that extends over many megabases. These results raise the important possibility that chromosome breaks may result in a more global change in DNA compaction across large segments of a chromosome.

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D.F. Callen (✉) · H. Eyre · S. McDonnell · K. Bhalla
Department of Cytogenetics and Molecular Genetics,
Women's and Children's Hospital, 72 King William Road,
North Adelaide, South Australia 5006, Australia

S. Schuffenhauer
Department of Paediatric Genetics, Ludwig-Maximilians
University, Munich, Germany

D.F. Callen
Present address: Bionomics Ltd., 31 Dalgleish Street,
Thebarton, South Australia 5031, Australia,
e-mail: dcallen@bionomics.com.au

Introduction

As mitosis proceeds the chromatin is progressively condensed. This process has been exploited in high-resolution G-banding. By analysing G-banding of chromosomes in late prophase to early metaphase an increased resolution is achieved due to the higher number of bands. The increase in the number of bands is related to less chromatin condensation resulting in more attenuated chromosomes. Studies of G-bands at progressively increasing chromosome condensation show that the relative compaction of the chromosome is not uniform. In the transition from prophase to metaphase the rate of contraction is higher in G-band-negative regions compared with G-band-positive regions (Francke and Oliver 1978). The banding pattern is thought to reflect the underlying folding of the chromatin. The presence of a consistent banding pattern and the consistency in the relative length of chromosomes suggests that the process of chromatin compaction is highly ordered (Koshland and Strunnikov 1996).

The basic organisation of chromatin is the nucleosome, which consists of 145 bp of DNA wound on an octomer of histones. Linker DNA, which varies in length, connects the nucleosomes (Kornberg and Lorch 1999). Various models (reviewed in Daban 2000) have been proposed for the folding of the chain of nucleosomes to form a chromatin fibre 30–40 nm in diameter. To allow further compaction of these chromatin fibres into metaphase chromosomes, additional folding is required. Again, a variety of models have been proposed (Daban 2000). A model containing loops arising from

scaffold attachment sites (SARs) is consistent with present observations (Hart and Laemmli 1998). The SARs are AT-rich regions of DNA and compaction is a consequence of association of these SARs in a linear array. The dark G-bands are AT-rich, contain fewer genes and are generally late replicating. The characteristics are consistent with more tightly coiled and folded DNA due to the greater frequency of the SARs. The characteristics of R-bands (pale G-bands), AT-poor, early replicating and gene-rich, are consistent with a more open chromatin conformation due to fewer SARs resulting in longer loops.

In this report, we present a unique chromosome rearrangement that is associated with a change in the packaging of chromatin over large physical distances. These observations have implications for models of chromatin compaction in metaphase chromosomes.

Materials and methods

Identification of clones

A bacterial artificial chromosome (BAC) contig containing b545E8, b3052B24, b441K8, b192K18 and b26O3 was constructed across the 16p13.3 breakpoint of the t(14;16) (Bhalla et al., unpublished). Two additional P1 bacterial artificial chromosomes (PAC) clones, p102J11 and p722G3, were isolated from screening the human genomic PAC filters of Genome Systems (St. Louis, Mo.) and BAC/PAC resources (Buffalo, N.Y.), respectively. The BAC clones located from 16p12.2 to 16p11.2 were from the physical map of Cao et al. (1999). Clones were purchased from Research Genetics (USA) and kindly provided by Dr. Norman Doggett (Los Alamos National Laboratories, Los Alamos, USA) and Dr. Mei Wang (California Institute of Technology, Pasadena, USA).

Isolation of DNA

Clones were grown at 37°C in LB medium containing 50 µg/ml of kanamycin (cosmids and PACs) or 30 µg/ml of chloramphenicol (BACs). DNA was isolated using Qiagen 100 columns (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Fluorescence in situ hybridisation (FISH)

For use as FISH probes, cosmids, PACs and BACs were labelled by nick translation with biotin-14-dATP (Gibco-BRL), SpectrumGreen or SpectrumOrange (Vysis). Metaphase chromosomes were harvested by standard methods from lymphoblastoid cultures. Fluorescence in situ hybridisation of single probes was performed as previously described (Callen et al. 1990), except that chromosomes were mounted prior to analysis in antifade containing propidium iodide (as counterstain) and 4′,6-diamidino-2-phenylindole (DAPI) (for chromosome identification). For dual-colour FISH, SpectrumOrange- and SpectrumGreen-labelled probes were codesiccated, resuspended in hybridisation mixture, then denatured and hybridised to metaphase chromosomes using a Hybaid PCR Express flat block (Integrated Sciences). For chromosome painting, commercially prepared whole chromosome paints for chromosome 14 [Cambio, digoxygenin (DIG)-labelled] and chromosome 16 (Cambio, biotin-labelled) were hybridised to metaphase chromosomes according to manufacturer's instructions and detected with tetramethylrhodamine isothiocyanate-anti-DIG for chromosome 14, and avidin-fluorescein isothiocyanate for chromosome 16. Images of metaphase chromosome preparations were captured using a cooled CCD camera using the Chromoscan image collection and enhancement system (Applied Imaging Corporation). The FISH signals and the DAPI banding patterns were merged for figure preparation. Measurement of distances between fluorescently labelled probes was achieved by capturing the image of the metaphase and using the appropriate Chromoscan supplied software. The distance was measured by drawing a line between the probes that bisected each of the lines joining the two chromatid signals for each probe. All metaphases were measured at the same magnification and distances were expressed in arbitrary units. The lateral position of probes was determined by assessing whether the position of the probe signal for each chromatid was external, medial or internal to the chromatid.

Case report

The male subject was born after an uneventful pregnancy and labour. During the first three months of life he had feeding difficulties and abdominal pain. He sat at 8 months and walked at 18 months. At 14 years of age his speech was normal and he was in a special school having been assessed as having mild mental retardation. He had grand mal seizure at the age of 5 and 7 years, and a further two from 7 to 14 years. Short absence seizures were also noted at about monthly intervals. Anticonvulsive therapy was initiated at age 7 years. At the age of 7 years he was in the 97th percentile for height, weight and head circumference. His motor abilities were retarded and there were behavioural difficulties. His EEG was normal. Clinodactyly of the 5th finger and mild syndactyly of the 2nd and 3rd toes were the only dysmorphisms.

Results

Resolution of rearrangement

Classical G-banding of chromosomes from a patient with moderate mental retardation demonstrated a de novo translocation between chromosomes 14 and 16, t(14;16) (q32;p13.3) (Fig. 1a). A mouse/human somatic cell hybrid, CY182, was constructed containing the der(16) (Callen et al. 1995). The order of somatic cell hybrid breakpoints has been refined to pter-23HA-CY196-CY197-CY182-CY177-CY168. Mapping of a variety of probes by PCR amplification of hybrid DNA was consistent with a location of the breakpoint at 16p13.3 between the genes DNL1 and CDG1, which are approximately 7 Mb from 16pter. Probes distributed throughout 16p were tested by either FISH to chromosomes of the patient or by PCR mapping of somatic cell hybrids, including the hybrid CY182 (Table 1). There was no evidence of any interstitial deletions of 16p proximal to the 16p13.3 breakpoint. Chromosome painting with chromosome 14 and 16 paints (Fig. 1b, c) supported the interpretation of an apparently reciprocal translocation.

This interpretation of the rearrangement was found to be incorrect when FISH was undertaken with the BAC b545E8, which is known to span the 16p13.3 breakpoint (Bhalla, unpublished). A single signal was observed at 16p13.3 on the normal chromosome 16. As predicted, the translocated 16 showed two signals, one on the der(14) and the other on the der(16). Unexpectedly, the signal of the der(16) was at 16p11.2 rather than 16p13.3 (Fig. 1d). Such a signal would be observed if the short

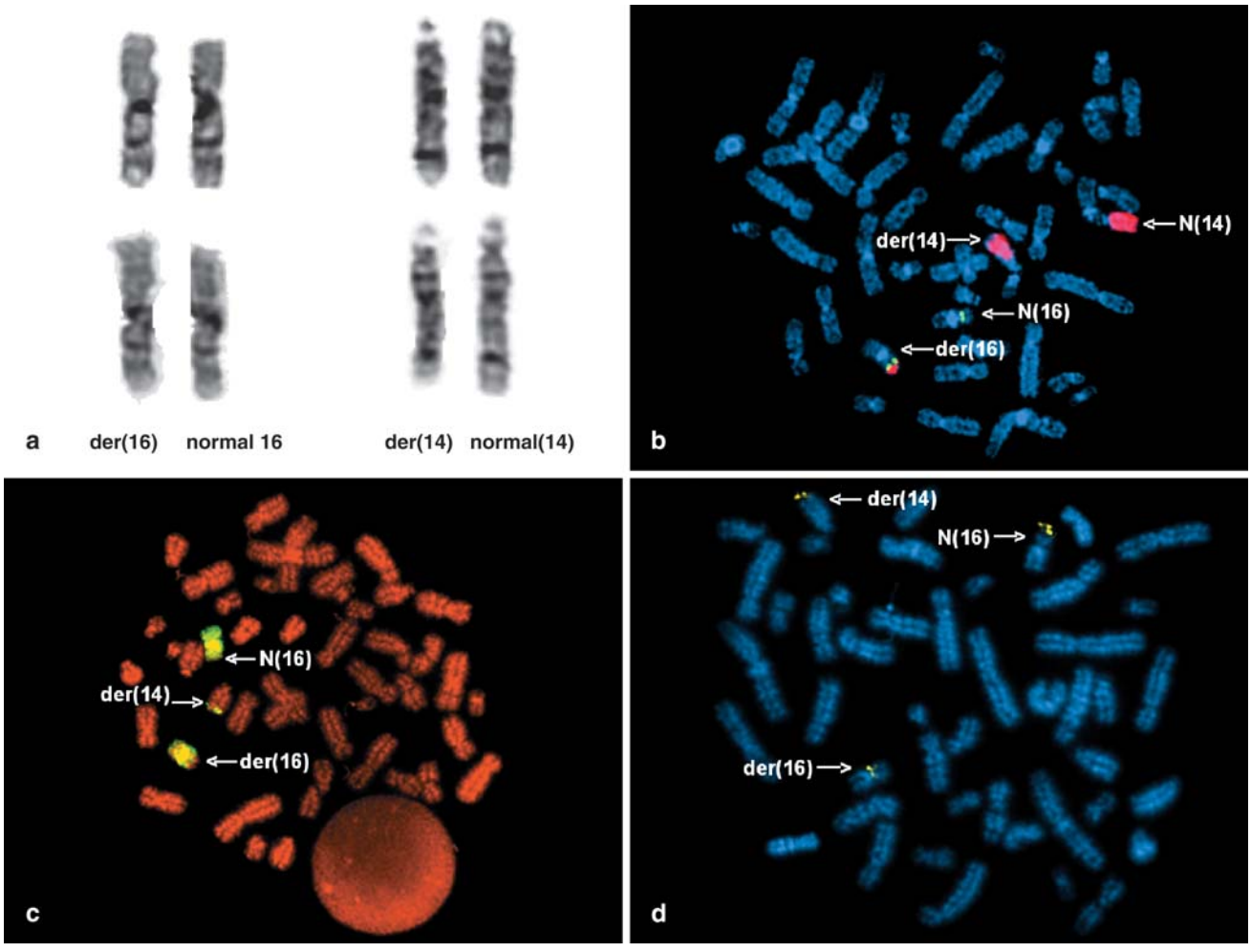

Fig. 1a–d Fluorescence in situ hybridisation (FISH) studies of the chromosome 16 rearrangement. **a** GTL-banding of the normal and derivative chromosomes 14 and 16. **b** Co-hybridisation of no. 14 paint and b268E9. No. 14 paint was detected with tetramethylrhodamine isothiocyanate (*red*). b268E9 was detected with fluorescein isothiocyanate (FITC) (*green*). **c** No. 16 paint detected with FITC (*yellow*). **d** Hybridisation signal from b545E8 on the normal chromosome 16, the derivative 16 and the derivative 14 (spanning the translocation breakpoint)

arm of chromosome 16 involved in the translocation was also involved in a paracentric inversion with breakpoints of the two rearrangements at p13.3 being either coincident or in close proximity. If not coincident the location of BACs in the region would suggest a distance between the breakpoints of less than 100 kb. The presence of an inversion was confirmed by FISH with a series of probes known to be distributed on the short arm of chromosome 16 from their location on the mouse/human somatic hybrid map (Callen et al. 1995) and from the BAC map of Cao et al. (1999) (Table 1). Five probes at 16p13.3 distal to the b545E8 were only present on the der(14).

A series of 17 probes between 16p13.3 and p11.2 were all located on the der(16) in positions relative to the centromere that were different from that on the normal 16p. These locations were consistent with the presence of a paracentric inversion. Four BACs at 16p11.2–12.1 were located at the same position on the normal and the der(16). Therefore, the proximal breakpoint of the inversion was located between the BACs A-670B5 and b2049O4. These data are consistent with a paracentric inversion of a portion of the short arm of 16 with the distal breakpoint either coincident or in close proximity to that of the translocation breakpoint at 16p13.3. The proximal breakpoint of the inversion is at 16p12.1. This complex rearrangement can be notated as t(14;16)(p32;p13.3) inv16(p13.3p12.1).

Demonstration of differential compaction

During the resolution of the 16p rearrangement by FISH it was noted that the probes at 16p12 were distributed over a smaller interval on the der(16) compared with the normal 16. It is possible that the relative spacing of probes was altered in the der(16), and this was further investigated by measurement of the distance apart of the FISH signals of three probes: A (b441K8), B (c307G2) and C (b268E9). On the normal chromosome 16, A was 25–75 kb proximal to the translocation breakpoint at

Table 1 Resolution of der(16) by fluorescence in situ hybridisation (FISH) and the polymerase chain reaction (PCR). The order of probes [b, bacterial artificial chromosomes (BACs); p, P1 BACs; c, cosmids] is given from 16pter to centromere. A, B and C refer to probes as discussed in the text. The somatic cell hybrids are as given in Callen et al. (1995). The band locations of probes were determined by direct mapping against somatic cell hybrids or by sequence homology to known mapped probes

Probe	Somatic cell hybrid interval	Chromosome 16 band	FISH signal[a]
RGS11	pter–CY14	p13.3[b]	
c77E8	CY14–CY190	p13.3	Distal
p102J11	23HA–CY196	p13.3	Distal
b375G12	23HA–CY196	p13.3	Distal
b315L9	CY196–CY197	p13.3	Distal
b118C2	CY197–CY182	p13.3	Distal
b545E8	CY197–CY182–CY177	p13.3	Spans
b441K8 (A)	CY182–CY177	p13.3	Proximal[c]
b3052B24	CY182–CY177	p13.3	Proximal[c]
b192K18	CY182–CY177	p13.3	Proximal[c]
b26O3	CY182–CY177	p13.3	Proximal[c]
c62F6	CY177–CY198	p13.2	Proximal[c]
p722G3	CY177–CY198	p13.2	Proximal[c]
c10B8	CY177–CY198	p13.2	Proximal[c]
bA-475D10	CY198–CY191	p13.13	Proximal[c]
D16S2613	CY191–CY180(D)	p13.12[b]	
D16S2570E	CY180(D)–CY19	p13.12[b]	
b962B4	CY19–CY185	p13.11	Proximal[c]
c37C6	FRA16A–CY183	p13.11	Proximal[c]
RPS15A	CY163–CY175	p12.3[b]	
c307G2 (B)	CY13–CY15	p12.3	Proximal[c]
bA-279B10	CY156–CY165	p12.2	Proximal[c]
bA-268E9 (C)	CY156–CY165	p12.2	Proximal[c]
c311D5	CY155–CY160(D)	p12.1	Proximal[c]
bA-218C7	CY155–CY160(D)	p12.1	Proximal[c]
bA-485G10	CY155–CY160(D)	p12.1	Proximal[c]
bA-670B5	CY160(D)–FRA16E	p12.1	Proximal[c]
b2049O4	FRA16E–CY12	p11.2p12.1	Proximal
bA-331G1	FRA16E–CY12	p11.2p12.1	Proximal
b761H5	FRA16E–CY12	p11.2p12.1	Proximal
bA-305A8	FRA16E–CY12	p11.2p12.1	Proximal

[a] Position of FISH signal on short arm of chromosome 16 relative to translocation breakpoint at 16p13.3. The FISH signal designated as "distal" was located on the der(14), while "proximal" was located on the der(16)

[b] Probes shown to be present on the der(16) by PCR of the somatic cell hybrid CY182. The band localisation of the probe was determined by mapping against a somatic hybrid panel of chromosome 16 (Callen et al. 1995)

[c] Signals located in a different position relative to the centromere on the der(16) compared with the normal chromosome 16 in the same metaphase

p13.3 while C was just distal to the p12.1 breakpoint of the inversion. In each metaphase measurements were made, on the normal and derivative 16, of the distance between probe A and probe C. From 32 metaphases the average distance between A and C on the der(16) was 21% smaller than the distance between the probes on the normal 16. This difference was significant (t_{31}=5.11, P<0.001). Each pair of measurements, that is the distances between probes A and C on the normal and on the der(16), are plotted in Fig. 2. As expected there is variation between metaphases in the relative length of chromosomes as a result of differential metaphase chromosome condensation. The differential compaction on the short arm of chromosome 16 was independent of chromosome condensation.

This compaction of the der(16) was investigated in more detail by using the same two probes, A and C, but including an additional probe, B, located between these two. Probe B was labelled in a different colour from the flanking probes. The relative position of B between the two flanking probes A and C on the normal and der(16) was then measured in 22 different metaphases. On the normal chromosome 16 the probe B was positioned closer to C than to A, while on the der(16) the probe B was about midway between A and C. This difference was significant (t_{21}=5.15, P<0.001). The measurements of probes are summarised in Fig. 3.

The measurements establish that in the same metaphase the region of the short arm of chromosome 16 inverted in the der(16), is reduced in size relative to the same region on the normal 16. In addition, this change in size is not uniform, with the proximal portion on the inverted chromosome showing almost half the relative length (38 from 69 relative units) while the distal portion is increased marginally in size (41 from 31 relative units). Therefore, the rearrangement of the short arm of 16 has changed the compaction of chromatin relative to the normal 16 in the same metaphase.

The G-banding pattern of the der(16) does support the existence of an inversion (Fig. 1a). However, the definition of bands on 16p is always relatively poor and it is difficult to determine whether there were any consistent differences in banding pattern associated with the observed changes in chromatin compaction.

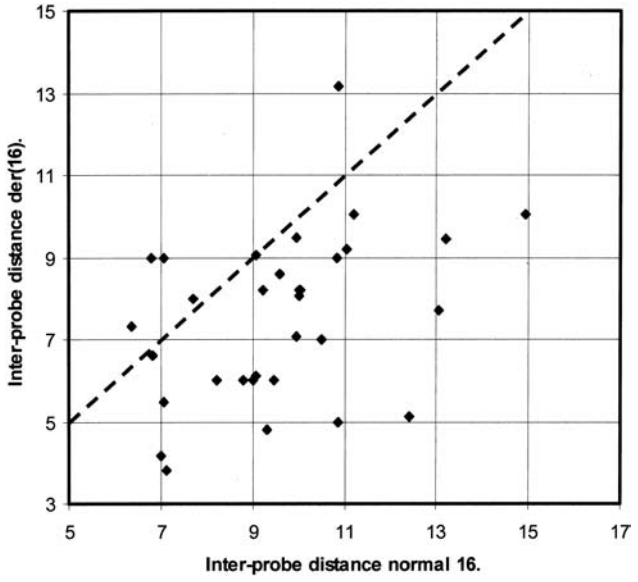

Fig. 2 Measurement of inter-probe distances. Each *point* represents a single metaphase with the relative distance between probes A and C on the normal chromosome 16 defined by the *x*-axis and the distance on the der(16) by the *y*-axis. The *dashed line* indicates the expected position if the distance between probes A and B were identical on both the normal and der(16)

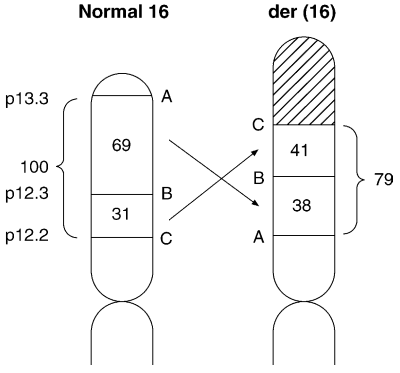

Fig. 3 Representation of differential compaction of der(16). The distances between probes A, B and C were determined by measurements from FISH to metaphase chromosomes of the t(14;16)inv(16). Measurements of the der(16) were relative to the normal chromosome 16 in the same metaphase where the distance between A and C is taken as 100 units. The *hatched region* on the der(16) represents the region derived from chromosome 14

Table 2 Lateral location of probes on chromatids. (ns, not significant)

Probe	Chromosome	Position of signal (%)		
		External	Medial	Internal
A	Normal 16	38 (52)	35 (48)	0
	der(16)	3 (4)	56 (78)	13 (18), $P<0.001$
B	Normal 16	4 (10)	37 (88)	1 (2)
	der(16)	5 (12)	36 (88)	0, ns
C	Normal 16	40 (33)	80 (67)	1
	der(16)	31 (25)	93 (75)	0, ns

Location of probes on sister chromatids

The lateral location of the probes on each sister chromatid on the normal and der(16) was scored for the probes A, B and C (Table 2). Probes B and C were located at similar positions. However, for probe A, 52% of the signal was external on the normal 16 but only 4% was external on the der(16) and the difference in position of the probe was statistically significant.

Discussion

A de novo chromosome rearrangement in a patient with epilepsy and mental retardation was initially described as a reciprocal translocation. There was no evidence of any deletion of 16p associated with the rearrangement. Subsequent studies (Bhalla et al., in preparation) are consistent with the phenotype being related to the disruption of a gene at the 16p13.3 breakpoint. Detailed FISH characterisation of the rearranged chromosome 16 demonstrated that the der(16) was a complex rearrangement that was derived from both a (14;16) translocation and paracentric inversion of 16p. The breakpoint of the translocation and one breakpoint of the inversion are within approximately 100 kb and are likely to be coincident on the assumption that a three-break rearrangement is more likely than a four-break rearrangement. The chromosome abnormality is notated as t(14;16)(q32;p13.3)inv16 (p13.3p12.1). Translocations and inversions of chromosome 16 have been described in the inv(16)(p13q22) associated with M4Eo acute myelomonocytic leukaemia (Maarek et al. 1999). However, of eight reported cases, the breakpoints of the inversion and translocation were distinct.

It is possible that this chromosome rearrangement is more common, but has not been previously detected due to the weaker resolution of conventional G-banding. The risk of phenotypic abnormality associated with de novo chromosome rearrangements is likely to be due to the chance of chromosome breakage disrupting a gene. Therefore this risk, 6.7% for a two-break rearrangement (Warburton 1991), would be expected to increase with an increase in the number of breakpoints. Therefore at prenatal diagnosis the non-detection of a three-break rearrangement associated with a simultaneous translocation and inversion would result in the determination of a lower risk than is the case.

When a translocation is associated with a particular disease this can provide an approach for positional cloning of the disease gene. The finding that an apparent reciprocal translocation is an unexpected complex chromosome rearrangement will complicate this approach. This has been found by Feil et al. (1991) where an X translocation in a patient with adrenoleukodystrophy was found also to involve two small deletions and an inversion >110 kb in size.

Measurement of probe position on the short arm of the chromosome 16 involved in the translocation inver-

sion demonstrated a change in chromatin compaction. Overall there was a 21% reduction in the relative size of the same region of the short arm in the inversion of the der(16) compared with the normal short arm. This reduction was not uniform since the inverted segment that corresponds with the proximal position of the region on the normal chromosome was compacted by 55%. Probe A (b441K8), which is situated in this highly compacted region on the der(16), also showed an alteration in the lateral position of the probe on the chromatin. In summary, this chromosome rearrangement results in a change in the normally predetermined process of chromatin compaction. Possible explanations are either a localised severe disruption of DNA packaging over relatively short distances (hundreds of kilobases) or a more generalised change that extends many megabases. The latter explanation is more likely as it is difficult to envisage a mechanism that would allow a localised event to cause sufficient impact on chromatin condensation.

Since the consistent banding pattern of chromosomes is thought to reflect the relative compaction of chromatin then the observation of a change in compaction would be expected to be observed as a change in G-banding. However, it is not possible to observe an obvious change in compaction on G-banding due to the complexity of the rearrangement, together with the lack of well-defined G-bands on the short arm of chromosome 16. The phenomenon of a change in chromosome compaction coincident with a chromosome rearrangement may be more widespread since anecdotal evidence from cytogenetics laboratories suggests that there can be alteration of the expected G-banding pattern at the breakpoints of translocations.

The first stage of chromatin compaction involves the formation of the nucleosomes and their subsequent compaction into a 30–40 nm fibre (Daban 2000). The consistent relative length of metaphase chromosomes, the consistent banding pattern of chromosomes and the consistent lateral position of DNA in the chromatid (Baumgartner et al. 1991) all suggest that the final stage of chromatin compaction is a highly ordered process. The chromosome rearrangement described in this report results in alterations in compaction over a region that may range from hundreds of kilobases to many megabases of DNA. This is further supported by the finding that the lateral position of DNA in the chromatid has been altered. Therefore higher order chromatin compaction may involve chromatin domains that extend over many megabases of DNA. These domains must ultimately be defined by DNA sequence since a single chromosome breakpoint can disrupt the organisation of the entire domain. These observations need to be considered in any models of chromatin folding for metaphase chromosomes.

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