ORIGINAL ARTICLE

Analysis of sex chromosome abnormalities using X and Y chromosome DNA tiling path arrays

A C Karcianas, K Ichimura, M J Mitchell, C A Sargent, N A Affara

Supplementary tables 1 and 2 are available online at <http://jmg.bmj.com/supplemental>

See end of article for authors' affiliations

Correspondence to: Professor N A Affara, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK; na@mole.bio.cam.ac.uk

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Background: Array comparative genomic hybridisation is a powerful tool for the detection of copy number changes in the genome.

Methods: A human X and Y chromosome tiling path array was developed for the analysis of sex chromosome aberrations.

Results: Normal X and Y chromosome profiles were established by analysis with DNA from normal fertile males and females. Detection of infertile males with known Y deletions confirmed the competence of the array to detect AZFa, AZFb and AZFc deletions and to distinguish between different AZFc lesions. Examples of terminal and interstitial deletions of Xp (previously characterised through cytogenetic and microsatellite analysis) have been assessed using the arrays, thus both confirming and refining the established deletion breakpoints. Breakpoints in iso-Yq, iso-Yp and X–Y translocation chromosomes and X–Y interchanges in XX males are also amenable to analysis.

Discussion: The resolution of the tiling path clone set used allows breakpoints to be placed within 100–200 kb, permitting more precise genotype/phenotype correlations. These data indicate that the combined X and Y tiling path arrays provide an effective tool for the investigation and diagnosis of sex chromosome copy number aberrations and rearrangements.

With the exception of trisomy 21, copy number variation and structural variants of the human sex chromosomes are found more frequently in the population than for autosomes.² In general, structural and numerical abnormalities of the Y chromosome are not life threatening, but impact primarily on pathways of sexual development and differentiation or gamete production, reflecting the gene content retained on a largely degenerate chromosome.^{3–4} Conversely, copy number aberrations of X chromosome sequence that can impact seriously on male health and viability are often ameliorated by random X-inactivation in the diploid female. The exception is Turner syndrome arising from partial or complete loss of X chromosome sequences, causing severe developmental abnormalities for the embryo and fetus, and frequently proving fatal before term.⁵

The application of comparative genomic hybridisation (CGH) using a tiling path array of large-insert genomic DNA clones provides a means of assessing copy number changes along the complete length of a chromosome.^{6–7} The resolution of the array is determined by the insert sizes of the genomic DNA clones contributing to the tiling path. For CGH arrays assembled from bacterial artificial chromosome (BAC) clones this is typically around 100–300 kb, permitting more precise genotype/phenotype correlations than conventional cytogenetic analysis or metaphase CGH. The application of a complete tiling path array for the X chromosome to the analysis of lesions leading to X-linked mental retardation has been described previously.⁸ The present study describes tiling path arrays that cover 94.4% of the X chromosome and the euchromatic region of the Y chromosome to analyse a wide range of sex chromosome abnormalities. Through comparison with both male and female

control samples, we have demonstrated the ability of the arrays to characterise rapidly (a) altered dosage of entire chromosomes, (b) Y deletions in infertile males, (c) partial deletions of the X chromosome, (d) breakpoints in isochromosomes of the Y and X–Y translocations and (e) abnormal X–Y interchange in sex-reversed XX male individuals. The findings suggest that an initial screen of the genome or suspected individual chromosomes by means of array-based CGH will rapidly reveal chromosomal regions of interest for more detailed molecular analysis. In addition to applications in research where array CGH can be used for detailed genotype/phenotype correlations, this array will also be useful for routine diagnostic applications to define sex chromosome abnormalities.

MATERIALS AND METHODS

Clinical materials and DNA extraction

DNA was extracted by standard procedures from the following cases: (a) one fertile male (proven father—sample ID 2), (b) two males with X and Y chromosome aneuploidy (sample IDs 3 and 4), (c) a male with an X/Y translocation (sample ID 5), (d) a male and a female with Y chromosome rearrangements (sample IDs 6 and 7), (e) four infertile males with various Yq deletions (sample IDs 8–11), (f) two females with X chromosome deletions (sample IDs 21 and 18) and (g) four Y-positive XX males (sample IDs 14–17). Table 1 summarises the karyotypic analysis of each case and the cytogenetic location

Abbreviations: BAC, bacterial artificial chromosome; CGH, comparative genomic hybridization; PAR, pseudoautosomal region; STS, sequence-tagged site

Table 1 DNA samples from individuals included in this study

Sample ID	Karyotype	Clone at breakpoint as defined by array CGH
1	46,XX	N/A
2	46,XY	N/A
3	48,XXXXY	N/A
4	47,YYY	N/A
5	46,Xt(X;Y)(p22.3;q11)	RP11-292P9
6	45,X/46,X,idel(Y)(q11.21)	RP11-434F12
7	48,XX,idel(Y)(p11.2),idel(Y)(p11.2)	RP11-62H15
8	46,X,del(Y)(q11.21q11.221)*	RP11-558M10 - RP11-218F6
9	46,X,del(Y)(q11.221q11.222)*	RP11-509B6 - RP11-207L19
10	46,X,del(Y)(q11.223)*	RP11-178M5
11	46,X,del(Y)(q11.221q11.223)*	RP11-537C24
21†	46,X,del(X)(p21.1p11.3)	RP11-147E15 - RP11-377B2
18†	46,X,del(X)(p11.3)	RP11-126D17
14	46,Y,der(X),t(X;Y)(p22.33;p11.2)*	RP11-172A6 on X, RP11-108I14 on Y
15	46,Y,der(X),t(X;Y)(p22.3;p11.2)*	RP11-501G22 on X, RP11-62H15 on Y
16	46,Y,der(X),t(q22.33;p11.2),inv(Y)(p11.2)*	RP11-105N19 on X, RP11-218E11 - RP11-105F19 on Y
17	46,Y,der(X),t(p22.33p11.2),inv(Y)(p11.2)*	RP11-74L17 on X, RP11-62H15 - RP11-309M4 on Y

CGH, comparative genomic hybridisation

*Breakpoints are derived from array-CGH data.

†Sample ID is according to Lachlan *et al*¹

of BAC clones determined by CGH to be located at breakpoint boundaries in this study.

Construction of chromosome X and Y tile path arrays

The chromosome X and Y clone sets (derived from the Golden Path tile set) were obtained from the Sanger Institute, Cambridge, UK. To complete gaps, 112 clones were included from the 32K BAC clone set.⁹ The nucleotide sequences and positions for the clones were according to the Ensembl database (V.33.35e, September 2005, http://www.ensembl.org/Homo_sapiens). The X chromosome tiling path consists of 1708 clones (1083 BACs, 517 PACs, 86 cosmids and 22 fosmids), providing 94.4% coverage (see supplementary tables 1 and 2 available online at <http://jmg.bmj.com/supplemental> for clone lists and positions). The Y chromosome tiling path (195 BACs, four cosmids and two fosmids) covers 92.6% of the euchromatic region, excluding the pseudoautosomal region 2 (PAR 2—see supplementary table 1). The PAR1 region is represented by an identical set of clones for both chromosomes X and Y. The array also included (for the normalisation of copy number changes) a further 538 BAC clones covering all autosomes at approximately 5 Mb intervals and six *Drosophila* BAC clones (see supplementary table 2). Construction of the microarray was performed according to the published protocol.¹⁰ The PCR products for each clone were printed in duplicate onto a CodeLink slide (GE Healthcare, Little Chalfont, UK), using a MicroGrid II arrayer in 4×4 subarrays.

Labelling, hybridisation and analysis of array CGH

Labelling of 400 ng of test and reference DNA with Cy5-dCTP and Cy3-dCTP, respectively, hybridisation and washing were carried out according to the published protocol.¹¹ The reference DNAs were from a single fertile male and female. The arrays were scanned using a GenePix 4100A personal scanner (Axon Instruments, Union City, California, USA). The scanned images were quantified using BlueFuse software (BlueGnome, Cambridge, UK). Analysis and normalisation were carried out as published elsewhere.¹¹

Reference DNA samples

Seventeen fertile males were tested against each other in various permutations, in order to determine the individual with the least variation on the Y chromosome. This identified a

single individual with minimal variation of his Y chromosome, subsequently used as the male reference DNA for all experiments. A single normal female DNA was used as the universal reference.

Sequence-tagged site PCR analysis

Sequence-tagged site (STS) PCR analysis was carried out on selected Y chromosome sequences using two Y-specific markers, *DYS231* and *DYS212* (http://www.ensembl.org/Homo_sapiens), and standard PCR conditions.

RESULTS

Validation of sex chromosome tiling paths

The pattern of X–Y homology on the Y chromosome is complex, involving a combination of major sequence blocks covering up to 3.5 Mb of DNA and individual gene homologies interspersed with Y-specific sequence. This is illustrated schematically in fig 1A, together with a representation of the PAR1 and extra-PAR X–Y homology that yields significant signals on CGH analysis (PAR 2 is not included in the array). Figure 1B (sample ID 1) shows that female DNA against the male reference yields a ratio close to 2:1 on the X tiling path for all X-specific sequences, but a ratio of 1:1 for the major regions of X–Y homology (indicated in fig 1B, sample ID 1). On the Y tiling path, the majority of the clones are reduced in ratio, except for the known X–Y homology regions (1:1 ratio).

For array validation, a series of DNA samples from normal male and female individuals and X and Y aneuploidies were hybridised to the tiling paths against male and female control DNA. Figure 1C (sample ID 2) shows that for male DNA against the male control the expected 1:1 ratio is observed.

Figure 1D (sample ID 3) and E (sample ID 4) show the profiles of individuals with increased copy numbers of entire X and Y chromosomes (49,XXXXY and 47,YYY) tested against the normal male control. The quadruple X individual depicted in the figure (fig. 1D, sample ID 3) shows the expected increase in copy number across the entire X tiling path. A 1:1 ratio is observed across the Y tiling path (fig. 1D, sample ID 3), with the exception of the PAR1 and X/Y homology block, which are present in a ratio of 5:2, as a result of the additional copies contributed from the multiple X chromosomes. The 47,YYY individual showed the expected 1:1 ratio for the X profile, except for the Yp11.2/Xq21.3 homology block where additional

Figure 1 Array validation using normal individuals and whole chromosome aneuploidies. (A) Schematic of the X and Y chromosomes showing the major X and Y blocks of homology. (B) X and Y chromosome profiles of a normal female hybridised against a normal male. PAR1 and centromere are designated P1 and C, respectively, and are shown shaded in light grey, and the major X/Y homology blocks are indicated by a horizontal line. The Y-specific SRY region (lying between the PAR1 and the major Yp X/Y homology block) is also shown and is depressed in ratio. (C) X and Y chromosome profiles of a normal male hybridised against the normal male control. (D) X and Y chromosome profiles of a 49,XXXXY male hybridised against the normal male control. (E) X and Y chromosome profiles of a 47,XYY male hybridised against the normal male control.

copies have been contributed by the extra Y chromosomes. A ratio close to 2:1 is observed for the Y chromosome profile. The fact that the log₂ ratio for this individual does not quite reach 1 may be attributable to mosaicism. These analyses confirm that the X and Y tiling path arrays can determine the copy number status in the expected manner.

Complex Y rearrangements

Complex structural rearrangements of the sex chromosomes investigated using the normal male control included an XY translocation, an iso Yp and an iso Yq chromosome (fig 2). The female individual depicted in fig 2A (sample ID 5) shows the profile of an X:Y translocation (46,X,t(X;Y)(p22.3;q11)) (from cytogenetic chromosome analysis) and illustrates that the translocated segment of Yq (breakpoint at Yq11.221, clone

RP11-292P9) is in a 1:1 ratio. The 1:1 ratio against the male control for X chromosome clones extending from Xp22.31 to the telomere indicates a hemizygotic terminal deletion of Xp. The 1:1 ratio of the major homology block on Yp11.2 is due to the presence of two copies of homologous Xq sequence. The reduced ratio of PAR1 clones reflects the loss of one PAR region from the X chromosome bearing the translocation.

Figure 2B (sample ID 6) shows a male with an iso-dicentric Yp chromosome (45,X/46,X,idic(Y)(q11.21) by cytogenetic analysis). The Y chromosome profile shows that the Y breakpoint is in Yq11.21 (clone RP11-434F12), and that the retained region of the Y chromosome is present in an elevated copy number ratio compared with that in the male control. The failure to observe a 2:1 ratio (log₂ values not quite reaching 1) can be attributed to mosaicism in the individual.¹² The elevated

Figure 2 Y chromosome abnormalities. (A) X and Y chromosome profiles of a female with an X/Y translocation (46,X,t(X;Y)(p22.3;q11)) hybridised against the normal male control. The clone lying at the breakpoint is shown by an arrow. A schematic representation of the normal X chromosome and the X chromosome with the translocation is shown on the right. (B) X and Y chromosome profiles of a male with an isoYp chromosome (45,X/46,X,idel(Y)(q11.21)) hybridised against the normal male control. The clone lying at the breakpoint is shown by an arrow. A schematic representation of these chromosomes is shown on the right. (C) X and Y chromosome profiles of a female with two isodicentric Yq chromosomes (48,XX,idel(Y)(p11.2),idel(Y)(p11.2)) hybridised against the normal male control. The clone located at the breakpoint is indicated by an arrow. A schematic representation of these chromosomes is shown on the right.

ratios of PAR1 and the Yp11.2/Xq21.3 homology block sequences can be attributed to the contribution of two copies from the isochromosome in addition to the copies from the X chromosome.

The female individual shown in fig 2C (sample ID 7) displays an isodicentric Yq chromosome and has a non-mosaic karyotype of 48,XX,idel(Y)(p11.2),idel(Y)(p11.2). The chromosome Y profile shows the presence of the Y sequences in four copies from the breakpoint at Yp11.2 (clone RP11-62H15) to Yq. The PAR1 and the Yp11.2/Xq21.3 X/Y homology block on both the X chromosome and Y chromosome profiles are present in the expected 1:1 ratio, as these regions are deleted from the isochromosomes.

Y deletions leading to male infertility

The utility of the sex chromosome arrays to detect Y deletions associated with male infertility was assessed using known deletions of the AZFa, b and c regions of Yq previously characterised by PCR analysis with STS markers. Figure 3A (sample ID 8) and B (sample ID 9) show AZFa and AZFb deletions, respectively, and demonstrate the ability of the Y tiling path to detect discrete interstitial deletions of Yq. Figure 3C (sample ID 10) shows an individual with the common b2/b4 AZFc deletion (one of two brothers with identical patterns). Figure 3D (sample ID 11) shows a complete AZFb deletion extending into but not removing the entire AZFc region. Also shown on the chromosome Y profiles are STS-PCR data generated to confirm the presence or absence of Y chromosome sequences in view of the variable copy number ratio pattern observed (possibly due to incomplete and variable suppression of repeats), arising from its complex Y-specific repeat structure. Single-copy STS markers have been selected in clones expected to be deleted or present in these individuals. The absence of a band in the female control and its presence in the male control confirms that the STS PCR primers are Y-specific. From fig 3D (sample ID 11), it can be seen that

markers in clones exhibiting different copy number ratios indicating deletion are indeed at least partially deleted when assessed by PCR, whereas other markers outside the deletion (fig 3C, sample ID 10) remain present.

X chromosome deletions

Figure 4A (sample ID 21) shows the X chromosome profile (against the normal female control) of a female individual with an interstitial Xp deletion (46,X,del(X)(p21.1p11.3)) between clones RP11-147E15 and RP11-377B2. Figure 4B (sample ID 18) shows the profile of a female individual with a terminal Xp deletion (46,X,del(X)(p11.4), from clone RP11-126D17. In both cases, the deleted segment is present in one copy.

Interchanged XX males

Four examples of Y-positive XX males (from the analysis of a cohort of 10 cases) arising from illegitimate X–Y interchange in the father¹³ and with varying degrees of loss of Xp have been analysed against the normal male control (fig 5). Where the Yp11.2/Xq21.3 X–Y homology block has been transferred in an interchange, a ratio of 3:2 is expected (two from Xq and one from Yp). A 1:1 ratio is observed for the distal Xp/Yq homology block (due to two copies from the X and absence of the homologous Yq sequences). It should be noted that on the Y chromosome plot, a number of data points do not deviate from the log₂ ratio of zero. This is due to small segments of X–Y homology in individual clones distributed throughout the Y chromosome, where the presence of two X chromosomes ensures a 1:1 ratio. A previous molecular analysis of these patients¹⁴ has revealed that different extents of Yp have been transferred to the interchanged X chromosome, reflected in the different location of the major ratio change on the Y tiling path array (marked by an arrow). The individuals represented in fig 5A (sample 14, 46,Y,der(X),t(X;Y)(p22.3;p11.2)) and B (sample ID 15, 46,Y,der(X),t(X;Y)(p22.3;p11.2)) show a contiguous transfer of Yp sequences to the X chromosome

Figure 3 Infertile males. Chromosome Y profiles of various infertile males. Clones defining the deletion breakpoints are indicated by an arrow. In all cases, the patients were hybridised against the normal male control. (A) An infertile male with an AZFa deletion. (B) An infertile male with an AZFb deletion. (C) An infertile male with an AZFc deletion. This individual is one of two brothers, and the deletion pattern is identical in both, as seen from the enlarged superimposed AZFc deletion profile from both brothers. A gel photograph of sequence-tagged site-PCR analysis is also shown. F, M and P indicate female control DNA, male control DNA and patient DNA, respectively. (D) An infertile male with an AZFb and partial AZFc deletion. A gel photograph of STS-PCR analysis is shown, as already described in fig 3C.

(3:2 ratio), with the former transferring almost all Yp sequences. Figure 5C (sample ID 16, 46,Y,der(X), t(q22.33;p11.2)), inv(Y)(p11.2)) and D (sample ID 17, 46,Y,der(X), t(p22.33p11.2), inv(Y)(p11.2)) show individuals who appear to have a non-contiguous presence of Yp sequences, consistent with the molecular analysis using Yp markers.¹⁵ Analysis of the X chromosome profiles shows that each XX male has a different breakpoint on the interchanged X. For the individuals in fig 5B (sample ID 15) and C (sample ID 16), the breakpoint lies outside of PAR1 (observed in a 1:1 ratio on both the X and Y chromosome profiles). The individuals in fig 5A (sample ID 14) and D (sample ID 17) have the breakpoint within PAR1, since there is a change in ratio among clones representing the PAR1 region. It is apparent from both the X and Y chromosome profiles that only the transferred portion of the PAR1 is present in a 3:2 ratio.

DISCUSSION

The application of array CGH has been primarily in the analysis of chromosome gain and loss in tumour DNA, to delineate the location of potential oncogenes and tumour suppressor genes.⁶ This paper has assessed the application of array-based CGH for the analysis of sex chromosome aberrations.

Many of the structural abnormalities in these patients have been characterised previously, both cytogenetically and through the use of a range of molecular markers.^{12–17} In addition, the regions of X–Y homology on both the X and Y chromosomes have been described in detail in a number of other studies.^{18–23} The breakpoints and chromosome regions participating in copy

number change flagged by CGH can be assessed for consistency with previous analyses. In this respect, the use of both X and Y tiling paths has proven to be very powerful in the analysis of abnormalities involving exchanges between the sex chromosomes and in providing an accurate detailed profile of a range of different chromosomal aberrations. This paper is the first report use of a Y chromosome tiling path for this purpose.

X–Y HOMOLOGOUS REGIONS

As schematically indicated in fig 1A, there are extra PAR regions of X–Y homology between the sex chromosomes. The largest of these is the Yp11.2/Xq21.3 block, which is human-specific and spans 3.5 Mb. This forms one contiguous block on the X chromosome and two separate blocks on the Y short arm, as a result of a major rearrangement on the Y chromosome, although the order of loci on Yp homologous to the X chromosome has been conserved to a high degree.²⁴ The behaviour of the PAR1 and Yp11.2/Xq21.3 regions is readily observed when there is a dosage change in the X or Y chromosomes, since they form monolithic homology segments covered by several BAC clones that respond as a cohort. A good example of this is the female against male hybridisation experiment (fig 1B), where the homologous regions remain in a 1:1 ratio, regardless of dosage changes elsewhere on the chromosomes. In experiments where there is a duplication or deletion involving an X–Y homologous segment, ratios across these regions are altered specifically, as seen in the XX males for the Yp11.2 X–Y homology block. Other homology regions (such as Yq11.2 and Xp11.4 and Xp22.3) are more dispersed on

Figure 4 X chromosome deletions. (A) X chromosome profile of a female with an interstitial X deletion hybridised against the normal female control. The clones lying on either side of the breakpoints are indicated by an arrow. (B) X chromosome profile of a female with a terminal deletion hybridised against the normal female control. The clone lying at the deletion breakpoint is indicated by an arrow.

Figure 5 Interchanged XX males. Chromosome X and Y profiles of sex-reversed XX males. Clones defining the deletion breakpoints are indicated by an arrow. The major X/Y block of homology Yp11.2 is shown on each Y chromosome profile, indicated by a horizontal line. In all cases, the patients were hybridised against the normal male control. (A) X and Y chromosome profiles of a normal female hybridised against a normal male. PAR1 and centromere are designated as P1 and C, respectively, and are shown shaded in light grey. (B) An interchanged XX male. (C) An interchanged XX male. (D) An interchanged XX male. The non-contiguous presence of Yp sequences can be seen. (E) An interchanged XX male. The non-contiguous presence of Yp sequences can be seen.

both the X and Y chromosomes and involve individual or small groups of BAC clones whose ratio change requires focused dissection from the background variation.

Effects of mosaicism on array profiles

Frequently, individuals with structurally abnormal chromosomes (eg, isochromosomes) are found to be mosaic, where only a proportion of the cells examined cytogenetically bear the abnormality. This mosaicism is detected by array-based CGH as a departure from the expected copy number ratios. An example of this is clearly seen in the case of the individual with the Yp isochromosome (fig. 2B) where a 2:1 ratio against a normal male reference is expected for the regions of the Y chromosome included in the isochromosome. The ratio is intermediate between 1:1 and 2:1. This variable intermediate ratio has been observed in the analysis of several patients (data not shown) with Yp isochromosomes (all mosaic for a 45X cell line¹²). It may be possible to calibrate these varying ratios to get an estimate of the percentage of mosaicism.

Detecting Yq deletions leading to male infertility

The CGH analysis of infertile men indicates that the sex chromosome tiling path arrays will provide a rapid means for the diagnosis of Y deletions associated with defective spermatogenesis phenotypes. The deletions at the AZFa,^{22 25-28} AZFb^{25 29-31} and AZFc^{25 32-35} intervals have been characterised

in some detail, and thus can be compared with the regions detected by array CGH. For the AZFa and AZFb intervals, discrete deletions involving a relatively small number of clones containing the appropriate candidate genes have shown reduced copy number. In contrast, the pattern of copy number reduction within the AZFc region is complex and varies from one clone to the next. The non-recombining region of the Y chromosome is densely packed with intrachromosomal repetitive elements³⁶ known as amplicons, and these are usually arranged into palindromic structures with high sequence identity (>99.9%) between palindrome arms.^{33 37} Such structures are particularly prominent in the AZFc region of the Y chromosome, and can result in deletions, duplications, inversions and gene-conversion events due to homologous recombination between direct and inverted repeats.^{30 38 39} The variable extent of deletion exhibited by various clones across the interval is likely to reflect, in part, the incomplete removal of both arms of a palindrome and/or suppression of Y-specific repeat sequences present elsewhere on the chromosome. The use of PCR primers for STS markers designed from single-copy regions has confirmed that sequences contained in some clones that show partial deletion are in fact deleted.

Deletions provoked through recombination events between different direct repeats in AZFc palindromes have defined divergent categories of AZFc interval loss.^{35 39} The AZFc deletion patterns observed in the different individuals investigated in

this study correlate with the common b2/b4 deletion spanning 3.5 Mb of the Y chromosome long arm.^{32–40} Through the analysis of different categories of AZFc deletion, it should be possible to identify diagnostic AZFc deletion signatures. Not all copy number changes in the AZFc region cause infertility, as revealed in this study by two normal fertile males showing in one case deletion and in the other case amplification of AZFc sequences (data not shown). This may be because the dosage of key genes has not exceeded a lower or upper critical threshold. It is interesting to note that amplification of AZFc sequences is not associated with infertility, consistent with reports on the effect of DAZ gene copy number.⁴¹

Detection of X–Y interchange in sex-reversed XX males

Deletion maps using a series of Yp DNA probes have shown that there is considerable variation in the transfer of Y sequences in XX males.^{14–16,42} This is apparent from the four XX males presented in this study, where not only have different portions of the Y chromosome been exchanged, but variation is also seen in the loss of chromosome X material. Furthermore, there appears to be some discontinuity in the interchanged Yp sequences in two of the cases studied, reflecting findings from molecular analysis.¹⁴ This pattern may represent true complex discontinuous deletions, although this is more likely to be a result of inversion polymorphisms within the Y chromosome population, believed to be common in the non-recombining part of the Y chromosome where a strict order is not maintained by homologous pairing and recombination.¹² An example of this has been described by Vogt *et al.*,⁴³ where the order of the TSPYA and TSPYB loci on Yp is reversed in some individuals. Following X–Y interchange in the father of the XX male bearing the inversion polymorphism, the Y sequences present in the XX male seem to come from discontinuous blocks. In two patients, the interchanged X breakpoint has occurred within the PAR1, leading to a triplication of the portion of the PAR1 that is proximal to the X breakpoint (fig 5A). The observed 1.5:1 ratio is consistent with the 3:2 ratio for proximal PAR1 that would result from its triplication. As expected, the remainder of the PAR1 is present in a 1:1 ratio owing to the contribution of the distal segment from the interchanged Y. A similar triplication of the Yp11.2/Xq21.3 X–Y homologous sequences can also be observed where the appropriate Yp regions have been included in the interchange leading to the XX male.

The generation of complete chromosome tiling path arrays for the sex chromosomes based on large-insert clones of 100–200 kb permits far greater resolution of breakpoints associated with chromosome aberrations than is possible with conventional cytogenetic approaches. This not only has great potential in studies of genotype/phenotype correlations but also has powerful applications in the diagnostic setting to define precise sex chromosome aberrations. Complete tiling path arrays have some advantages over targeted arrays designed to deliver information on preselected genomic regions. The latter may fail to detect chromosomal perturbations that give rise to a similar phenotype because of their incomplete genome coverage of a given chromosome. Complete chromosome tiling path arrays can thus be used to perform an initial rapid screen using appropriate arrays to locate the site of an aberration, and to confirm any alterations using high-resolution cytogenetic analysis, fluorescence in situ hybridisation (FISH) or PCR techniques. Subsequent to identification of interesting regions on the sex chromosomes, construction of a region-specific array of higher resolution (using oligonucleotides) may lead not only to the detection of finer lesions but also to the elucidation of more subtle and complex dosage changes within smaller aberrations.

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Authors' affiliations

A C Karcianias, C A Sargent, N A Affara, Department of Pathology, University of Cambridge, Cambridge, UK

K Ichimura, Department of Pathology, Division of Molecular Histopathology, University of Cambridge, Cambridge, UK

M J Mitchell, INSERM U.491, Faculté de Médecine, Marseille, France

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