Advanced genome-wide screening in human genomic disorders

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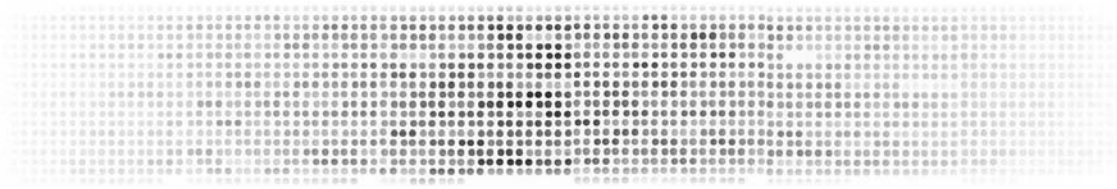
Chapter 5

Array-CGH detection of a cryptic deletion in a complex chromosome rearrangement.



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Array CGH detection of a cryptic deletion in a complex chromosome rearrangement

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Abstract

Balanced complex chromosome rearrangements (CCR) are extremely rare in humans. They are usually ascertained either by abnormal phenotype or reproductive failure in carriers. These abnormalities are attributed to disruption of genes at the breakpoints, position effect or cryptic imbalances in the genome. However, little is known about possible imbalances at the junction points. We report here a patient with a CCR involving three chromosomes (2;10;11) and eight breakpoints. The patient presented with behavioural problems as the sole phenotypic abnormality. The rearrangement, which is apparently balanced in G-banding and multicolour FISH, was shown by genomic array analysis to include a deletion of 0.15–1.5 Mb associated with one of the breakpoints. To explain the formation of this rearrangement through the smallest possible number of breakage-and-reunion events, one has to assume that the breaks have not occurred simultaneously, but in a temporal order within the span of a single cell division. We demonstrate that array comparative genomic hybridisation (CGH) is a useful complementary tool to cytogenetic analysis for detecting and mapping cryptic imbalances associated with chromosome rearrangement.

Introduction

Complex chromosome rearrangement (CCR) is a general term designating any structural rearrangement involving more than two breakpoints and/or chromosomes (Pai et al. 1980). Individuals with balanced CCRs are most often ascertained through fertility problems, recurrent miscarriages, or by congenital anomalies in newborn offspring. These individuals are otherwise normal, and their problems are a direct consequence of either meiotic failure or imbalanced chromosome segregation. However, some carriers of apparently balanced complex translocations present phenotypic abnormalities and/or mental retardation (Batanian and Eswara 1998; Batista et al. 1994; Joyce et al. 1999; Phelan et al. 1998). The abnormal phenotype in these cases is thought to originate from disruption of genes or cryptic imbalances in the genome (Batista et al. 1994). While disruption of genes at breakpoints is well documented and has been instrumental in the mapping of several disease genes, such as, DMD, NF1 and mesomelic dysplasia (Ledbetter et al. 1989; Spitz et al. 2002; Zatz et al. 1981), cryptic deletions or duplications in the genome in apparently balanced translocations have only sporadically been demonstrated (Borg et al. 2002; Kumar et al. 1998).
Here we report a 5-year-old patient who exhibited behavioural changes and delay in speech development as the only phenotypic abnormalities, and was found to carry an apparently balanced CCR on the basis of Giemsa banding and multicolour FISH. The rearrangement, which involved three chromosomes and at least eight breakpoints, was studied at high resolution by multicolour FISH and genomic array, and a cryptic imbalance was detected.

Clinical description

The patient is a Caucasian 5-year-old male, the first child from healthy and non-consanguineous parents. Pregnancy was unremarkable and the Apgar score at birth was 8/9. At 19 months of age, delayed speech, hyperactivity and attention deficit were noted, and he underwent a neurological evaluation. Results of EEG, audiogram and screening for inborn metabolic errors were all in the normal range.
At 5 years of age, he was referred for genetic and cytogenetic evaluation. His intelligence was apparently normal (IQ evaluation was denied), but hyperactivity and attention deficit were noted. The child is still under treatment for psychological and speech difficulties. However, because the child is raised in a tri-lingual environment, the significance of the speech delay is difficult to evaluate.

Materials and methods

Molecular and classical cytogenetic studies

Peripheral blood lymphocytes from the patient and his parents were cultured for 72 h according to standard procedures. Cytogenetic analysis was performed on GTG-banded chromosomes, and 25 cells from the patient and 20 cells of each parent were fully analysed.

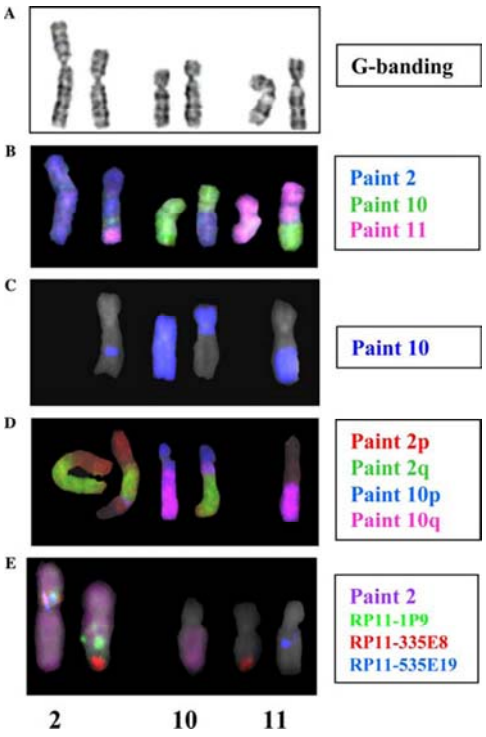

Fig. 1a–e Rearranged chromosomes and their normal homologues present in the patient carrying a complex chromosome rearrangement. a G-banding image shows a three-way translocation (2;10;11) and an insertion of 2p in der(2)(q); b 24-colour hybridisation confirms the exchange of material between (2;10;11), and identifies an additional weak signal on der(2)(q). c Painting with a chromosome 10 library shows that the small insertion on 2q is derived from chromosome 10. d Hybridisation with chromosomearm paintings for chromosome 10 and 2 shows that the material from 10q into der(2)(q) maps proximal to 2p material. e Chromosome 2 painting hybridised together with three BAC probes on 2p. The three signals are present on the normal chromosome 2. The other green and blue signals map to der(2)(q) and der(11), respectively (see map in Fig. 2). The red-labelled BAC is deleted and does not present other signals on 2p material. Note that the red-labelled BAC also presents a secondary signal on 11qter

Metaphases of the patient were analysed by the combinatorial binary ratio labelling (COBRA-FISH approach; Tanke et al. 1999), allowing the identification of each arm by a different colour combination. Labelling, hybridisation and immunostaining were performed as previously described (Tanke et al. 1999). For chromosomes that were found by multicolour FISH to be rearranged, libraries labelled with single fluorochromes were hybridised to metaphases of the patient to confirm the identity of the rearranged chromosome segments.

Array comparative genomic hybridisation (CGH)

Slides containing triplicates of ~3,500 BAC/PAC DNA targets spaced at ~1 Mb intervals were produced in the Leiden University Medical Center. The BAC set used to produce these arrays was received from the Wellcome Trust Sanger Institute (UK), and information regarding the full set is available at the Sanger Center mapping database site, Ensembl (http://www.ensembl.org/). Protocols for BAC DNA preparation and amplification, spotting on the slides and hybridisation were previously described (Knijnenburg et al. 2005), and based on protocols optimised by the group of Dr. N. Carter (Carter et al. 2002; Fiegler et al. 2003; Tanke et al. 1999). Slides were scanned with a GenePix Personal

4100A scanner, and the spot intensities measured by the GenePix Pro 4.1 software (Axon Instruments, Westburg BV, Leusden, The Netherlands). Further analyses were performed using a home-made routine developed in Microsoft Excel 2000, and spots outside the 20% confidence interval of the average of the replicates were excluded from the analyses. Unbalances of the targets were determined based on log 2 ratios of the average of their replicates, and we considered sequences as amplified or deleted when outside the ±0.3 range.

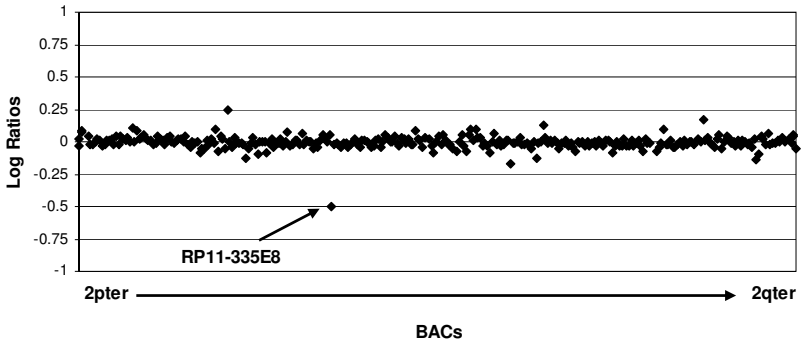

Fig. 2 Array CGH profile of chromosome 2. The graphic shows the log 2 values of the array CGH test/reference ratios for BACs in chromosome 2. The arrow shows the deleted BAC on 2p

Results

G-banding analyses revealed a CCR, which involved a three-way translocation t(2;10;11)(q23;q22;q23), plus an additional intrachromosomal rearrangement involving the translocated chromosome 2 and resulting in the insertion of segment 2p11.2-p15 into 2q22 (Fig. 1a). Karyotypic analysis of the parents was normal.

Multicolour FISH analyses confirmed the insertion of 2p material into the long arm of chromosome 2, and revealed that the exchange between chromosomes 2, 10 and 11 was more complex than a straight three-way translocation: in addition to the previously detected segment of 11q on the der (2) long arm, a small insertion of 10q material was also present (Fig. 1b–d).

Array CGH results revealed a cryptic deletion on 2p involving BAC RP11-335E8, whose localisation was cytogenetically compatible with the proximal breakpoint of the insertion (Fig. 2). To confirm this deletion and investigate how it related to the rearrangement, we hybridised both the deleted and its two flanking probes to metaphase spreads of the patient. One signal of each one of the three probes localised to the proximal short arm of the normal chromosome 2. In accordance with the array results, RP11-335E8 did not produce any other signal on 2p material. However, RP11-335E8 produced secondary signals on 11qter, which is typically seen in normal controls (data not shown): in the cells of this

patient, one of these secondary signals localised to the normal chromosome 11, and the other to the terminal region of der(2) (red-labelled, Fig. 1e). The two flanking probes hybridised also to the long arms of der(2), (RP11-1P9, green-labelled, proximal BAC) and der(11) (blue-labelled, distal BAC: RP11-535E19), respectively (Fig. 1e). Accordingly, the karyotype of the patient is currently regarded to be as follows (Mitelman 1995): 46,XY,der(2)(2pter → 2p15::2p11 → 2q22::10q22 → 10q23::2p13 → 2p15::11q23 → 11qter),der(10)(10pter → 10q22::2q22 → 2qter),der(11)(11pter → 11q23::2p12 → 2p12::10q23 → 10qter.
Figure 3 shows a map of the rearranged region on chromosome 2p, the location of the FISH probes on chromosome 2, and indicates to which of the derivatives these probes map.
A representation of the rearranged chromosomes, and a proposed sequence of events invoking the minimum number of exchanges leading to this karyotype within a single cell cycle, are depicted in Fig. 4.

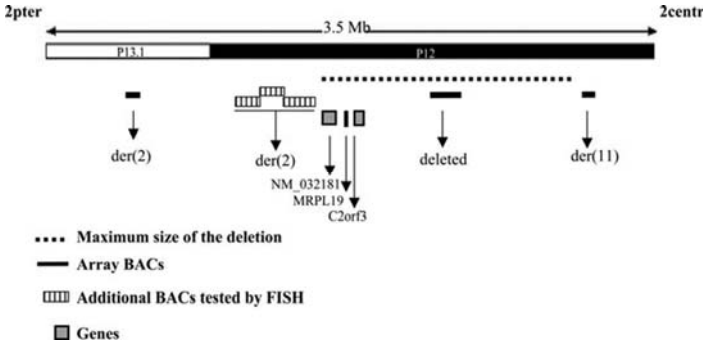

Fig. 3 Scheme of the rearranged region on 2p. The figure is a representation of the 2p region containing the deleted BAC and the breakpoint between der(2) and der(11). The figure shows the BACs present on the array, the additional BACs investigated by FISH, the maximum possible size of the deletion, and the genes that might be absent

Discussion

Initially, the complex rearrangement described here was interpreted as balanced, and derived from a three-way translocation between chromosomes 2, 10 and 11, and an insertion from the short into the long arm of der(2). However, 48-colour chromosome arm painting revealed that the rearrangement was far more complex than originally estimated, involving a larger number of breaks. Furthermore, array CGH analyses showed a cryptic deletion associated with one of the breakpoints. Given the ~1 Mb resolution of the array used, the occurrence of further imbalances of small size cannot be excluded. Until recently, the detection of imbalances associated with apparently balanced chromosome rearrangements depended on sequencing across the breakpoints, and have only sporadically been described (Borg et al. 2002; Kumar et al. 1998). The

combination of array CGH and FISH, as described here, enormously facilitates such analysis.

It is unclear how the phenotype of our patient relates to his chromosome rearrangements, but it is likely that either the 2p deletion, or one of the many breakpoints, or a combination of these, had a role in this. None of the breakpoints present in our patient are compatible with gene locations believed to be involved in attention deficit-hyperactivity disorder (ADHD), except maybe ADRA2 (10q25.2) (http://www.ensembl. org/), which is relatively close to the cytogenetically estimated breakpoints on 10q (10q22 and 10q23). The BAC on 2p that is deleted in our patient does not contain any identified gene, but the surrounding area that might also be deleted (in between the two adjacent BACs) includes a number of genes (http://www.ensembl. org/). We showed by FISH that the majority of them map to the der(2) (Fig. 3), and only few of them map to the possibly deleted area, namely NM_032181, MRPL19 and C2orf3. NM_032181 is a gene of unknown function, but C2orf3 is known to be a regulator involved in transcription repression (Johnson et al. 1992), while MRPL19 codes for the 60S ribosomal mitochondrial protein L19 (http://www.ensembl.org/). Although the phenotypic effect of hemizygosity for these genes is unknown, the gene functions are broad enough to consider their possible involvement in the abnormal phenotype of the patient.

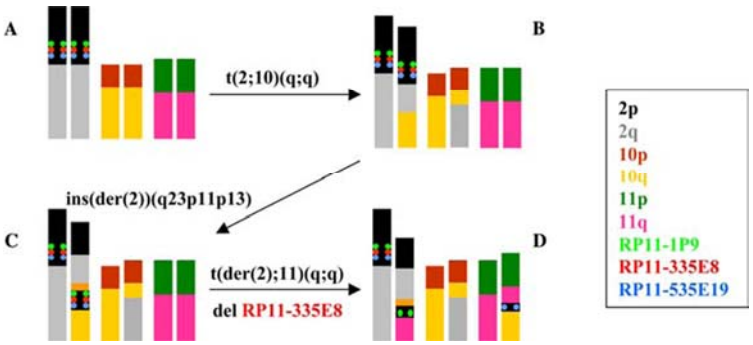

Fig. 4a–d Proposed model for the formation of this complex chromosome rearrangement. The proposed sequence of events invokes a minimum number of exchanges leading to the reported rearrangement. a–d Each of the steps in temporal order, from the normal karyotype (a) to the observed configuration of the rearranged chromosomes after G-banding, multicolour FISH and array CGH analysis (d). For the present model, we have to assume that the recombination events have occurred in a chronological order within the span of a single cell division

The sequence of events that gave rise to the CCR in our patient is not easy to determine. We propose a chronological sequence in Fig. 3 that basically involves two translocations and one insertion, which appears to be the minimum number of events required for such rearrangement. It is reasonable to conceive that a ‘‘catastrophic’’ event simultaneously producing multiple chromosome breakages gave rise to this and other CCRs. A complication of our model for the present

rearrangement, is that the events proposed must have occurred sequentially; i.e. first, a translocation, followed by an insertion, and finally by another translocation, in which a segment would be lost in the breakpoint region. In cancer, complex chromosome rearrangements are assumed to result from alterations accumulated during many cell divisions. However, we found no evidence of the presence of different clones containing precursor karyotypes in our patient, suggesting that his CCR originated either at gametogenesis or at fertilisation. Because of the absence of detectable mosaicism, we are forced to assume that the recombination events have probably occurred in a temporal order within the span of a single cell division.
The present study illustrates that array CGH, combined with other molecular cytogenetic methodologies, will not only improve the description of rearranged chromosomes, but also challenge our interpretation of their mechanisms of origin.

Acknowledgements

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