High-resolution karyotyping by oligonucleotide microarrays : the next revolution in cytogenetics

Gijsbers, A.C.J.

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Chapter 3.4

Mosaicism with a normal cell line and an unbalanced autosomal reciprocal translocation; three new cases and review of the literature

Antoinet CJ Gijsbers, Johannes Dauwerse, Cathy AJ Bosch, Ed Aanhane, Wilco van den Ende, Sarina Kant, Kerstin MB Hansson, Martijn H Breuning, Egbert Bakker and Claudia AL Ruivenkamp

Center for Human and Clinical Genetics; Leiden University Medical Center (LUMC), Leiden, The Netherlands

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Abstract
Mosaicism involving a normal cell line and an unbalanced autosomal translocation are rare. In this study we present three new cases detected by Single Nucleotide Polymorphism (SNP) array analysis in our routine diagnostic setting. These cases were further characterized using Fluorescence *in situ* Hybridisation (FISH) analysis and conventional karyotyping.

The first case is a mentally retarded male who carries an unbalanced translocation in 87% of his cells. Remarkably, the phenotypically normal mother carries the balanced form of the translocation in all her cells. The second case is a phenotypically normal female who has an unbalanced translocation in 52% of her cells. She passed the unbalanced translocation to her daughter who has mild mental retardation and serious behavior disturbance. The third case is a female referred for Rubinstein-Taybi syndrome who carries a complex unbalanced translocation in 60% of her cells. Her mother showed a normal karyotype.

The mechanisms that might be responsible for these mosaic karyotypes are discussed. Furthermore, we demonstrate that high-resolution whole-genome array is a powerful tool to reveal cryptic unbalanced translocations and mosaicisms, including the more rare cases.

Introduction
Mosaicism is the presence of genetically different cell lines in one individual derived from a single zygote. Mosaicism can be caused by several mechanisms, including chromosomal abnormalities and DNA mutations (reviewed by Youssoufian and Pyeritz, 2002). Whether a mosaicism is disease-causing depends upon the abnormality, on which tissue is abnormal and on how much of a tissue is affected. If only a fraction of the soma is abnormal (somatic mosaicism), the phenotype is likely to be normal and will probably never be recognized (Gardner et al., 1994). If it involves a substantial part of the soma, it can cause dysmorphisms and malformation, and if the brain is included, mental retardation (MR). Abnormality involving only a part of the gonad (gonadal mosaicism) is not associated with an abnormal phenotype of the carrier and will usually only be recognized after two siblings are born with the same '*de novo*' abnormality (Youssoufian and Pyeritz, 2002).

Chimerism is the presence of genetically different cell lines in one individual derived from two or more different zygotes. If both cell lines contain the same sex chromosomes, conventional karyotyping is generally not able to distinguish between mosaicism and chimerism. Support for the mechanism causing two or more different cell lines as a result of chimerism can be obtained by comparison of DNA microsatellite markers (Cotter and Hirschhorn, 1998).

Somatic mosaicism with an abnormal cell line can be missed by conventional karyotyping if masked by a high percentage of normal cells or even dismissed as a culture artefact (Ballif et al., 2006). Additionally, healthy individuals with a mosaicism will in most cases not be investigated. The prevalence of mosaicisms is therefore difficult to establish. Lebbar et al. (2008) reviewed the literature for mosaicism with a normal cell line and a balanced rearrangement and reported 35 cases. Most of these carriers have a normal phenotype and were referred due to recurrent miscarriages, infertility or the birth of an abnormal child. Furthermore, they described the first two cases of mosaicism for a normal cell line and a complex chromosome rearrangement (CCR) in patients ascertained through infertility. Zaslav and colleagues (1999) reported

23 cases of mosaicism for a normal cell line and an unbalanced autosomal structural rearrangement (N/UASR). These UASRs included duplications, deletions, insertions, isochromosomes, and derivative chromosomes. In total they reported only 4 cases with a mosaicism for a normal cell line and an autosomal derivative chromosome (Zaslav et al., 1999). To our knowledge, only 3 additional mosaicism cases with a normal cell line and an unbalanced autosomal translocation have been described (Stallings et al., 1997; Kulharya et al, 2002; Petkovic et al., 2003).

The application of high-resolution whole-genome array technology to cytogenetic testing has improved the ability to detect smaller abnormalities and mosaicisms (Ballif et al., 2006; Gijsbers et al., 2009). We report on three unrelated cases with a mosaicism for a cryptic unbalanced reciprocal translocation detected by Single Nucleotide Polymorphism (SNP) array analysis and Fluorescence *in situ* hybridisation (FISH). Our results illustrate that array technology is a sensitive method for the detection of mosaicisms, unbalanced translocations and, as described here, a combination of these two.

Material and methods

Patients

This study is based on results obtained from patients with MR submitted to our laboratory for SNP array screening. In this report we include three cases (two patients with MR and one healthy parent) in which a mosaicism with a normal cell line and an unbalanced autosomal translocation was detected. Pedigrees are shown in Figure 3.4.1.

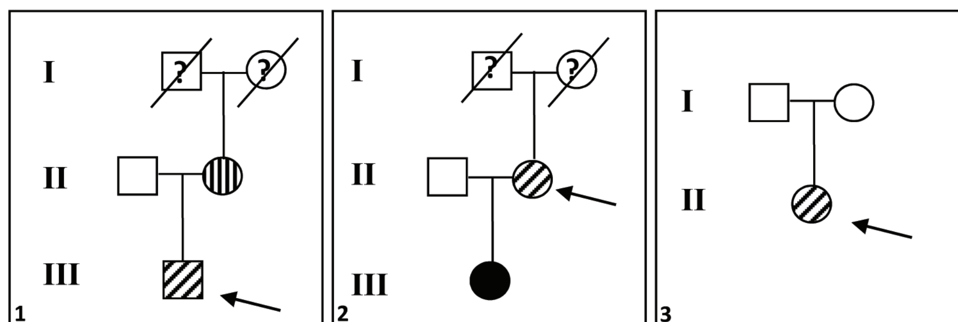

Figure 3.4.1 Pedigrees of the three cases with a normal cell line and an unbalanced autosomal translocation. Arrow indicates case with the mosaicism.

Conventional karyotyping and FISH

Conventional cytogenetic analysis on GTG-banded chromosomes from cultured lymphocytes was performed according to standard techniques. FISH analysis was carried out by standard procedures as described (Dauwerse et al., 1990). Bacterial artificial clones (BAC) mapping to the affected chromosome regions were selected based on their physical location within the affected region (http//: www.ensembl.org, Ensembl release 54 - May 2009).

SNP arrays

DNA was extracted from whole blood by a Gentra Puregene DNA purification Kit (Gentra Systems, Minneapolis, USA), following the manufacturer's instructions. The Affymetrix GeneChip Human Mapping 262K *NspI* array (Affymetrix, Santa Clara, CA, USA) was performed following the manufacturers' instructions and data was analyzed

as described previously (Gijsbers et al., 2009). The Affymetrix Genome-Wide Human SNP Array 6.0 contains more than 1.8 million markers for genetic variation, including more than 906,600 SNP probes and more than 946,000 probes specific for the detection of copy number variation (Affymetrix). 500 ng of genomic DNA was processed according to the instruction provided in the Affymetrix Cytogenetics Copy Number Assay user guide. SNP copy number was assessed in the patient using Affymetrix Genotyping Console 2.1 software. Aberrations of at least five consecutive probes were considered as significant.

Results

Case 1

The patient was a 42-year old male with MR and dysmorphic features including coarse hair, small dysplastic low-set ears, deep-set eyes, hypotelorism and short 4th metatarsal of the left foot. He also had scoliosis, aortic stenosis, coarctation of the aorta, hypertension, anal stenosis, sensineural deafness and distal symphalangism of toes.

SNP array analysis with the Affymetrix Genome-Wide Human SNP Array 6.0 detected a terminal duplication of 10.11 Mb on the long arm of chromosome 10 and a 7.51 Mb deletion on the long arm of chromosome 11 (Fig. 3.4.2a and b). Additional FISH analysis showed in 87 of the 100 metaphases an unbalanced translocation between the long arm of chromosome 10 and the long arm of chromosome 11 (Fig 3.4.2c. The karyotype of this patient was 46,XY,der(11)t(10;11)(q26.13;q24.2) [87]/46,XY [13]. DNA microsatellite markers showed no extra alleles.

FISH analysis of the parents identified that the mother was a carrier of the balanced translocation between chromosomes 10 and 11.

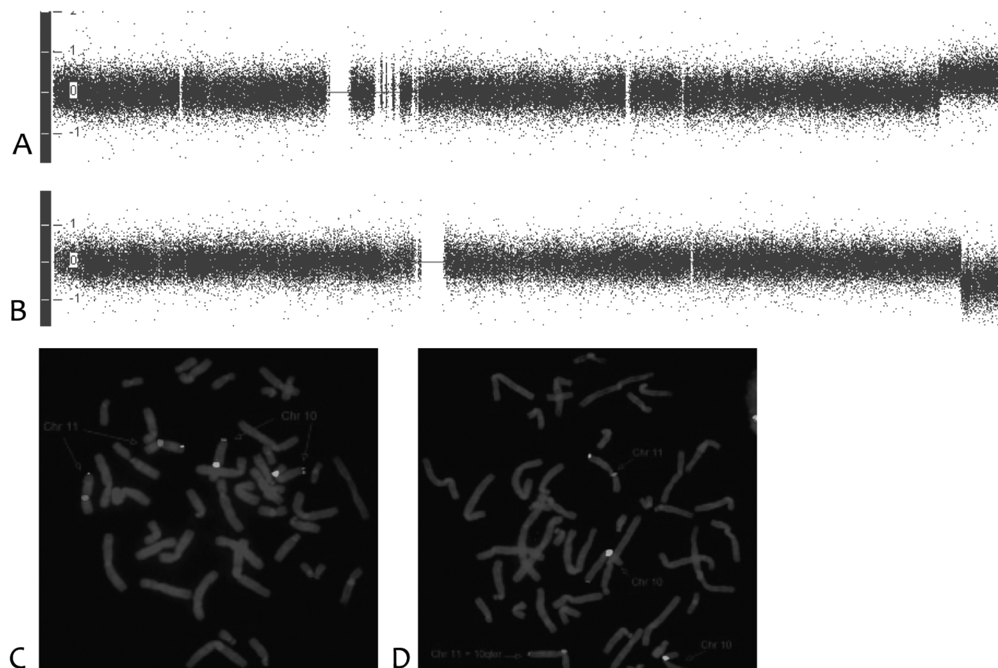

Figure 3.4.2 Case 1. (a) SNP array analysis revealed a duplication of 10.11 Mb on chromosome 10 and (b) a 7.51 Mb deletion on chromosome 11. (c) FISH analysis results showing a normal cell line and (d) a cell line with the der(11)t(10;11)(q26.13;q24.2).

Case 2
Case 2 was a 51-year old healthy female. The daughter of case 1 was referred because of developmental delay and serious behavioral problems.

SNP array analysis with the Affymetrix GeneChip Human Mapping 262K *NspI* array on the daughter of case 2 revealed a terminal duplication of 12.87 Mb on the long arm of chromosome 8. FISH analysis identified an unbalanced translocation between the long arm of chromosome 8 and the short arm of chromosome 22. Additional SNP array analysis for case 2 showed the same duplication, however with a lower intensity and FISH analysis confirmed this was due to a mosaic unbalanced t(8;22) resulting in a karyotype of 46,XX,der(22)t(8;22)(q24.2;p10) [15]/46,XX [14]. DNA microsatellite markers showed no extra alleles.

The parents of case 2 were not available for testing

Case 3
The patient was a female referred for Rubinstein-Taybi syndrome. FISH and sequence analysis for the *CREBBP* gene excluded the presence of a deletion or a mutation.

SNP array analysis with the Affymetrix GeneChip Human Mapping 262K *Nsp*I revealed a terminal duplication of 6.8 Mb on the long arm of chromosome 6 and a terminal deletion of 11.2 Mb on the long arm of chromosome 11. Additional FISH analysis in the patient confirmed the presence of an unbalanced translocation between the long arm of chromosome 6 and the long arm of chromosome 11 in 6 of the 10 cells. The karyotype of this patient was 46,XX,der(11)t(6;11)(q26;q24.1) [6]/46,XX [4]. DNA microsatellite markers showed no extra alleles. In addition, the SNP analysis showed large stretches of homozygosity, suggesting consanguinity of the parents.

FISH results for the parents were normal. The parents are first cousins once removed.

Discussion
Mosaicism involving a normal cell line and an unbalanced autosomal translocation is rare. To our knowledge, only 7 cases have been reported (Table 3.4.1) (Stallings et al., 1997; Kulharya et al, 2002; Zaslav et al., 1999; Petkovic et al., 2003). Zaslav et al. (1999) reported a mosaicism for three cell lines, present in fibroblasts and lymphocytes and a patient with mosaicism for two cell lines in both lymphocytes and fibroblasts. Two cases are described with normal cell line in lymphocytes and mosaicism in fibroblasts (Stallings et al., 1997; Kulharya et al, 2002). The last three cases are mentioned with mosaicism in their lymphocytes only (Zaslav et al., 1999; Petkovic et al., 2003). The inheritance was unknown for three cases (Zaslav et al., 1999; Petkovic et al., 2003), three were *de novo* (Tsien et al., 1991; Stallings et al., 1997; Zaslav et al., 1999), and for one case the mother was carrier of the balanced translocation (Kulharya et al, 2002). In this study we reported three new cases with a normal cell line and an unbalanced autosomal translocation identified in lymphocytes. Other tissues were not analyzed.

The presence of a normal cell line and an unbalanced translocation can arise in several ways. 1) A mitotic exchange of nonhomologous chromatids followed by the loss of one of the translocated chromatids, subsequent segregation would result in a normal and an unbalanced cell line (Zaslav et al., 1999). 2) An unbalanced zygote followed by loss of the abnormal chromosome, a subsequent monosomy rescue event and a duplication of the normal chromosome. This would result in an isodisomy for this chromosome (Cotter and Hirschhorn, 1998). 3) 3:1 segregation with the derivative

Table 3.4.1 Mosaicism with a normal cell line and an unbalanced autosomal reciprocal translocation

Reference	Karyotype	Parents
Stallings et al., 1997	46,XX (blood)-46,XX/46,XX,der(15)t(3;15)(q11;p11) (fibro)	normal
Zaslav et al., 1999	46,XX,der(4)t(4;15)(q35;q22)/46,XX	normal
	45,XX,-15,-18,+der(18)t(15;18)(q13;q23)/46,XX	n/a
	47,XX,+mar/47,XX,+der(9)t(9:9)(p13;q34) (blood) - 47,XX,+der(9)t(9:9)(p13;q34)/46,XX (fibro)	normal
	45,XY,-7,-15,+der(15)t(7;15)(q34;q13)/46,XY	n/a
Kulharya et al., 2002	46,XY,der(22)t(11;22)(q23.3;q11.2)/46,XY (fibro)- 46,XY (blood)	mother carrier balanced
Petkovic et al., 2003	46,XX,der(10)t(6;10)(p11;q11)/46,XX	n/a
Present cases	46,XX,der(22)t(8;22)(q24.2;p10) [15]/46,XX [14]	n/a
	46,XY,der(11)t(10;11)(q26.13;q24.2) [87]/46,XY [13]	mother carrier balanced
	46,XX,der(11)t(6;11)(q26;q24.1) [6]/46,XX[4]	normal

chromosome and two normal associated chromosomes. Loss of a normal chromosome in one cell and loss of the derivative chromosome in the other cell will result in two different cell lines (Kulharya et al, 2002). Depending on the stage of development the abnormal cell line involves a greater or lesser fraction of the embryo for these three mechanisms. 4) Chimerism (Nyberg et al, 1992).

Here, we report on three unrelated cases of mosaic unbalanced translocations detected by SNP array and FISH analysis. Case 1 inherited the unbalanced translocation from his mother who carried the balanced translocation. To our knowledge this is only reported once (Kulharya et al., 2002). This mosaicism could only be explained by events 2, 3 or 4. No extra alleles were detected with DNA microsatellite marker study and the genotype information was not suggestive for isodisomy. Since there was approximately in only 10% of the cells a normal karyotype this might be under the detection level of both techniques, and therefore mechanism 2 and 4 could not be excluded. For case 2, we were not able to determine the inheritance of the unbalanced translocation since the parents were not available for testing. Case 3 showed a *de novo* mosaic unbalanced translocation. The origin of these mosaicisms is most likely postzygotic. Chimerism was excluded for both cases and genotype information derived from SNP array analyses showed no allele differences suggestive for isodisomy. The first mechanism seems to be most likely in these cases.

To detect low-level mosaicisms with conventional karyotyping, large cell numbers need to be examined. Therefore, mosaicism screening is time consuming and expensive (Ballif et al., 2006). The application of high-resolution whole-genome arrays in a diagnostic setting has the potential to improve the identification of mosaicisms (Ballif et al., 2006; Gijsbers et al., 2009). Furthermore, the increased resolution of arrays enables the detection of smaller deletions and duplications. The gains and losses detected in this study were all larger than 5 Mb, which should be identifiable with conventional karyotyping. This highlights that the subtelomeric regions are more difficult to characterize by conventional karyotyping due to their G-negative staining. Therefore, unbalanced translocations could be easily masked and missed by routine cytogenetic testing.

This study reports three new cases of a mosaicism with a normal cell line and an unbalanced autosomal translocation, which is a rare phenomenon. The exact mechanism responsible for these karyotypes is not clear. We demonstrate that high-resolution whole-genome arrays will reveal more mosaicisms, unbalanced translocations and a combination of both.

Acknowledgements

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