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Microdissection and Reverse Painting. Reevaluation of Three Cases with Structural Abnormalities in Chromosome 13

Purnima Kumarathas[1], Peter K.A. Jensen[2], Mette Houman[1] and Ursula Friedrich[1]

1. Department of Human Genetics, University of Aarhus, Denmark
2. Department of Clinical Genetics, Aarhus University Hospital, Denmark

KEY WORDS Microdissection; deletion of chromosome 13; phenotype-genotype correlation.

ABSTRACT Three patients with different deletions of chromosome 13 were reexamined. The abnormal chromosomes were microdissected. The DNA from these chromosomes was DOP amplified, labelled with Biotin and used for reverse banding by FISH. The precise breakpoints in chromosome 13 were defined which is a prerequisite for delineation of the different deletion phenotypes.

INTRODUCTION

Microdissection of chromosomes followed by reverse painting has proved to be an excellent tool for rapid and precise characterisation of structurally abnormal chromosomes. Not only the chromosomal contents but also the exact segment involved can be clarified.

Chromosome no 13 has a characteristic banding pattern. But nevertheless, it may be difficult precisely to decipher which band is deleted. Even more challenging is the diagnosis of small ring chromosomes. Reverse painting with microdissection probes is a rapid method which may be used to pinpoint a specific chromosomal region in which further research for small deletions can be carried out on DNA level by specific DNA probes.

We have reexamined three patients with deletions of chromosome no 13. We were able to revise the previous cytogenetic diagnosis which were performed using different banding methods. We have reviewed the literature in an attempt to further delineate regions of developmental importance on chromosome no 13.

MATERIAL AND METHODS

Case Histories

Case 1: The mother was 31 years, gravida 2. There was 1 previous provoked abortion. In gestation week 19 extreme hygroma colli and subcutaneous edema of the fetus were seen by routine ultrasound. Labour was induced and a hydropic fetus was delivered. Autopsy revealed a male fetus, 176 gr, 19 cm, crown-rump length 12.5 cm, head circumference 14 cm, a small occipital encephalocele, a dilated fourth ventricle with cerebellar hypoplasia and hypoplastic vermis. Both lungs had two lobi.

Case 2: The mother was 21 years, primi gravida. In the 29th gestation week an ultrasound examination was performed due to intra uterine growth retardation. An anencephalic fetus was diagnosed. Labour was induced and a dead male child was born in breech position. Autopsy showed a child of 650 gr, length 31 cm. There was anencephaly, hypoplasia of left thumb, undescended testes and imperforate anus without atresia. Examination of the internal organs revealed a gall bladder replaced by fibrous strings, but the ducti were present. Both ureteres had a stenosis and the adrenal glands were hypoplastic.

Case 3: This boy was born at term. Birth weight 2550 gr, length 50 cm, head circumference 32 cm, normal apgar score. His mother was 22 years and his father 25 years. He had a 3 years older brother. The pregnancy was complicated by contractions and threatening abortion. The newborn boy did not thrive. At 4 months he had an operation due to inguinal hernia on the left side. Because of psychomotoric retardation and a peculiar appearance he was further examined. He did not smile consciously, he was hypotonic and could not hold his head. He had a narrow sloping forehead, a short upturned nose, a hypoplastic philtrum and a thin upper lip. There was a high-arched palate and receding chin. The ears were large, not well-modulated, there were pits and tags in front of the right ear. Musculus pectoralis seemed to be hypoplastic on both sides and the nipples were widely spaced. On the right side there was an inguinal hernia. There were atypical simian creases in both hands and the thumbs were placed proximally. An eye

Correspondence to: Ursula Friedrich, Department of Human Genetics, The Bartholin Building, University of Aarhus, DK-8000 Aarhus C, Denmark
Fax: +458612 3173 *E-mail:* uf@humgen.au.dk

examination including ultrasound revealed a small tumour in the right eye and a very large tumour in the left eye. A bilateral retinoblastoma was diagnosed. CT scanning of thorax and abdomen, as well as MR scanning of the cerebrum did not disclose metastases. The left eye was enucleated at the age of 6 months, and the right eye tumour was treated radiologically.

Method

The methods have been described in detail by Friedrich et al (2001). In the present cases we have microdissected 3 to 5 abnormal chromosomes no 13. We amplified the dissected chromosomes by using a degenerate oligo-nucleotide primed-polymerase chain reaction (DOP-PCR). We labelled the PCR products with Biotin in a further PCR reaction and used them as probes for FISH. We hybridised to the patients' chromosomes to check the probe and to chromosomes from a normal male for reverse painting.

RESULTS

Case 1: Chromosome examination from fibroblast cultures revealed a terminal deletion of the long arm of chromosome 13. The karyotype was 46,XY,del(13)(q22). Chromosome examination in both parents showed a normal karyotype 46,XY in the father and a balanced translocation between chromosome 13 and 20 in the mother. Her karyotype in Q-banding was 46, XX,t (13;20) (q22;q13). The fetus thus had an unbalanced translocation of maternal origin involving a deletion of chromosome 13 and a partial trisomy of chromosome 20q.

Reverse painting with the microdissection probe and counter staining with DAPI localised the breakpoint in chromosome 13 at band q21.2. A tiny signal was also seen on chromosome 20 from q13 to qter. The revised karyotype therefore is: 46,XY, -13,+der(13) t(13;20)(q21.2;q13) mat (Fig 1).

Case 2: Chromosome examinations from cultures of fibroblasts showed a small ring chromosome 13. Neither in Q-banding nor in prometaphase examination was it possible to precisely localise the breakpoints in chromosome 13. Reverse painting with the microdissection probe and counter staining with DAPI clearly showed a signal from the centromere region to band q14 on chromosome 13. In the ring chromosome the entire long arm with the bright fluorescence in DAPI staining was missing as was the short arm of chromosome 13 distal to the centromere region. The karyotype is: 46,XY, r(13)(p12→q14). The ring did not differ in size. Both parents had normal chromosomes (Fig 1).

Case 3: Prometaphase examination from peripheral blood showed an interstitial deletion of chromosome no 13. The breakpoints could not be definitely described in banded preparations. The karyotype was 46,XY,del(13)(q12.?2q21.3). Both parents had normal chromosomes.

FISH with a probe from the locus for retino-

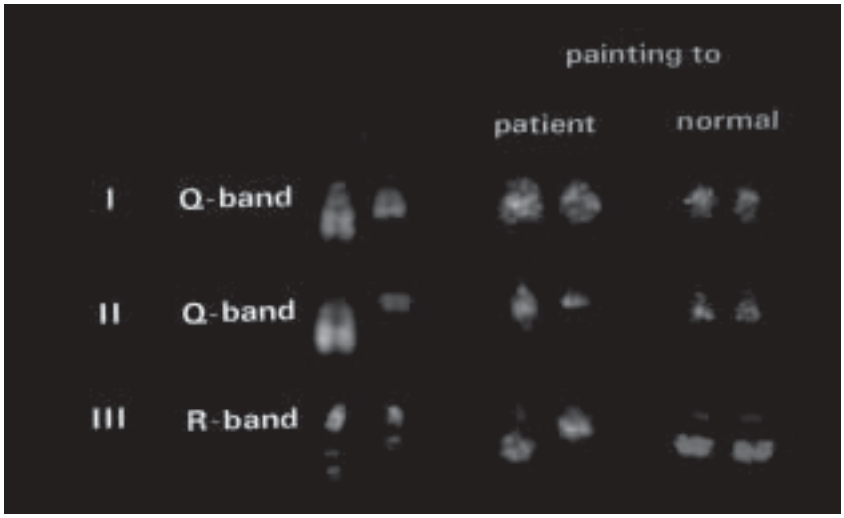

Fig. 1. Conventional banding, forward painting and reverse painting with microdissection probe

blastoma, which is located at 13q14, showed a signal at the normal chromosome 13. The deleted chromosome 13 did not have the signal. This indicated that the deletion included the retinoblastoma locus at q14.

Reverse painting with the microdissection probe and counterstaining with DAPI disclosed the breakpoints at q14.1 and q32. The previously described deletion is thus larger than originally suspected. The karyotype of the patient is: 46,XY,del(13) (pter→q14.1::q32→qter) (Fig 1).

DISCUSSION

Reverse banding with microdissection probes is a quick and reliable method for the description of structural chromosome abnormalities. We reanalysed the deleted chromosomes no 13 from three patients who were previously examined using different banding techniques. In all three cases the diagnosis was revised: in the first case the breakpoint was more proximally placed resulting in a larger deletion of chromosome 13 and a partial trisomy on chromosome 20 was shown; in the second case of a small ring chromosome it was impossible to localise the breakpoints using the conventional banding techniques. It was, however, easy when using reverse banding with the microdissection probe which disclosed a rather large deletion of chromosome 13; the third case had an interstitial deletion where the proximal breakpoint was corrected from q12.?2 to q14.1 and the distal from q21.3 to q32.

The precise description of the breakpoints is a necessary prerequisite for delineation of the different deletion phenotypes.

Depending on the size of the deletion of chromosome 13, the phenotype of these patients may comprise the following symptoms: developmental delay, growth retardation, microcephaly, neural tube defects, hypertelorism, broad nasal bridge, micrognathia, hypoplastic or absent thumbs, hypoplastic kidneys, ambiguous genitalia and imperforate anus (for review see: Luo et al., 2000).

Several authors have tried to correlate phenotype with genotype, and several critical regions have now been localised. Luo et al. (2000) performed a review of the literature and a molecular genetic analysis of one patient. Neural tube defects were seen in those terminal deletions or translocations which had lost the region spanning 13q33 to 13q34. Some confusion still exists in regard to ring chromosome 13 cases. In most of these cases the breakpoint on the long arm of chromosome 13 was rather distally placed, for example at band q33 to 34. But unfortunately, the precise distal breakpoint in these cases has often not yet been mapped by molecular genetic methods. The only conclusion so far is that deletions from q33 to qter are correlated with NTD. Luo et al. (2000) proposed the hypothesis that haplo insufficiency as well as trisomy which means a dosage of genes in this region, play a crucial part in neural tube development.

At region q32 the ZIC2 gene is localised. In mouse embryonic development ZIC2 expression plays a major part in brain and distal limb development (Nagai et al. 1997). In humans, heterozygous mutations may lead to the severe developmental brain anomaly holoprosencephaly (Brown et al. 1998). The part of ZIC2 in human limb development could not be clarified since several of the patients described by Brown et al. (1998) did not have thumb aplasia. Two of the present patients with deletions in this region had thumb anomalies described as left sided thumb hypoplasia (case 2) or proximally placed thumb (case 3).

Deletions in region q32 lead to severe malformations (Brown et al. 1995). In the present study cases 1 and 2 include this region. Both children were severely affected and died during pregnancy. In case 3 the distal breakpoint of the interstitial deletion lies in band q32, but does not involve a deletion. This boy has a deletion of q14.1 leading to bilateral retinoblastoma. Beside this, he has several symptoms which can be described as neurocristopathy (Warburg and Friedrich 1986). The crista neuralis is one of the earliest developmental fields. Gains and losses of chromosomes as well as other genetic imbalances or teratogenic events influence the crista neuralis. The neural crest cells undergo a series of transformations involving induction and proliferation of the cells followed by migration and eventual differentiation. Each of these steps is under genetic control. Depending on the environment in which these cells settle, the resulting phenotypes may become quite variable as seen in our case 3 and the cases described in the literature.

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