Disclosure of five breakpoints in a complex chromosome rearrangement by microdissection and FISH

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Abstract

Microdissection and fluorescence in situ hybridisation (FISH) were used to elucidate the nature of a complex chromosome translocation, after GTG banding failed in the complete characterisation of the structural rearrangement between chromosomes 6 and 12. These chromosomes were painted with chromosome specific paints and one of the chromosome regions involved in the translocation was isolated by microdissection. Ten copies of the microdissected region were collected with microneedles from GTG banded metaphases, transferred to a collecting drop, and amplified by means of DOP-PCR. The PCR product was labelled with biotin-14-dATP and used as a FISH probe for hybridisation to normal metaphase chromosomes and metaphase chromosomes of the patients (microFISH). FISH with this chromosome region specific painting probe and with chromosome band specific probes enabled the characterisation of a complex chromosome rearrangement with five breakpoints in two chromosomes. This resulted in the following karyotype: 46,XY,t(6;12)(6pter→

6q12::12q24.1→12qter;12pter→12q13.3::6q16.2→6q26::12q13.3→12q24.1::6q12→6q16.2::6q26→6qter).

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Key words: chromosome microdissection; complex chromosome rearrangement (CCR); degenerate oligonucleotide primer-PCR (DOP-PCR).

A complex chromosome rearrangement (CCR) has been defined as an interchange comprising more than two chromosome breaks and reciprocal exchange of segments.¹ Since interpretation of a CCR is often difficult when only standard GTG banding techniques are used, FISH with whole chromosome paints, developed by Pinkel *et al*² and Lichter *et al*,³ is frequently used for characterisation of a CCR.⁴⁻⁸ However, in the case reported here, the application of GTG banding and whole chromosome painting still did not characterise the CCR completely. MicroFISH is the physical dissection of (GTG) banded chromosomes followed by DOP-PCR and subsequent FISH with the probe obtained. This method, developed by Meltzer *et al*⁹ and Deng *et al*,¹⁰ is successfully used to generate whole

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Figure 1 Partial karyotype presenting normal GTG banded chromosomes 6 and 12 (left) and derivative chromosomes (right). Arrows indicate breakpoints, asterisks indicate points of reunion.

Figure 2 Der(12) chromosome (A) before, (B) during, and (C) after microdissection. The arrow points to the chromosome portion that is used for DOP-PCR.

chromosome painting probes,¹¹ region specific probes,^{9,12,13} and band specific probes.^{14,15} In clinical cytogenetics microFISH is used to identify marker chromosomes¹⁶⁻¹⁹ and unidentifiable chromosome abnormalities.²⁰

We describe the use of microFISH in a case in which chromosomes 6 and 12 are involved in a CCR with five breakpoints (fig 1). Whole chromosome painting and microFISH were very beneficial in identifying the rearranged segments. Additionally, FISH with centromere specific and band specific probes was essential for the complete characterisation of the CCR.

Material and methods

CASE REPORT

The patient was an 18 year old male whose chromosomes were re-examined because of a strong suspicion of an unbalanced karyotype. Cytogenetic analysis at 3 years of age had shown a reciprocal translocation (6;12)(q12;q24). At that time he was referred because of moderate delay in psychomotor development. Now, at the age of 18 years, he is a moderately mentally retarded male with a normal phenotype. The parents of the patient had normal karyotypes.

CYTOGENETICS

Chromosomes were prepared from peripheral blood lymphocyte cultures using a modification of the synchronisation method of Dutrillaux and Viegas-Pequignot²¹ by treatment overnight with thymidine, followed by incubation with 5-BrdU for six hours and ethidium bromide for one and a half hours before harvest. High

resolution banding was performed by treatment with trypsin followed by staining with Giemsa to obtain a GTG banded pattern. A cell line is available.

MICRODISSECTION AND AMPLIFICATION OF CHROMOSOMAL DNA

For microdissection, routinely fixed (methanol:acetic acid 3:1) cell suspensions stored at -20°C were used to prepare metaphase spreads on coverslips. The slides were rinsed in water and stored in 98% ethanol at -20°C . Dissection of the distal region of the der(12) chromosome (fig 2) was performed with glass microneedles controlled by a Narishige micromanipulator (MO-202). Before use, the needles and centrifuge tubes were treated with UV light for 30 minutes. The dissected chromosome parts were transferred to a 20 μl collection drop (containing 250 $\mu\text{g}/\text{ml}$ proteinase-K) in a 0.5 ml centrifuge tube. A fresh microneedle was used for each dissection. Before DOP-PCR, the collection drop, containing 10 copies of the dissected region, was incubated at 37°C for 30 minutes (proteinase-K treatment) and at 90°C for 10 minutes (proteinase-K inactivation). PCR reactions were performed following the protocol of Guan *et al*¹² in a Biometra Personal Cycler with a heated lid. PCR products were analysed for yield and probe size (200–800 bp) on an agarose gel and stained with ethidium bromide. The PCR product was precipitated and purified with NH_4Ac (4 mol/l) and isopropanol, washed with ethanol (70%), and dissolved in 50 μl T0, 1E buffer (10 mmol/l TRIS-HCl/0.1 mmol/l Na_2EDTA , pH 8.0).

Chromosome specific probes used in the analysis

Chromosome region	Probe	Insert (kb)	Reference
Cen 6	p308	3.00	22
Cen 12	pa12H8	1.35	23
6q26	cCl6-60	40	Y Nakamura, personal communication
6q27	cCl6-107	40	Y Nakamura, personal communication

FLUORESCENCE IN SITU HYBRIDISATION (FISH)

The chromosome specific probes used are shown in the table.

Paints specific for chromosomes 6 and 12 and labelled with biotin-11-dUTP were purchased (Oncor) and painting was performed

Figure 3 (A) Metaphase chromosomes of the patient after FISH with a chromosome 6 specific paint. (B) Metaphase chromosomes of the patient after FISH with a chromosome 12 specific paint. (C) Metaphase of a normal male after FISH with the DOP-PCR probe generated from the der(12) chromosome. Arrows point to the signals on the q arm of chromosomes 6. (D) Metaphase of the patient after FISH with the DOP-PCR probe generated from the der(12) chromosome. Signals are on the q arm of chromosome 6 and on the q arm of the der(12) chromosome. (E) Metaphase of the patient after FISH with centromere probes p308 (6) and pa12H8 (12) together with probe cC16-60 specific for chromosome band 6q26. Arrows point to the signals on the q arm of chromosome 6 and the q arm of the der(12) chromosome. (F) Same as fig 3E with probe cC16-107 specific for chromosome band 6q27.

according to the manufacturer's specifications. FISH with the DOP-PCR product was performed following the protocol of Pinkel *et al*²⁴ with only minor modifications. The centromere

specific and band specific probes were labelled by nick translation with biotin-11-dATP. For FISH with these probes the protocol of Lichter *et al*²⁵ was followed. The slides were examined

with a Zeiss Axiophot microscope and photographed using Scotchchrome 640 ASA colour slide film. Fig 3D was imaged with a CCD camera (Metasystems).

Results

Cytogenetic re-examination of GTG banded metaphases showed a complex chromosomal rearrangement of chromosomes 6 and 12 in which at least four regions were involved (fig 1). Painting with a chromosome 6 and a chromosome 12 specific paint confirmed this conclusion (fig 3A, B). However, GTG banding combined with chromosome painting was not sufficient to characterise the distal region of the der(12) chromosome completely. Ten copies of the distal region of the der(12) chromosome were collected by microdissection (fig 2) and the probe that was generated by DOP-PCR was hybridised to normal metaphase chromosomes and to metaphase chromosomes of the patient. Normal metaphases showed a signal in the region 6q12 to 6q16.2 and a second signal in the distal region of chromosome 6 (fig 3C). On metaphase chromosomes of the patient, the same fluorescence pattern as described above was seen on the normal chromosome 6 and, as expected, a fluorescent signal was seen at the distal end of the der(12) chromosome (fig 3D). FISH with the probe cCI6-60 specific for chromosome band 6q26 showed that the probe hybridised at the distal end of the normal chromosome 6. In the der(12) chromosome the corresponding fluorescent spot was detected in the middle of the q arm (fig 3E). Probe cCI6-107 specific for chromosome 6q27 hybridised at the distal end of the normal chromosome 6 and at the distal end of the der(12) chromosome (fig 3F).

Discussion

High resolution GTG banding is usually sufficient to identify chromosome translocations. However, for the clarification of complex chromosome rearrangements, FISH with chromosome painting probes is nowadays almost indispensable. At the moment painting probes are available for all chromosomes and their use has led to a reinterpretation of the karyotype in at least one other previously reported case with a CCR.⁵ In the present case, the combined data of GTG banding and chromosome painting still left some doubt about the structure of the der(12) chromosome. Microdissection has provided a means for characterisation of chromosome bands and chromosome regions. Several laboratories have previously used microdissection of GTG banded metaphase chromosomes to generate region specific libraries. The first studies used restriction endonuclease digestion and DNA ligation steps.²⁶⁻³⁰ Later, Meltzer *et al.*⁹ developed a technique that eliminated the microchemistry procedure by making use of a DOP-PCR and cloning procedure. Recently, Guan *et al.*^{11,13,15} used DOP-PCR probes generated from microdissected GTG banded chromosomes for chromosome (region) painting.

In our case, whole chromosome painting disclosed that at least six regions (and four breakpoints) were involved in this complex rearrangement. However, microFISH of the distal region of the der(12) chromosome showed that this region originated from two different segments of chromosome 6, namely 6q12-6q16.2 together with a distal region of 6q, thus disclosing a fifth breakpoint. FISH with probes specific for chromosome bands 6q26 and 6q27 further characterised this fifth breakpoint in chromosome 6 between these bands. The patient's karyotype is:

46,XY,t(6;12)(6pter→6q12::12q24.1→12qter; 12pter→12q13.3::6q16.2→6q26::12q13.3→12q24.1::6q12→6q16.2::6q26→6qter).

As loss or gain of chromosomal material was not detected, the mental retardation of our patient can best be explained by a small sub-microscopic deletion or by disruption of a gene or regulatory sequence caused by one (or more) of the five breakpoints. This assumption is supported by the increased risk of mental retardation and phenotypic abnormalities in carriers of apparently balanced de novo reciprocal translocations³¹ or de novo CCRs^{32,33} when compared with familial cases.

In summary, the use of chromosome painting, microFISH, and FISH with band specific probes showed a CCR of chromosomes 6 and 12 in which five breakpoints were involved. The application of these techniques provides a powerful tool in the characterisation of constitutional and acquired complex structural aberrations. Furthermore, the results of our study show that the generated DOP-PCR probe contains DNA sequences that span a breakpoint. Microdissection in combination with DOP-PCR and subsequent cloning will enable the isolation of sequences from translocation breakpoints associated with genetic diseases or specific forms of cancer that can be used in metaphase and interphase cytogenetics and in genome analysis.

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