Chromosome 2 aberrations in clinical cases characterised by high resolution multicolour banding and region specific FISH probes

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The field of human cytogenetics has been through many different stages of development, each of them improving the characterisation of structurally abnormal and/or supernumerary chromosomes. The era of reliable identification of human chromosomes started with the invention of the banding method by Dr Lore Zech in 1968.¹ The introduction of fluorescence in situ hybridisation (FISH) techniques in human cytogenetics by Pinkel *et al*² in 1986 allowed specific staining of chromosomes and chromosomal subregions. Even though G banding³ is still the gold standard against which all molecular cytogenetic techniques are measured, this technique based on alternating light and dark bands can lead to equivocal chromosome breakpoints.⁴ The development of multicolour FISH⁵ in 1996, multiplex FISH (M-FISH),⁶ and spectral karyotyping (SKY),⁷ allowing the simultaneous and specific painting of all 24 human chromosomes in different colours, was helpful in overcoming these problems in part. However, they are not suited for the detection of inversions or duplications or for more precise determination of chromosome breakpoints. Several FISH based techniques that are capable of solving this problem have been developed in the last decade: the application of chromosome arm specific probes,⁸ the use of chromosome bar codes,^{9,10} the cross species colour banding (RX-FISH) approach,¹¹ and the high resolution multicolour banding technique (MCB).^{12,13} The latter approach can cover the entire karyotype with human DNA probes without leaving any gaps.

To illustrate the power of the MCB technique, clinical cases with five different kinds of aberrations identified by conventional banding techniques, that is, translocations (four cases), deletions (two cases), duplications (three cases), inversions (two cases), and small supernumerary marker chromosomes (one case), were reinvestigated. In 9/11 cases (~80%), the chromosome breakpoints were redefined by MCB and these results have been confirmed by locus specific FISH probes.

MATERIAL AND METHODS

Fixed suspensions of peripheral blood from 11 patients with different chromosome 2 aberrations were included in the present study (table 1). Chromosome preparation and analysis was performed using routine cytogenetic procedures. As the studied cases were chosen according to their cytogenetic aberrations in chromosome 2, they form a clinically heterogeneous group. The corresponding clinical signs and symptoms and their cytogenetic aberrations are summarised in table 1.

High resolution multicolour banding (MCB) based on 10 microdissection derived, region specific libraries for chromosome 2 was performed as described previously.^{12,13} Additionally, the MCB probe sets for human chromosomes 8 and 11 were applied in cases 1 and 2, respectively, to characterise the corresponding translocation breakpoints. The MCB probe sets are specified in table 2 and in Mrasek *et al*.¹³ Region specific

YAC, BAC, and cosmid probes for chromosomes 2 and 9 were used in combination with the MCB probe sets or separately in one and two colour FISH experiments¹⁴; these probes are listed in table 2. Moreover, a probe for all human telomeres (DAKO) was hybridised on chromosomes of case 11 and a probe specific for the short arms of all human acrocentric chromosomes (midi 54, described in Mrasek *et al*¹³) was applied in case 4. CGH¹⁵ was performed in cases 1, 6, and 7.

Metaphase spreads were analysed using a fluorescence microscope (Axioplan 2 mot, Zeiss) equipped with appropriate filter sets to discriminate between a maximum of five fluorochromes and the counterstain DAPI (diaminophenylindol). Image capturing and processing were carried out using an isis mFISH imaging system (MetaSystems, Germany) for the evaluation of one and two colour FISH experiments, CGH, and MCB.

RESULTS

MCB pattern

The MCB pseudocolour pattern for chromosome 2 consists of 26 different bands (fig 1A). However, three bands (blue, red, yellow-green) are present twice in an identical sequence at 2p22-p21 and in the subcentromeric region (2q12-14.1). This is because of identical fluorochrome profiles in these two regions. The repetition could not be deleted by changing the labelling scheme of the five fluorochromes used. The knowledge of this small identical MCB pattern sequence did not influence the evaluation of the results in the present study.

The bands of MCB 2 were assigned to their corresponding light and dark G bands (fig 1A) via the inverted DAPI banding pattern, precisely located YAC probes, and the analysis of the fluorescence intensity profiles of the microdissected region specific probes (data not shown). The resolution achieved by the pseudocolour pattern corresponds to a 400 band resolution. Nonetheless, owing to the different colours of each of the MCB bands, apart from the three previously mentioned ones, it was possible to obtain more information out of this banding level than by simple black and white banding at this resolution.

Studied cases

Table 1 summarises the results obtained by the re-examination of chromosomal breakpoints for the 11 cases with chromosome 2 aberrations. In two cases (18%), MCB

Abbreviations: M-FISH, multicolour FISH; SKY, spectral karyotyping; RX-FISH, cross species colour banding; MCB, multicolour banding; YAC, yeast artificial chromosome; BAC, bacterial artificial chromosome; CGH, comparative genomic hybridisation; SMC, supernumerary marker chromosome

Table 1 Sex (M/F), clinical features, and karyotypes of the 11 studied cases after G banding (GTG) and multicolour banding (MCB)

Case No (sex)	Clinical signs and symptoms	Result after cytogenetics and MCB
<i>Translocation</i>		
1 (M)	Severe primary mental retardation Multiple dysmorphic features (eg, craniofacial dysmorphism, progressive kyphoscoliosis, muscular atrophy)	GTG: der(2)t(2;8)(q37;q22) MCB: der(2)t(2;8)(q37.3;q23.3) Translocation maternally inherited
2 (F)	Increased bone fragility	GTG: t(2;11)(q22.3-23.1;p15.1-15.2) MCB: identical to GTG De novo
3 (M)	Primary mental retardation Severely delayed speech development	GTG: der(9)t(2;9)(q24.2;p24.3) MCB: t(2;9)(q24.2;p24.3) De novo
4 (M)	Primary mental retardation Brachydactyly E Anaemia	GTG: add(2)(q37) MCB: der(2)t(2;acro)(q37.2;p11.2) De novo
<i>Deletion</i>		
5 (M)	Primary mental retardation Delayed speech development Craniofacial dysmorphism Sandal gap	GTG: del(2)(q32-33) MCB: del(2)(q31-32.1) De novo
<i>Duplication</i>		
6 (F)	Cerebral dysmorphism Pulmonary stenosis	GTG: dup(2)(p16.1p12) MCB: dup(2)(p16.3p12) De novo
7 (F)	Microphthalmia, microgenia, and cleft palate at birth Pierre-Robin syndrome-like clinical features	GTG: dup(2)(p15p13) MCB: dup(2)(p15p13.2) De novo
8 (M)	Healthy Infertility problems	GTG: dup(2)(p12) MCB: dup(2)(p13.2-q11.2) No family history available
<i>Inversion</i>		
9 (M)	Healthy	GTG: inv(2)(p11.2q13.1) MCB: identical to GTG Inversion detected in case 9 and in his unborn daughter
10 (M)	Primary mental retardation Tendency to seizures Craniofacial dysmorphism Adiposity	GTG: inv(2)(p11q23)+dup? or inv(2)(p21q24.1)+del? MCB: inv(2)(p15q24.3) No family history available
<i>Small supernumerary marker</i>		
11 (F)	BOR syndrome-like symptoms (ie, craniofacial dysmorphism and dysplasia of the kidney)	GTG: +mar MCB: +r(2)(p11.2q11.1) De novo

analysis confirmed the GTG breakpoints, in four cases (36%) one, and in five further cases (46%) both breakpoints and/or the nature of the rearrangement were redefined by MCB.

Translocations

Four cases with translocations of chromosome 2q and another autosomal chromosome were studied. In one of these cases, the translocation breakpoints determined by GTG could be confirmed by MCB using probes for chromosome 2 and 11 and FISH using two breakpoint flanking YAC probes (case 2, results not shown; all YAC probes are specified in table 2). The G banding result of case 1 could be refined by application of MCB probe sets for chromosomes 2 and 8 (fig 1B). CGH characterised the breakpoint in chromosome 8 as 8q22 (results not shown). By hybridising a subtelomeric probe for chromosome 2q, it could be shown, as well, that no obvious partial monosomy 2 was present in addition to the partial trisomy 8q23.3-24.3, as determined by MCB (fig 1B). In case 3, it could be shown by MCB and YAC or BAC probes that a reciprocal translocation between chromosomes 2 and 9 was present; the breakpoints were assigned correctly by G banding before, but a cytogenetically non-balanced situation was suggested. The additional material present at the tip of the long arm of chromosome 2q in case 4 could be characterised as material derived from one of the short arms of an acrocentric chromosome (results not shown) using a specific microdissection derived probe (midi 54). This translocation is combined with a

deletion of 2q37 material which could be visualised using a subtelomeric probe for 2q. This deletion was not visible on GTG banding. It was not clearly visible in the MCB pattern applied in the present study, even though the last blue band in 2q was clearly diminished in the derivative chromosome 2. The deletion could be made visible by introducing a subtelomeric 2q probe, which resulted in two additional bands in the MCB pattern of the normal chromosome 2 (fig 1B).

Deletions

Case 4 showed a translocation combined with a deletion (see above). Another deletion visible with GTG and MCB was studied in case 5 (fig 1C). Applying the MCB pattern used throughout the present study, a reduction of the blue-grey band corresponding to 2q31.2-32.1 to about 50% of its original size was observed in the derivative chromosome 2. In parallel, the YAC probes 894H9 and 956G4 could be seen to flank the breakpoint, while YAC 762E6 was located within the deleted region. Using YAC 762E6 and the MCB probe set for chromosome 2 simultaneously, an additional violet pseudocolour band was obtained proximal to the blue-grey band in the normal chromosome 2. This band was absent in the chromosome with the deletion (fig 1C). CGH performed on this case showed a similar result to MCB: .rev ish del(2)(q32).¹⁵

Duplications

Two of the three cases studied with duplications in chromosome 2 were analysed in parallel by MCB and by CGH. Very

Table 2 YAC, BAC, cosmid, and the microdissection derived MCB probes for chromosomes 2, 8, 9, and 11 used in the present study. Their chromosomal localisation and in which of the 11 cases (table 1) they have been applied are given

Probe	Localisation	Applied in case No
MCB probe 2-1	2pter-2p23	1-11
MCB probe 2-2	2p23-2p21	1-11
MCB probe 2-3	2p21-2p13	1-11
MCB probe 2-4	2p15-2p12	1-11
MCB probe 2-5	2p14-2q10	1-11
MCB probe 2-6	2p10-2q14.2	1-11
MCB probe 2-7	2q14.1-2q24.2	1-11
MCB probe 2-8	2q21.3-2q31	1-11
MCB probe 2-9	2q24.3-2q34	1-11
MCB probe 2-10	2q34-2qter	1-11
MCB probe 8-1	8pter-8p22	1
MCB probe 8-2	8p22-8p12	1
MCB probe 8-3	8p21.1-8q11.23	1
MCB probe 8-4	8q11.21-8q21.1	1
MCB probe 8-5	8q21.1-8q23.2	1
MCB probe 8-6	8q21.3-8q24.1	1
MCB probe 8-7	8q24.1-8qter	1
MCB probe 11-1	11pter-11p15.1	2
MCB probe 11-2	11p15.2-11p11.2	2
MCB probe 11-3	11p11.2-11q14.1	2
MCB probe 11-4	11p11.1-11q13.3	2
MCB probe 11-5	11q14.1-11q23.3	2
MCB probe 11-6	11q23.2-11qter	2
YAC 816E9	2p21	10
BAC 82K13	2p21	9
YAC 957B10	2p16.3	6
YAC 850A4	2p16.1-15	10
BAC 498O5	2p15	7
YAC 635D7	2p15-13.3	9, 10
YAC 856E4	2p12	6
YAC 850A4	2p12	8
YAC 953D7	2p12	8, 11
YAC 894F8	2p13	7, 8, 11
YAC 894F8	2p13	11
YAC 809C8	2q11.1-12	9
YAC 858D1	2p11	11
YAC 774E9	2q11	11
YAC 765E4	2q12	11
YAC 902B12	2q22	2
YAC 748E2	2q23	2
YAC 744G6	2q24	3, 10
YAC 952F10	2q24	10
YAC 938B4	2q24.2-24.3	3
BAC 26B22	2q24.3	3
YAC 894H9	2q24.3-31.1	5
YAC 762E6	2q31-32.1	5
YAC 956G4	2q32.1-32.2	5
cos210E14	Subtelomeric probe for 2q	1, 4
YAC 757A10	9q21-22	4
YAC 806f02	9q21.3-22.1	4
YAC 945f5	9q21.3-22.1	4

similar results were obtained, differing only slightly in one breakpoint each. Case 6 had a duplication of 2p16.3-p12 according to MCB and FISH using YAC probes (fig 1D) and CGH resulted in .rev ish dup(2)(p16.1p12). In case 7, a dup(2)(p15p13) was detected by CGH and a dup(2)(p15p13.2) was characterised by MCB. This result was confirmed by the breakpoint spanning YAC 894F8. The duplication in case 8 was the smallest included in the present study. Apart from the characterisation by MCB, the duplication was visible after FISH with YAC 850A4, YAC 953D7, and BAC 4C8. Moreover, YAC 894F8 spanned the breakpoint (results not shown).

Inversions

One of the two cases studied with pericentric inversions had the well known constitutive variant of inv(2)(p11.2q13.1). It

(case 9) was successfully characterised by MCB, but owing to flaring effects¹³ an additional orange plus brown band and an extra orange band appeared in the short and long arm of the derivative chromosome 2, respectively (fig 1E, case 11, red arrowheads). Case 10 had a large pericentric inversion, not clearly visible by G banding. MCB clearly characterised this inversion. The results were confirmed by a panel of YAC probes (table 2), of which YAC 850A4 and YAC 744G6 were the nearest to the breakpoints within the inverted regions (fig 1E, case 10).

SMC

One case with a supernumerary marker chromosome (SMC) derived from chromosome 2 was also characterised by MCB. The SMC of case 11 was characterised previously by cenM-FISH and was present in about 70% of the metaphases.¹⁶ According to the MCB pattern, the SMC is derived from 2p11.2-q11.1, which was confirmed by a positive signal of BAC 4C8 (fig 1F). As no telomeric signals were detectable on the SMC, it was assumed that it must be a ring chromosome.

DISCUSSION

Technical aspects

MCB allows high resolution analysis of the fine structure of chromosomes at various band levels. In the present study, a 400 band level was chosen for chromosome 2; however, higher resolutions are also possible as all the pseudocoloured bands are fluorescence ratio specific and can freely be assigned using the ISIS software (MetaSystems, Altlußheim, Germany). For example, different banding resolutions for chromosome 5 have been used in a research paper on leukaemia breakpoints (24 different pseudocoloured bands¹⁷) compared to a study on α ray induced aberrations (12 different pseudocoloured bands¹⁸). The same MCB probe set was applied in both studies. In the present study, additional bands were created successfully in another way, by including additional region specific probes (case 4, fig 1B and case 5, fig 1C).

The present study was not hampered by the fact that three bands in 2p22-p21 and in 2q12-14.1 had an identical pseudocolour sequence. This problem, which is based on identical fluorochrome profiles of these two regions, could only be solved by introducing more fluorophores, which is hampered mainly by the availability of additional reliable fluorochromes. Only in one case with a small pericentric inversion (case 9) did problems appear because of flaring effects.⁶ Similar effects have been seen before in a minority of rearrangements studied with the MCB technique and have been discussed before.^{12 13 17} In general, chromosomal aberrations that cause artefacts while using the MCB pseudocolours can be clarified by carefully analysing the fluorescence ratios.^{12 13 17}

Chromosomal breakpoints were reinvestigated by MCB in at least 10 metaphase spreads per case. The breakpoints always appeared within the same coloured bands, which underlines the reproducibility of the method.¹²

Studied cases

In 10 of 11 studied cases, the chromosomal breakpoints were redefined by MCB, which is a similar rate to other previous MCB studies.^{12 17} MCB was shown to be more precise than the CGH method and the results obtained were in concordance with the conventional banding pattern. As FISH using breakpoint flanking or spanning YACs, BACs, or cosmids is the method of choice to define the chromosomal breakpoints exactly on a molecular level, these probes have been used additionally in each of the 11 studied cases. It could clearly be seen that the MCB results are also in agreement with the additionally used region specific probes, suggesting that MCB is a reliable tool for the definition of chromosomal breakpoints. No similar cases to our cases 1, 2, and 3 with translocations involving chromosomes 2 and 8 and 11 and 9 and an

Figure 1 FISH images were captured on a Zeiss Axioplan microscope (Zeiss Jena, Germany) using a XC77 CCD camera with on-chip integration (Sony); the MCB images were created with the IKAROS and ISIS digital FISH imaging system (MetaSystems, Altlußheim, Germany). In fig 1B-F chromosomal breakpoints are marked with blue arrowheads. (A) Chromosome 2 is depicted schematically according to the GTG banding on the left of this figure. The MCB pseudocolour pattern consisting of 26 different bands is shown beside the GTG scheme for two normal chromosomes 2. Each pseudocolour has been aligned with the corresponding GTG bands. (B) Two cases with translocations involving one chromosome 2 are presented. Case 1 has a translocation of chromosomes 2 and 8. The material of chromosomes 2 and 8 is coloured in light and dark grey, respectively, on the der(2). The fact that no chromosomal material was lost on the der(2) was proven by the application of a subtelomere 2q (subtel 2q) probe. In case 4, additional material derived from one of the short arms of an acrocentric chromosome (results not shown) was translocated to the tip of the long arm of chromosome 2 and is combined with a deletion of 2q37 material which could be visualised using a MCB 2 probe set in combination with a subtelomeric probe for 2q (subtel 2q, small white arrowhead). For further details refer to the text. (C) Another case with a deletion (case 5, deletion in 2q31-32.1) could be visualised by MCB and MCB in combination with a region specific probe (YAC 762E6, small white arrowhead). (D) Two cases with pericentric inversions of chromosome 2 were studied by MCB. Case 9 has the well known constitutional inversion inv(2)(p11.2q13.1); in MCB a flaring effect led to additional false pseudocolours (red arrows). For further details refer to the text. In case 10, a large inversion could be characterised by MCB. The results have been confirmed by single colour FISH using specific YAC probes (YAC 850A4, 2p16.1-15 and YAC 744G6, 2q24). (E) A small supernumerary marker chromosome (SMC) derived from chromosome 2 was characterised by MCB, region specific YAC and BAC clones, and a telomeric probe (see text).

Key points

- Precise localisation of breakpoints is of major interest in clinical cytogenetics. However, conventional banding techniques often fail to characterise the exact nature of chromosomal rearrangements.
- In order to improve the definition of chromosomal breakpoints, the high resolution multicolour banding (MCB) technique was applied to identify human chromosome 2 breakpoints from 11 clinical cases presenting with five different kinds of aberration: translocations, deletions, duplications, inversions, or small supernumerary marker chromosomes (SMC).
- The results of MCB were aligned successfully with other molecular cytogenetic techniques, like CGH or FISH, using locus specific probes. In nine of the 11 cases, at least one breakpoint was redefined, indicating that MCB markedly improves chromosomal breakpoint definition.
- The highly reproducible MCB pattern can be used to characterise abnormalities that remain cryptic or unresolvable by G banding analysis.

acrocentric chromosome, respectively, have previously been published. However, a fragile site in chromosome 11p15.1 has been described, which is near the breakpoint in chromosome 11 of case 2,¹⁹ and the breakpoint in chromosome 9 of case 3 was involved in a familial complex chromosome rearrangement as well.²⁰ A deletion of the chromosomal band 2q37.3 as in case 4 has been reported previously in similar cases with brachydactyly E and Albright hereditary osteodystrophy (AHO)-like disorder.²¹ Translocations of 2q37 and other chromosomes have been reported.^{22–25}

Case 5 has the smallest interstitial deletion involving the region 2q31–32.1 reported so far. There are two similar published cases also with symptoms like the case reported here.^{24–25} The duplications present in cases 6 and 7 are also smaller than those published for the corresponding regions,^{26–27} while a duplication as reported for case 8 has not previously been published.

The constitutional inversion inv(2)(p11.2q13) as in case 9 is well known and was studied intensely at the beginning of the banding era.²⁸ Similar large inversions like that of case 10 have been published.^{29–30} Interestingly, as the patient suffers from seizures, a gene for spastic cerebral palsy has recently been mapped in the region of the breakpoint in 2q.³¹

Similar cases to case 11 with a SMC have been described before and have been summarised by Crolla.³² Either autistic behaviour or no abnormalities were reported in one spontaneous and one familial case, respectively.³²

The present study showed that the MCB technique is able to resolve any kind of chromosomal aberration. For this, the results of the MCB technique were aligned with the GTG banding results and confirmed by locus specific YAC, BAC, and cosmid probes. As shown in the discussion of each of the 11 cases, only a small number of or even no comparable published cases were available, for example, cases 6 and 10. Only after more cases have been characterised in more detail, including the exact localisation of their breakpoints, can conclusions about candidate genes and genotype-phenotype correlations be drawn.

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