LETTER TO JMG

Subtelomere specific microarray based comparative genomic hybridisation: a rapid detection system for cryptic rearrangements in idiopathic mental retardation

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Mental retardation (MR) occurs in 2–3% of the general population, and more than half of MR patients are categorised as idiopathic—that is, the cause is unknown.[1 2] Patients with idiopathic MR are presumed to be affected with certain genetic disorders or undetectable chromosomal abnormalities. MR may also be caused by environmental factors independently or by their interaction with genetic factors. Subtelomeric rearrangements comprise about half of segmental aneusomies,[3] and are one of the major causes of MR.[4 5] A recent review showed that subtelomeric rearrangements were detected in 131 (5.1%) of 2585 children with MR.[1 4–6] Conventional cytogenetic analysis can detect many, but not all, rearrangements, depending on its powers of resolution.[4] Other methods, such as fluorescent in situ hybridisation (FISH) using a complete set of subtelomeric probes, multicolour FISH (M-FISH), comparative genomic hybridisation (CGH), spectrum karyotyping, multiple amplifiable probe hybridisation, primed in situ labelling, and genotyping have been designed to detect subtelomeric rearrangements, but none of them is ideal in terms of sensitivity and/or efficiency.[4 6] Microarray based CGH is a promising, high throughput method of detecting subtelomeric rearrangements.[4] Veltman *et al* recently reported a microarray CGH system using crude bacterial/plasmid derived artificial chromosome (BAC/PAC) DNA for the analysis of subtelomeric aberrations, and suggested that degenerate oligonucleotide primed (DOP)-PCR products could also be used instead of crude clone DNA, although the performance of DOP-PCR products might be less sensitive.[7] We have developed a microarray CGH system to identify rearrangements involving a subtelomeric region, using DOP-PCR that amplifies subtelomeric BAC/PAC DNA. Here we describe details of the method and the results of microarray CGH analyses of five cases of Wolf-Hirschhorn syndrome (WHS) associated with terminal 4p deletions as positive controls, and of 69 patients with idiopathic MR with or without multiple congenital anomalies (MCA).

MATERIALS AND METHODS
Target DNA for microarray
BAC/PAC clones used in this study are shown in table 1. We selected subtelomeric clones based on the second generation set of human chromosome specific subtelomeric probes reported by Knight *et al*.[8] Five clones (GS-124I4 for 16p, GS-48O23 and GS-325I23 for 19q, GS-82O2 for 20p, and GS-202M24 for Xq) that showed signals appearing at sites different from those originally reported were excluded from this study. Three clones (GS-99K24, CITD-3018K1 for 22q, and GS-225F6 for XqYq) were unavailable. New clones (RP11-359B7 for 19q, RP11-238J15 for 20p, RP3-402G11 for

Key points

- We have developed a microarray based comparative genomic hybridisation (CGH) assay using bacterial and plasmid derived artificial chromosome clones mapped uniquely to each subtelomeric region of human chromosomes.

- To confirm the reliability of detecting cryptic subtelomeric rearrangements, we analysed five cases of Wolf-Hirschhorn syndrome (WHS) that were known to have a distal 4p deletion. The microarray CGH system successfully enabled us to reveal that two of the five WHS cases had 5q or 10q trisomy in addition to the 4p deletions present in all.

- We then analysed 69 subjects with idiopathic mental retardation (MR), whose karyotype was normal by conventional G banding analysis. The microarray successfully disclosed cryptic subtelomeric rearrangements in 4 (5.8 %) of the 69 patients.

- All these abnormalities showed a fluorescent signal intensity ratio (Cy3/Cy5) beyond the 2SD range, and were subsequently confirmed by conventional fluorescent in situ hybridisation using subtelomere specific BAC/PAC clones. Their karyotypes were ish dup(12)(p13.3p13.3)mat; ish del(9)(q34.3)de novo; ish der(5)t(5;20)(p15.33;p13)pat; and ish der(10)t(10;12)(q26.3;p13).

- The microarray CGH we have developed is a rapid and high throughput system and provides a promising way to find subtelomeric rearrangements in patients with idiopathic MR or MCA of unknown cause.

22q, and RP11-431N21 for XqYq) were obtained from online databases, the human telomere mapping and sequencing project (http://www.wistar.upenn.edu/Riethman/) and the human genome browser (http://genome.ucsc.edu/cgi-bin/hgGateway? Db = hg11). Although RP11-431N21 mapped to Xq/Yq showed unequal signal intensities between Xq and

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Table 1 BAC/PAC clones used in subtelomeric microarray CGH

Locus	Clone ID	Clone type
1pter	GS-232B23	BAC
1qter	GS-160H23	PAC
2pter	GS-8L3	BAC
2qter	CITB-172I13	BAC
3pter	CITB-228K22	BAC
3qter	GS-56H22	BAC
4pter	GS-36P21	PAC
4qter	GS-31J3	BAC
5pter	GS-189N21	PAC
5qter	GS-240G13	PAC
6pter	GS-62I11	PAC
6qter	GS-57H24	PAC
7pter	GS-164D18	PAC
7qter	GS-3K23	PAC
8pter	GS-77L23	BAC
8qter	GS-261I1	BAC
9pter	GS-43N6	PAC
9qter	GS-135I17	BAC
10pter	GS-306F7	PAC
10qter	GS-137E24	PAC
11pter	GS-44H16	PAC
11qter	GS-26N8	PAC
12pter	GS-124K20	BAC
12qter	GS-221K18	PAC
13qter	GS-163C9	PAC
14qter	GS-200D12	PAC
15qter	GS-154P1	PAC
16pter	CITB-191K2	BAC
16qter	GS-240G10	PAC
17pter	GS-68F18	BAC
17qter	GS-50C4	BAC
18pter	GS-52M11	P1
18qter	GS-75F20	BAC
19pter	CITB-129F16	BAC
19qter	RP11-359B7	BAC
20pter	RP11-530N10	BAC
20qter	RP11-238J15	BAC
21qter	GS-63H24	PAC
22qter	RP3-402G11	PAC
XpterYpter	GS-98C4	PAC
XqterYqter	RP11-431N21	BAC
Xp22.32	RP11-267O9	BAC
Xp22.3	RP4-617A9	BAC

RP11-267O9 and RP4-617A9 cover the steroid sulphatase gene (*STS*) and the arylsulfatase E gene (*ARSE*), respectively.

Bio). PCR was cycled 30 times at 94°C for 30 seconds, at linear ramping of 37–72°C over 10 minutes, and at 72°C for 1 minute. Successfully amplified PCR products, usually 0.2–2 kb in size, were used for subsequent experiments. Using DOP-A, DOP-B, or DOP-C, each PCR product (100 μl) was mixed with the same volume (300 μl) of 4×print buffer (1 mol/l sodium phosphate/0.001% *N*-lauroyl sarcosine, pH 8.5) and concentrated to a final volume of 30 μl using Microcon 100 (Millipore, Bedford, MA, USA). The products were mixed with DMSO and ddH$_2$O to a final concentration of 1×print buffer/20% DMSO and were ready for prints.

Subject DNA

Genomic DNA from 69 cases of idiopathic MR, whose karyotype was normal by G banding chromosome analysis at the 400 band level, was analysed. MCA was seen in all subjects except one, but recognisable syndromes were ruled out by at least one of the co-authors. We also used DNA from five patients with WHS (WHS1–5), who had been diagnosed as having various deletions for distal 4p, as positive controls to examine the reliability of the microarray CGH system described below (table 2), and that from normal controls as reference DNA for the system.

Microarray CGH

Each product was spotted in triplicate onto CodeLink™ activated slides (Amersham Bioscience, Buckinghamshire, UK) using Stampman (Nippon Laser and Electronics Laboratory, Nagoya, Japan) with a centre to centre distance of 1 mm between adjacent spots. The same triplicate spots were printed twice to block A and block B on the same slide. The slides were then pretreated,[10] denatured, and stored in a desiccator until use. Subjects' DNA was labelled with Cy3, and control DNA with Cy5 (CGH 1). Reverse labelling (patient DNA with Cy5, and control DNA with Cy3) was also performed (CGH 2). *Bam*HI digested genomic DNA (1 μg) was labelled with Cy5 or Cy3 dCTP by random prime labelling. Differentially labelled DNA was mixed with 67.5 μg CotI DNA (Invitrogen, Tokyo, Japan), and suspended with 60 μl hybridisation buffer (50% formamide, 10% dextran sulfate, 0.1% Tween 20, 2×SSC, 10 mmol/l Tris-HCl; pH 7.4) with 60 μg yeast tRNA (Roche, Basel, Switzerland). After prehybridisation with herring sperm DNA and human CotI DNA using an in situ frame (Eppendorf, Hamburg, Germany)

Yq (FISH signals of Xq were twice as intense as those of Yq), we included it in our microarray because no other suitable clones could be obtained. In addition, we added two X chromosome specific clones (RP11-267O9 on Xp22.32 and RP4-617A9 on Xp22.3) as internal controls. Clones were purchased from either Incyte Genomics (Polo Alto, CA, USA) or Invitrogen (Carlsbad, CA, USA). DNA was extracted using QIAgen Plasmid Midi kits (QIAgen, Hilden, Germany) from 200 ml bacterial culture with appropriate antibiotics according to the manufacturer's protocol.

Degenerate oligonucleotide primed PCR

DOP-PCR was performed to amplify target clone DNA using three different 5'-aminolabelled PCR primers, DOP-A (5'-CCGACTCGAGNNNNNATGTGG-3'), DOP-B (5'-CCGACTC GAGNNNNNNTTCACC-3'), and DOP-C: (5'-CCGACTCGAG NNNNNNAACAGG-3'). The sequence of DOP-A is the same as that of 6MW by Telenius *et al*.[9] Two other primers were randomly designed and confirmed as capable of amplifying clone DNAs as effectively as DOP-A. Primers were purchased from Hokkaido System Science Co Ltd (Sapporo, Japan). DOP-PCR was performed in a 100 μl cocktail containing 50 ng BAC/PAC DNA, 1 μmol/l of a DOP primer, 3 mmol/l MgCl$_2$, 200 mmol/l of each dNTP, 1× Ex*Taq* buffer (Takara Bio, Ohtsu, Japan), and 0.5 U Ex*Taq*™HS enzyme (Takara

Table 2 Summary of chromosomal aberrations detected in this study

Patient	Previous karyotype	Confirmed karyotype by combined results of microarray CGH and FISH
WHS		
WHS1	46,XY,del(4)(p16)de novo	46,XY.ish der(4)t(4;10)(p16;q26)de novo
WHS2	46,XY del(4)(p16)	46,XY,der(4)t(4;5)(p15.3;q35.1)pat
WHS3	46,XX,del(4)(p16.1).ish del(4)(p16.3)(D4S96-)	Same as above
WHS4	46,XY,del(4)(p15.3)	Same as above
WHS5	46,XX,del(4)(p15.2)de novo	Same as above
MR		
N02R245	Normal	46,XX.ish dup(12)(p13.33p13.33)mat
N03R003	Normal	46,XY.ish del(9)(q34.3)de novo
N03R017	Normal	46,XY.ish der(5)t(5;20)(p15.33;p13)pat
N03R088	Normal	46,XX,der(10)t(10;12)(q26.3;p13.1)

WHS, Wolf-Hirschhorn syndrome; MR, mental retardation.

surrounding each block on a slide in a hybridisation chamber (Genetix, New Milton, UK), labelled probe mixtures of CGH1 and CGH2 were simultaneously applied to blocks A and B, respectively. Slides were incubated at 37℃ for 72 hours with gentle shaking (5 rpm), and then washed twice with solution A (1×PBS with 0.05% Tween 20) for 10 minutes at room temperature, twice with solution B (2×SSC with 50% formamide) at 43℃ for 15 minutes, and finally with solution A at room temperature for 10 minutes. Slides were scanned with GenePix 4000B (Axon Instruments Inc, Union City, CA, USA) and analysed with GenePix Pro 4.0 software (Axon Instruments Inc).

The intensity ratio between patient and control DNA was calculated from the data of the single slide experiment, using the ratio of means formula. Average normalised inter-locus fluorescence ratio (ANILFR)[11] was calculated for each CGH. We had previously performed five sets of CGH experiments with two different normal DNA samples in order to evaluate the thresholds for copy number gain and loss, which showed that the average ANILFR of each clone ranged from 0.86 to 1.18, with an SD of 0.06. Based on this, the normal ANILFR range was defined as numbers within 2SD. This configuration was verified by the fact that the ANILFR of all clones excluding unique X chromosomal clones were within 2SD (fig 1A). If the mean signal ratio on each spot of clone was more than 2SD out of range, FISH analysis was subsequently performed.

FISH analysis

Subtelomeric BAC/PAC DNA labelled with either SpectrumOrange™ dUTP or SpectrumGreen™ dUTP (Vysis, Downers Grove, IL, USA) by nick translation was used for FISH analysis. Probes were denatured and hybridised to metaphase chromosomes of patients at 37℃ for 16 hours and washed. Photoimages were captured using a monochrome CCD camera (Carl Zeiss, Jena, Germany) on an Axioplan 2 fluorescence microscope (Carl Zeiss) with appropriate filters, and were analysed with ISIS software (version 3.4.5; MetaSystems, Altlussheim, Germany). All five WHS cases were analysed using all subtelomeric clones to confirm the results of the microarray analysis. In MR patients, specific subtelomeric probes were used for FISH analysis where a deletion or a duplication was suspected.

RESULTS

Patients with WHS

Five cases of WHS were analysed as positive controls by microarray CGH and FISH using all subtelomeric probes. As expected, using microarray CGH with the 4p subtelomeric clone, GS-36P21, the signal ratios for the patients were beyond the 2SD range (fig 1B–F), and all were confirmed to have a deletion at the GS-36P21 site. In addition, other subtelomeric aberrations were suspected by microarray analysis—that is, a 10q duplication in WHS1 and a 5q duplication in WHS2 (fig 1B, C). FISH using respective subtelomeric clones confirmed these aberrations. The parents of WHS1 and the mother of WHS2 were normal by FISH studies, but the father of WHS2 had a balanced translocation between 4p and 5q. High resolution G banding analysis indicated his karyotype to be 46,XY,t(4;5)(p15.3;q35.1). Thus, the karyotypes of WHS1 and 2 were interpreted as 46,XY.ish der(4)t(4;10)(p16;q26)de novo and
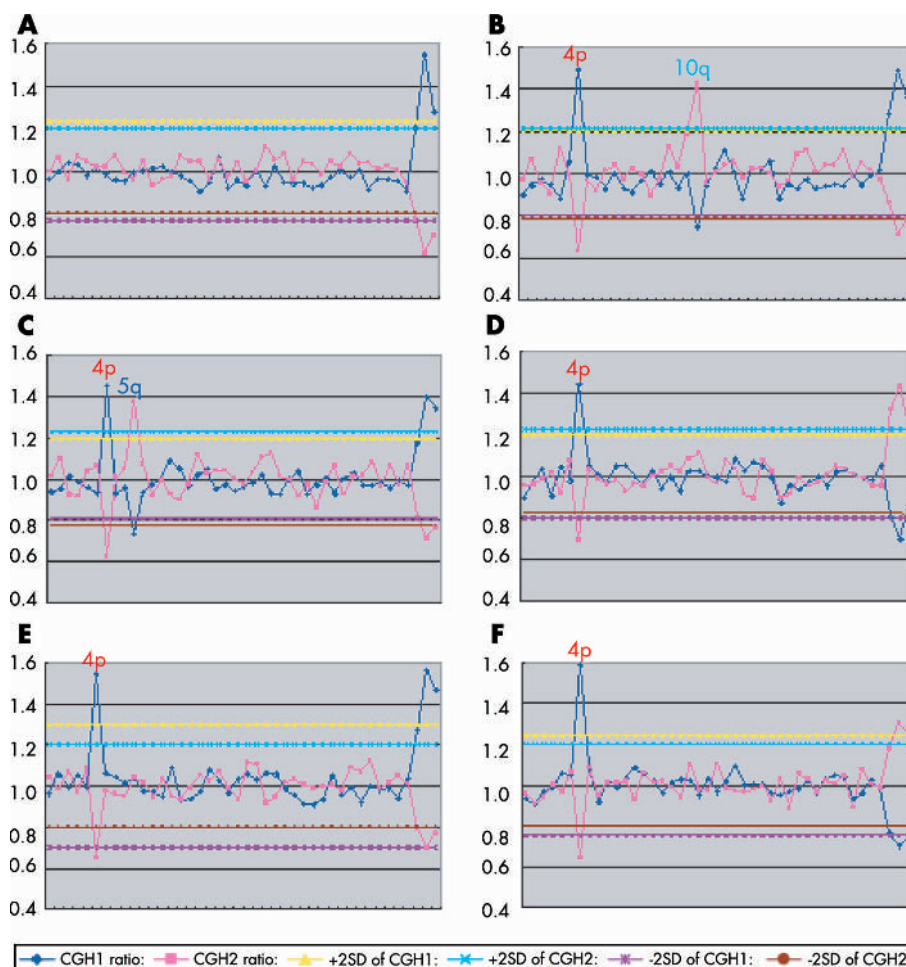
Figure 1 Microarray CGH profiles in a normal control and five cases of WHS. (A), (B), (C), (D), (E), and (F) indicate microarray CGH profiles in a normal male and in WHS1, 2, 3, 4, and 5, respectively. Horizontal axis, left to right: subtelomeric clones mapped to 1p, 1q, 2p, 2q, 3p, 3q, 4p, 4q, 5p, 5q, 6p, 6q, 7p, 7q, 8p, 8q, 9p, 9q, 10p, 10q, 11p, 11q, 12p, 12q, 13q, 14q, 15q, 16p, 16q, 17p, 17q, 18p, 18q, 19p, 19q, 20p, 20q, 21q, 22q, Xp/Yp, Xq/Yq, and clones covering *STS* at Xp22.32 and *ARSE* at Xp22.3. Vertical axis showed the CGH ratio (Cy5/Cy3) at each locus. CGH1 was performed by hybridisation of Cy3 labelled test DNA (patient) and Cy5 labelled reference DNA (normal control of opposite sex). CGH2 was hybridised with Cy5 labelled test DNA (patient) and Cy3 labelled reference DNA (normal control of opposite sex). Chromosomal gains and losses found were shown as blue and red characters, respectively. All rearrangements were clearly detected as ratios beyond the 2SD range. All WHS cases clearly showed their terminal 4p deletions (B–F). WHS1 and 2 also showed subtelomeric duplications of 10q and 5q, respectively (B, C). Internal reference clones in the X chromosome were also indicated as ratios beyond the 2SD range because of being the opposite sex.

Legend: CGH1 ratio: — CGH2 ratio: — +2SD of CGH1: — +2SD of CGH2: — -2SD of CGH1: — -2SD of CGH2

46,XY,der(4)t(4;5)(p15.3;q35.1)pat, respectively (table 2). In order to verify the accuracy of these micoarray CGH data, all subtelomeric regions were investigated by FISH in the five WHS cases, which confirmed the CGH data and supported the reliability of the method (table 2).

Idiopathic MR patients

We analysed 69 cases of idiopathic MR with or without MCA by microarray CGH. Subtelomeric rearrangements were detected in the following four cases (5.8%): N02R245, N03R003, N03R017, and N03R088 (table 2).

N02R245

A 2 year old girl was the second child of non-consanguineous healthy parents without any family history of MR or congenital anomalies. Her birth at 38 gestational weeks was uneventful, with a birthweight of 3130 g (+0.3 SD), length of 50 cm (+1SD) and Apgar score of 9 at 5 minutes. She showed moderate developmental delay. She could control her head at 5 months of age, and sit at 10 months, but could neither walk nor speak any meaningful words at 19 months. At the age of 21 months, her height was 80 cm (25th percentile), weight 9.6 kg (25th percentile), and head circumference 49 cm (90th percentile). Minor facial anomalies noted included epicanthal fold, depressed nose, low nasal bridge, and thin upper lip. A brain MRI study at 10 months did not reveal any abnormalities. A chromosomal gain of a 12p subtelomeric region was detected by microarray CGH (fig 2A). FISH analysis on metaphase chromosomes using the 12p subtelomeric clone GS-124K20 revealed that a signal on one homologous chromosome 12 was twice as large as that on the other (fig 3A), and duplicated signals and a separate single signal were detected on interphase cells (fig 3A), suggesting that tandem duplication of a 12p subtelomeric region had occurred. A similar finding was observed in her mother, whose phenotype was normal, but it was not observed in her father. High resolution G banding analysis of the patient and her mother did not reveal any differences in chromosome 12 (fig 4A). In order to see whether the 12p duplication was a normal variant, FISH analysis using GS-124K20 as the probe

was performed on 50 normal Japanese controls, none of which showed the duplication.
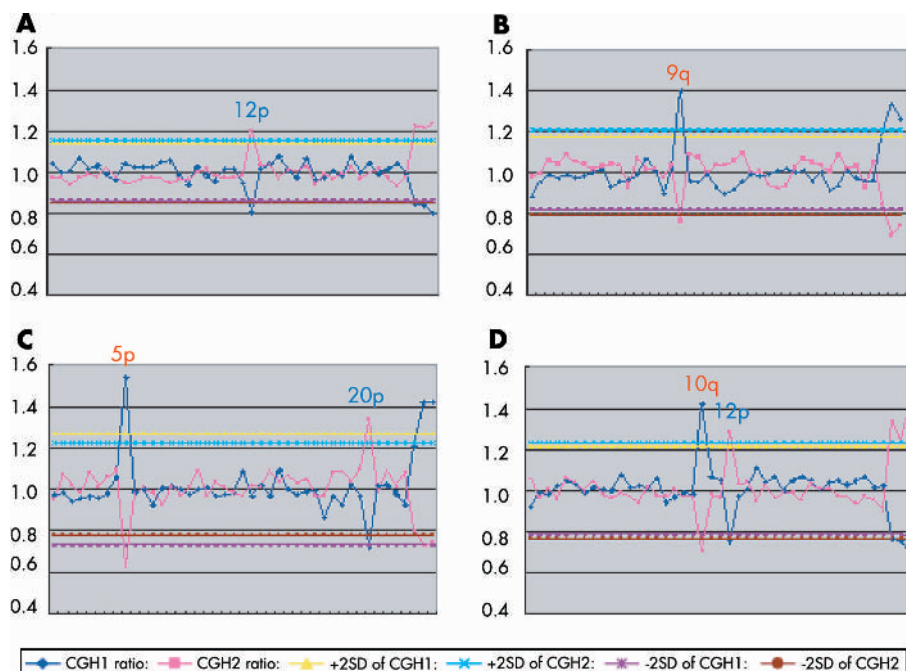
N03R003

An 8 year old boy had severe MR, seizures, microcephaly, dysmorphic face, and other anomalies. Detailed clinical information has been described elsewhere as patient 2.[12] Chromosomal deletion of a 9q subtelomeric region was detected by micoarray CGH (fig 2B), and was confirmed by FISH using a 9q subtelomeric probe as described previously[12] (fig 3B). As FISH analysis confirmed that neither of his parents had such a deletion, it was considered a de novo mutation. G banding chromosome analysis could not identify the deletion in the patient (fig 4B).

N03R017

A 15 month old boy was the first child of non-consanguineous healthy parents without any family history of MR or congenital anomaly. His birth at 39 gestational weeks was uneventful. His birth weight was 3046 g (+0.25 SD), birth length 52 cm (+1.5 SD), and Apgar score 9 at 5 minutes. He presented with severe developmental delay. He could control his head at 5 months of age, and sit at 12 months, but could neither walk nor speak any meaningful words at 14.5 months old. At 1 year of age, his height was 75 cm (50th percentile), and weight 7.8 kg (3rd percentile). Minor craniofacial anomalies were microcephaly, flat occiput, epicanthal folds, depressed nasal root, long philtrum, low set ears, and micrognathia. Infantile leukaemia was observed at the age of 10 months, and chemotherapy was performed. A subtelomeric chromosomal deletion of 5p and a gain of 20p were detected by microarray CGH (fig 2C), and were confirmed by FISH (fig 3C). FISH analysis of his parents revealed that his phenotypically normal father showed a balanced translocation between 5p and 20p, but no morphological difference between homologous chromosomes 5 and 20 was observed by G banding analysis (fig 4C).

N03R088

A 6 year old girl was born at 41 gestational weeks uneventfully. Her birth weight was 3020 g (−1 SD), birth

Figure 2 Microarray CGH in idiopathic MR cases. (A), (B), (C), and (D) show the results of microarray CGH on N02R245, N03R003, N03R017, and N03R088, respectively. Chromosomal gains and losses identified are shown as blue and red characters, respectively. Abnormal rearrangements were clearly detected as a CGH ratio beyond the 2SD range of ANILFR. (A) Duplication of 12p; (B) deletion of 9q; (C) deletion of 5p and duplication of 20p; (D) deletion of 10q and duplication of 12p.
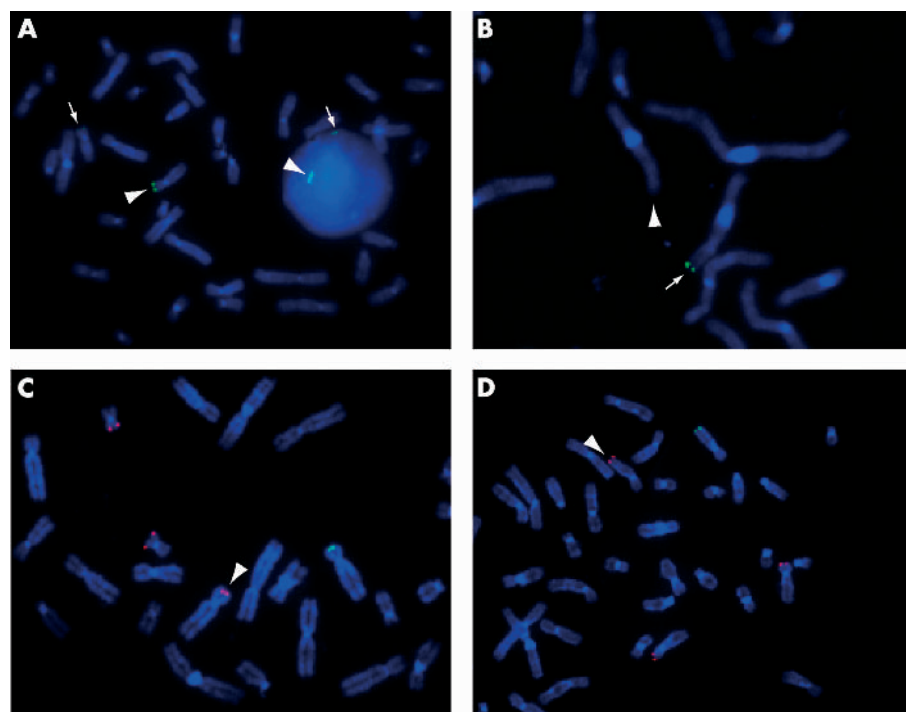
Figure 3 FISH studies confirming chromosomal abnormalities that were detected by microarray CGH in idiopathic MR cases. (A), (B), (C), and (D) show the results of the FISH analysis to confirm the microarray CGH detected rearrangements. (A) A subtelomeric clone for 12p (GS-124K20; green) was hybridised to metaphase chromosomes from N02R245. The arrow and arrowhead indicate a signal of a normal chromosome and that of its counterpart chromosome with a duplication, respectively. (B) A 9q subtelomeric clone (GS-135I17; green) was hybridised to chromosomes of N03R003. The arrow indicates a signal on chromosome 9, and the arrowhead shows a deletion. (C) Subtelomeric clone of 5p (GS-189N21; green) and subtelomeric clone for 20p (RP11-530N21; orange) were hybridised to chromosomes of N03R017. The arrowhead indicates a derivative chromosome, ish der(5)t(5;20)(p15.33;p13)pat. (D) Subtelomeric clones for 10q (GS-137E24; green) and for 12p (GS-124K20; orange) were hybridised to chromosomes of N03R088. The arrowhead indicates a derivative chromosome, der(10)t(10;12)(q26.3;p13.1).

length 47 cm (-1.5 SD), and Apgar score 9/10 at 1 and 5 minutes. She was the second child of non-consanguineous healthy parents. She showed general hypotonia and severe developmental delay. She could control her head at 6 months of age, roll over at 9 months, and sit at 4 years, but could neither walk nor speak any meaningful words at 6 years of age. She had febrile seizures that occurred at 1 year of age, but never developed epilepsy. Craniofacial abnormalities were noted: flat occiput, square shaped face, hypertelorism, low set ears, downturned corners of the mouth, cleft soft palate, and micrognathia. Brain MRI revealed mild dilatation of the lateral ventricles, cavum septum pellucidi, and cavum vergae. A sacral dimple was identified, but MRI could not reveal any gross abnormalities. Microarray CGH analysis detected a subtelomeric chromosomal deletion of 10q and a gain of 12p (fig 2D), which were subsequently verified by FISH (fig 3D). High resolution G banding analysis revealed that her karyotype was 46,XX,der(10)t(10;12)(q26.3;p13.1) (fig 4D). A parental origin of the unbalanced translocation was not confirmed because parental materials were unavailable.

DISCUSSION

Molecular studies focusing on cryptic subtelomeric rearrangements in idiopathic MR patients have been performed mainly by FISH using subtelomere specific probes, and genotyping using hypervariable genetic markers.[1 4–6] Microarray CGH, a high throughput technology, was developed to identify changes in copy number of chromosomal segments or genes not only in somatic events (neoplasms), but also in germline derived events (constitutional chromosomal changes).[13–18] Recently, Veltman et al showed the efficiency of subtelomere specific microarray CGH in detecting known and novel cytogenetic abnormalities.[7] We have developed subtelomere specific microarray CGH for MR. Its accuracy has been confirmed using five cases of WHS as positive controls, and we applied it to analysis of 69 patients with idiopathic MR. All the WHS cases showed a 4p

subtelomeric deletion, and two were found to have either a 10q or a 5q duplication by microarray CGH. Four (5.8 %) of 69 cases of idiopathic MR were found to have subtelomeric rearrangements, the frequency being consistent with the data from recent reports.[1 4–6] The accuracy of our microarray CGH was verified by FISH using all subtelomeric probes in the five WHS cases; the genomic rearrangements were not overlooked by our microarray CGH in any of them. ANILFR of clones with either a deletion or a duplication, and in those without changes of the copy number were 0.628, 1.433, and 0.995, respectively, in all the patients. The two former ratios were beyond the 2SD range, while all other clones were within the range (figs 1 and 2). We used three different DOP-PCR primers, DOP-A (the original primer developed by Telenius et al and named as 6MW[9]), DOP-B, and DOP-C (both originally designed by us). DOP-PCR products using these primers should represent original clones more faithfully than those using a single DOP primer. Similarly, Fiegler et al[10] adopted DOP-PCR using three different primers designed to amplify human specific DNA instead of contaminated bacterial DNA, and showed efficient detection of gains and deletions in a cancer cell line. Although our primers, DOP-B and DOP-C, were not designed for human specific DNA, DOP-PCR using the three primers was suitable for generating DNA for microarray. In addition, using the two simultaneous, independent hybridisations, CGH1 and CGH2, on one glass slide can eliminate conflicting results due to different conditions in each hybridisation, and detect genomic gains and deletions easily, because the data complement each other.

As well as the common 4p deletion, additional chromosomal abnormalities were detected in two (WHS1 and 2) of five WHS patients. Both showed a typical WHS phenotype, suggesting that the WHS critical region could be the "master" regulatory sequence on clinical manifestations with a strong pleiotropic effect,[19] or that the effects of 10q26 duplication and 5q35.3 duplication could be minimal.

Cryptic subtelomeric rearrangements were detected in 4 of the 69 cases of idiopathic MR (table 2). A 9q34.3 terminal
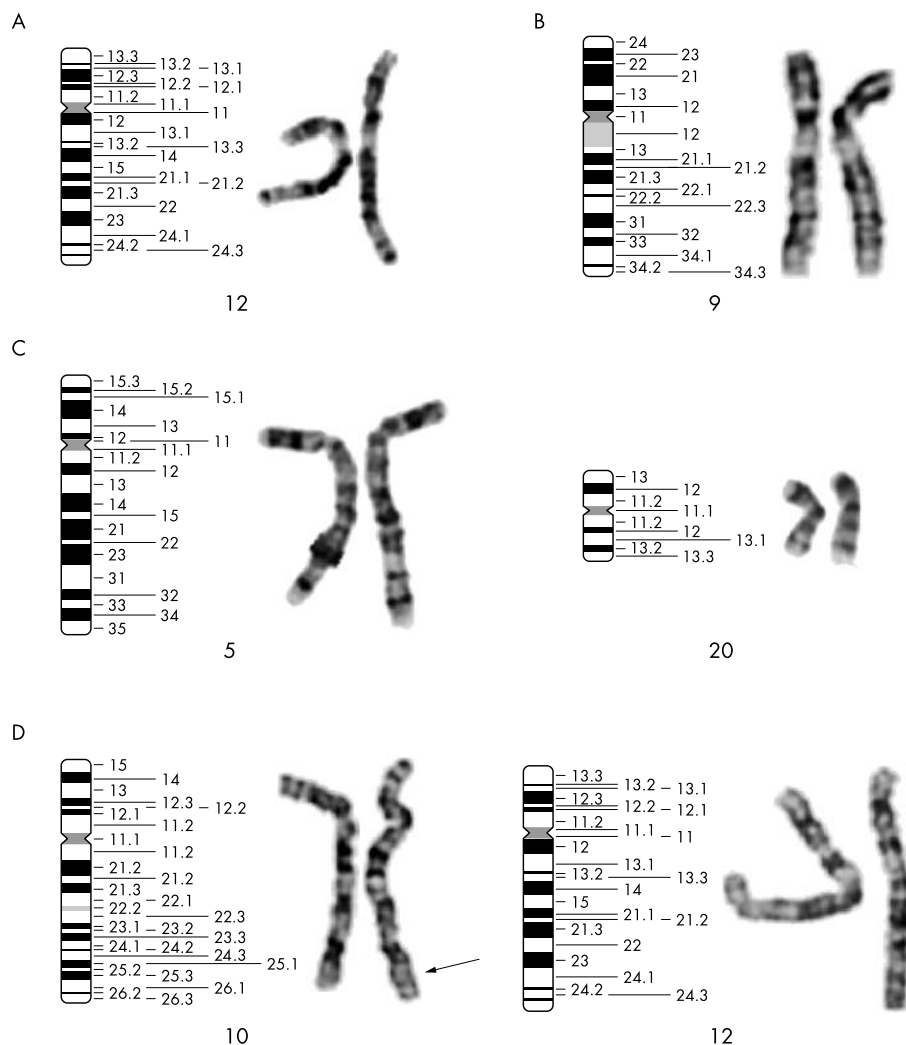
Figure 4 Partial karyotypes of rearranged chromosomes detected in MR cases. (A), (B), (C), and (D) show G banded partial karyotypes of N02R245, N03R003, the father of N03R017, and N03R088, respectively. (A–C) No morphological changes were noted between homologous chromosomes; (D) an unbalanced translocation, der(10)t(10;12)(q26.3;p13.1), was recognised by high resolution G banding analysis. The arrow indicates a 10q26.3 breakpoint.

deletion was found in N03R003. This, together with the 4p terminal deletions identified in three WHS (WHS3, 4, and 5), might have originated from hidden unbalanced translocations that our microarray system could not detect. If so, breakpoints might exist distal to the subtelomeric clones that were used in microarray CGH. However, as each subtelomeric clone is located within 500 kb of the chromosomal end, a phenotypic effect of a hidden duplication derived from the unbalanced translocation may be subtle. A tandem duplication (ish dup(12)(p13.33p13.33)mat) detected in N02R245 may be a normal variant, because her phenotypically normal mother had the same abnormality, but we could not detect this duplication among 50 normal Japanese controls. Further FISH analysis using other BAC clones mapped at 12p13.33 showed that the duplicated region was mapped to a region 0–530 kb proximal to the 12p telomeric end according to the UCSC genome browser (data not shown), but the orientation (inverted or directed) of the tandem duplication was uncertain. Two unbalanced translocations, ish der(5)t(5;20) (p15.33;p13)pat in N03R017 and der(10)t(10;12)(q26.3;p13.1) in N03R088, were detected. The origin of the latter remained unknown because parental materials were not available. The 5p subtelomeric deletion in N03R017 merits comment; a BAC clone covering *CTNND2* encoding delta-2 catenin associated with the critical deleted region of cri du chat syndrome (CdCS)[20] was not deleted in this patient (Okamoto, in preparation), supporting the absence of any CdCS phenotypes and indicating that the 5p deletion was less

than 1.1 Mb distance from the 5p telomere. In N03R088, who had a 10q26.3 deletion, a triangular face was not observed as previously reported.[21] A five item checklist was proposed by de Vries *et al* to improve the detection rate of subtelomeric rearrangements in MR subjects.[22] Retrospective scoring using the checklist revealed that the mean score in our 69 cases was 5.8, and scores in N02R245, N03R003, N03R017, and N03R088, who had subtelomeric rearrangements, were 2, 6, 6, and 6, respectively. These figures support the previous findings, which showed that a score of 3 or higher had a sensitivity of 100% with rather low specificity of 27% and was therefore recommended as the cut off point.[22] Several types of chromosomal aberrations were recently reported to be related to the olfactory receptor (OR) gene clusters at 8p23 and 4p16. A common inversion polymorphism between two of these clusters at 8p23 exists in the normal population, and mothers heterozygous for the inversion may have a risk of bearing children with a complex rearrangement, inv dup del(8p).[23 24] A similar inversion between two OR clusters at 4p16 has also been found.[25] Mothers with double heterozygous inversions at 8p23 and 4p16 tend to have an increased risk of t(4;8)(p16;p23) in their offspring,[25] and children with der(4)t(4;8)(p16;p23) may suffer from WHS owing to distal 4p deletions.[26–28] However no such translocation was found in our cases. To detect all submicroscopic chromosomal abnormalities, construction of a high resolution genomic microarray covering the whole genome is required.[13 14 16] Buckley *et al* reported a full coverage microarray for

chromosome 22 facilitating successful detection of genomic aberrations in acral melanoma, dermatofibrosarcoma, DiGeorge syndrome, and neurofibromatosis type 2.[17] We are now developing a high resolution microarray system that covers the whole human genome and are planning to identify genes responsible for genetic disorders including MR and MR related syndromes as research bases. In the near future, such microarrays targeting the whole genome including subtelomeric regions may be used in clinical diagnosis.

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