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ORIGINAL RESEARCH

Design, Construction and Validation of Targeted BAC Array-Based CGH Test for Detecting the Most Commons Chromosomal Abnormalities

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Abstract: We designed a targeted-array called GOLD (Gain or Loss Detection) Chip consisting of 900 FISH-mapped non-overlapping BAC clones spanning the whole genome to enhance the coverage of 66 unique human genomic regions involved in well known microdeletion/microduplication syndromes. The array has a 10 Mb backbone to guarantee the detection of the aneuploidies, and has an implemented resolution for telomeres, and for regions involved in common genomic diseases. In order to evaluate clinical diagnostic applicability of GOLDChip, analytical validity was carried-out via retrospective analysis of DNA isolated from a series of cytogenetically normal amniocytes and cytogenetically abnormal DNA obtained from cultured amniocytes, peripheral blood and/or cell lines. We recruited 47 DNA samples corresponding to pathologies with significant frequencies (Cri du Chat syndrome, Williams syndrome, Prader Willi/Angelman syndromes, Smith-Magenis syndrome, DiGeorge syndrome, Miller-Dieker syndrome, chromosomes 13, 18 and 21 trisomies). We set up an experimental protocol that allowed to identify chromosomal rearrangements in all the DNA samples analyzed. Our results provide evidence that our targeted BAC array can be used for the identification of the most common microdeletion syndromes and common aneuploidies.

Keywords: aCGH, BAC clones, targeted array, aneuploidies, microdeletions, microduplications

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Introduction

Microscopic karyotype analysis has been the gold standard for prenatal diagnosis since the development of chromosome banding techniques in the late 1960s.[1] Although highly reliable for identifying aneuploidies as well as large chromosomal rearrangements, this procedure presents some limitations due to the low resolution (5–10 Mb) and to the long average time required to get analysis results.[2,3] In order to overcome these limitations, alternative molecular cytogenetic analysis based on FISH (Fluorescence in Situ Hybridization) and QF-PCR (Quantitative Fluorescence Polymerase Chain Reaction) techniques have been applied to prenatal diagnosis for a rapid screening of common aneuploidies.[4–7]

The major limitation of these methods is that they do not provide a genome wide screening. Consequently, these techniques have been applied to clinical samples in addition to, rather than replacing, conventional chromosomal analysis. The Comparative Genomic Hybridization (CGH) analysis was developed as a genome wide screening strategy for detecting DNA copy number imbalances, but its resolution level continued to be low like microscopic karyotype analysis.[8]

The array-CGH (array-based Comparative Genomic Hybridization) technique is similar in principle to conventional CGH,[9,10] but uses arrayed DNA sequences instead of metaphase chromosomes as probes for hybridization, thus providing a direct link between detected aberrations and the physical and genetic maps of the human genome. Array-CGH analysis has a number of significant potential advantages over conventional prenatal testing providing a technique that is not only sensitive and comprehensive, but could be amenable to automation, thus decreasing costs, and the reporting time of results. A classic array-CGH experiment is shown in Figure 1.

Whole-genome array-CGH analysis has already been shown to be a useful tool in clinical genetics for detecting cryptic deletions and duplications in patients with mental retardation or learning difficulties, but with apparently normal karyotype. Besides a custom designed microarray can be exploited to analyze specific chromosomal regions. This type of array contains a large number of probes in chromosome regions selected by operator.[11–13] Most of these custom arrays have been successfully constructed for all or parts of the human genome and are currently available for research use, but the genome-wide dense arrays would have potential disadvantages in clinical use. More array probes are likely to generate a higher number of false positives, and large arrays are more expensive to fabricate, quality control, and interrogate. Moreover, recent investigations showing significant levels of copy number polymorphism in normal populations[14,15] reinforces the desire to test only a limited number of clones, the results of which do not give rise to needless complications in interpretation.

A diagnostically useful microarray must be reliable, must accurately detect the chromosome abnormalities assayed, and must provide interpretable results. Additionally, clinical confidence must be established using microarrays that interrogate regions of known clinical relevance.

Therefore, in the last year targeted arrays have been developed for clinical approach, focusing on medically significant and relatively common chromosomal alterations.[16] Shaffer et al[17] applied targeted BAC array-CGH for the analysis of subtelomeric and pericentromeric regions and of genomic regions known as critical for microdeletion syndromes, and they reported the identification of abnormalities in a cohort of cases selected for a variety of medical problems including developmental delay, mental retardation, seizures, and various congenital anomalies. In another study, performed on product of conception (POC) samples from spontaneous miscarriages using a low density array, array-CGH analysis was able to detect all abnormalities previously identified by microscopic karyotype analysis, and revealed additional abnormalities in approximately 10% of cases.[18,19]

The technique therefore holds some promises of combining the speed, sensitivity, and potential for partial automation of a DNA-based test, with the genome screening characteristics of microscopic karyotyping. Although it is becoming accepted that array-CGH will have a place in clinical genetic testing, it is not well established how this will be best applied. Particularly, for prenatal screening when time for further investigation is limited and ambiguous results cause severe anxiety, the ideal array would contain the minimum number of clones that will deliver the required diagnosis.
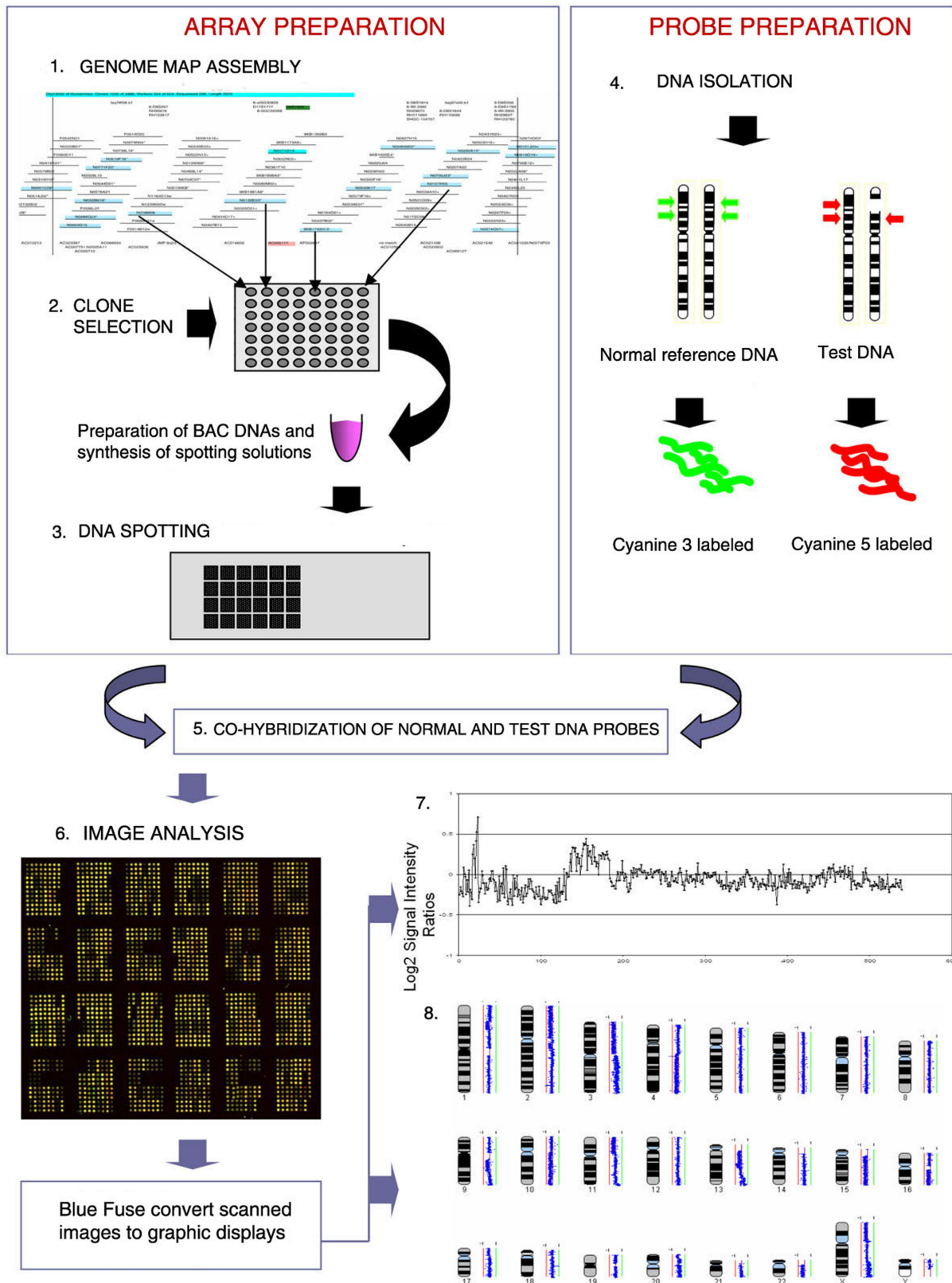
Figure 1. This figure shows the steps in BAC array CGH. (1) BAC clones are selected from a physical map of the genome. (2) DNA samples are extracted from selected BAC clones and their identity is confirmed by DNA fingerprinting or sequence analysis. (3) A multi-step amplification process generates sufficient material from each clone for array spotting. Each clone is spotted in replicate onto a solid support. (4) Reference DNA and test DNA are differentially labeled with cyanine 3 and cyanine 5 respectively. (5) The two labeled products are combined and hybridized onto the spotted slide. (6) Images from hybridized slides are obtained by scanning in two channels. Signal intensity ratios from individual spots can be displayed as a simple plot (7) or by using more complex software such as SeeGH, which can display copy number alterations throughout the whole genome (8).

We designed a new array-CGH microarray called GOLD (Gain or Loss Detection) Chip consisting of 900 non-overlapping BAC (Bacterial Artificial Chromosome) clones spanning the whole genome, concentrating on areas of known clinical significance with dense representation across 66 common microdeletion/microduplications syndromes critical regions (Table 1), and with a lower representation of about one clone per 10 Mb over the remainder of the genome to detect unexpected major chromosome imbalances.

We validated the microarray analyzing 47 cytogenetically abnormal DNA isolated from cultured amniotic fluid, peripheral blood samples and commercial cell lines (Table 2). The selected DNA represents 28 chromosomal abnormalities including common aneuploidies associated with Turner syndrome (45,X), Klinefelter syndrome (47,XXY), Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), Patau syndrome (trisomy 13), and several microdeletions associated, including Wolf-Hirschhorn syndrome (del4p16.3), Cri du Chat syndrome (del5p15.2), Williams syndrome, (del7q11.23), Prader-Willi and Angelman syndromes (del15q11–13), Smith-Magenis syndrome (del17p11.2), DiGeorge syndrome (del22q11.2). Our results provide evidence that

Table 1. Regions of the genome assayed by the GOLDChip.

Clinical relevance	Chromosomal region or Karyotype
Miller-Dieker lissencephaly syndrome	17p13.3
Alagille syndrome	20p12
Muscular dystrophy (Duchenne, Becker) DMD	Xp21.2
ATR-16	16pter-p13.3
Nail-Patella syndrome	9q33.31
Autism, X-linked, susceptibility to, 2, NLGN4	Xp22.33
Azoospermia factor a (AZFa)	Yq11.2
Azoospermia factor b (AZFb)	Yq11.2
Bruton agammaglobulinemia tyrosine kinase	Xq21.3–q22
Canavan disease (ASPA)	17pter-p13
Candidate gene for the testis-determining factor (TDF)	Yp11.3
Cat eye syndrome CECR1, CECR5, CECR6	22q11
Charcot-Marie-Tooth disease type 1A	17p11.2
Cornelia de lange syndrome CDLSI	5p13.1
Cri du chat syndrome	5p15.2
Dandy-Walker syndrome DWS (ZIC1; ZIC4)	3q24
DiGeorge syndrome (DGS)	22q11.2
DiGeorge syndrome critical region 2,	10p14-p1
Down syndrome 21q22.3	Xp11.23
Down syndrome critical region, GATA1	21q21–21q22.3
Early-onset Alzheimer disease/APP	21q21
Edwards syndrome	Trisomy 18
Feingold syndrome	2p24.1
Greig cephalopolysyndactyly syndrome, GLI3	7p13
Holoprosencephaly 1	21q22.3
Holoprosencephaly 3	7q36

(*Continued*)

Table 1. (*Continued*)

Clinical relevance	Chromosomal region or Karyotype
Barakat syndrome, GATA3	10p
Kallmann syndrome 2 (KAL2)	8p11.2-p11.1
Klinefelter syndrome	XXY
Langer giedion type II TRPS2	8q24.11–q24.13
Microphthalmia with linear skin defects	Xp22
Beckwith-Wiedemann syndrome	11p15.5
Brachydactyly-mental retardation syndrome, D2S2338	2q37
Neurofibromatosis 1 (NF1)	17q11.2
Neurofibromatosis 2 (NF2)	22q12.2
Ovarian dysfunction (FIMIANI; LAPERUTA)	Xq26
Patau syndrome trisomy 13	Trisomy 13
Pelizaeus-Merzbacher disease	Xq22.2
Potocki-shaffer syndrome	11p11.2–p12
Prader Willi syndrome/Angelman syndrome	15q11–q13
Retinoblastoma	13q14.1–q14.2
Rett syndrome (MECP2)	Xq28
Rieger syndrome type 1	4q25
Smith-Magenis syndrome	17p11.2
Sotos syndrome	5q35
Split-Hand/Foot Malformation 4	3q27
Steroid sulfatase deficiency (STS)	Xp22.31
Synpolydactyly/Syndactyly type II	2q31–q32
Tuberous sclerosis 2 (TSC2)	16p13.3
Turner Syndrome	45, X
Van der Woude syndrome	1q32–q41
Williams syndrome	7q11.23
Wolf-Hirschhorn candidate 1 (WHSC)	4p16.3
X-linked lissencephaly	Xq22.3–q23
Leri-Weill syndrome (dischondrosteosis Xp distal deletion SHOX)	Xpter-p22.32
1p36deletion (monosomy 1p36)	1p36
2q37 monosomy	2q37
Sex reversal deltion 9p	9p
Rubinstein-Taybi	16p13.3
Saethre-Chotzen Syndrome	7p21
Sex-determining region, SRY	Yp11.31
Simpson-Golabi-Behmel syndrome	Xq26
Split-Hand/Foot Malformation 3	10q24
WAGR syndrome (PAX6)	11p13
14q terminal deletion syndrome (van Karnebeek)	14q
Split-Hand/Foot Malformation 5	2q31

Table 2. Are shown the 28 disease tested in 47 cytogenetically abnormal DNA by GOLDChip (20 cell lines and 27 clinical samples). For each disease are shown OMIM, frequency, the number of sample analyzed, the gene map locus (or Karyotype if possible), and the gain(+) or loss (−) of DNA. ORPHA: data from ORPHANET Journal of Rare Diseases. nr = not reported. * = ECACC (European Collection of Cell Cultures): cell line name AL0053, catalogue number 97091501; ** = ECACC: cell line name AL0021, catalogue number 92102601; *** = ECACC: cell line name AG0847, catalogue number 94032208; **** = ECACC: cell line name AR0189, catalogue number 99012001.

Disease	OMIM	Frequency	Cell lines	Clinical samples	Total	Gene map locus or Karyotype	Gain/loss
Charcot-Marie-Tooth disease type 1A	118220	1/2.500	1		1	17p11.2	(+)
Cat eye syndrome	115470	1/5.000	1		1	22q11.2	(−)
Di George (22q12) Syndrome	188400	1/4.000	1	2	3	22q11.2	(−)
Di George (10p) syndrome	601362	<1/1.000.000	1		1	10p14–p13	(−)
Down syndrome	190685	1/1.000		2	2	47,XY,+21	(+)
Williams syndrome	194050	1/10.000	1	4	5	7q11.23	(−)
Smith-Magenis syndrome	182290	1/50.000		3	3	17p11.2	(−)
Patau syndrome	1660	nr		1	1	47,XX,+13	(+)
Duchenne syndrome	310200	1/35.000		1	1	Xp21.2	(−)
Miller-Diecker syndrome	247200	1/25.000	1		1	46,XY.ish del(17)(p13.3p13.3)(D17s379-)	(−)
XYY	158250	1/900		1	1	47,XYY	(+)
Klinefelter's syndrome (XXY)	278850	nr		2	2	47,XXY	(+)
Edwards Syndrome	601161	1/6.000		1	1	47,XY,+18	(+)
Prader Willi/Angelman syndrome	600161	1/20.000		2	2	15q11–q13	(−)
Ichthyosis-Mental retardation-Kallmann Syndrome	30870	1–9/100.000	1	1	2	Xp22.3	(−)
Monosomy 1p36	607872	1/5.000	1	2	3	1p36	(−)
Brachydactyly-Mental retardation syndrome	600430	>1/1.000.000	1	2	3	2q37	(−)
Cri du Chat syndrome	123450	1/50.000	1	1	2	5p15.2–>pter	(−)
Del 20p	ORPHA1611	<1/1.000.000	1	1	2	20pter*	(−)
Wolf-Hirschhorn syndrome (WHS)	194190	1/50.000	1		1	4p16.3–>pter	(−)
Del 6q	ORPHA96151	<1/1.000.000	1		1	6qter**	(−)
X-linked Ichthyosis	308100	1/6.000	1		1	46,X,del(X)(p22p32)	(−)
del(3)(p25)->pter	ORPHA1618	<1/1.000.000	1		1	3p25->pter***	(−)
Autism/Asperger syndrome	300497	1–5/10.000	1		1	46,X,del(X)(p22.13p22.31)	(−)

(*Continued*)

Table 2. (*Continued*)

Disease	OMIM	Frequency	Cell lines	Clinical samples	Total	Gene map locus or Karyotype	Gain/loss
Alagille syndrome	118450	1/100.000	1		1	46,XX,del(20)(p11.23p12.2)	(−)
Turner syndrome	158250	1/2.500	1		1	45, X****	(−)
Azoospermia	415000	1–5/10.000	1		1	Yq11.2	(−)
Dandy-Walker syndrome	220200	1/25.000	1		1	3q24	(−)

GOLDChip can be used for the identification of the most common microdeletions syndromes and common aneuploidies.

Methods
Array design and production
The targeted-array described in this study was developed using published protocols.[20] Briefly, large insert bacterial and plasmid artificial chromosome (BAC and PAC) clones were chosen from the public databases (UCSC, NCBI and Ensembl) to cover each chromosome at a resolution of one clone every 10 Mb. Additional clones were selected for the major common microdeletion syndrome critical regions consulting DECIPHER, OMIM and Orphanet databases, as far as possible covering identified critical regions and microdeletion breakpoints with overlapping clones. Isolated clone DNA was first amplified by degenerate oligonucleotide primed PCR (DOP-PCR), followed by secondary PCR with an amine modified primer. Array clones were spotted in two areas in six replicates onto Aldheyde slides (Genetix).

Degenerate oligonucleotide primed (DOP)-PCR
Degenerate oligonucleotide primed-PCR (DOP-PCR) was performed to amplify target clone DNA using three different PCR primers (DOP 1 primer: CCGACTCGAGNNNNNNCTAGAA; DOP 2 primer: CCGACTCGAGNNNNNNTAGGAG; DOP 3 primer: CCGACTCGAGNNNNNNTTCTAG). PCR was started at 94 °C for 3 min, then cycled first 10 times at 94 °C for 1 min30sec, at 30 °C for 2 min 30 sec and 72 °C for 3 min, then 30 times at 94 °C for 1 min, at 62 °C for 1 min 30 sec and 72 °C for 2 min, finally at 72 °C for 8 min. Gel electrophoresis

was carried out as quality control on PCR products. Successfully amplified PCR products, usually 0.2–2 kb in size, were used as template for PCR with 5' aminolink primer (NH2-GGAAACAGCCCGACTC-GAG). The process was started at 95 °C for 10 min, then cycled 34 times at 95 °C for 1 min, at 60 °C for 1 min 30 sec and 72 °C for 7 min, finally at 72 °C for 10 min. PCR products obtained were purified with Wizard SV-96 PCR Clean-up kit (PROMEGA), quantified with Nanodrop and diluted in water to a final concentration of 300 ng/ul. The products were mixed 1:1 with Aldehyde spotting solution (Genetix) and were ready for prints.

Microarray spotting
Amplified DNA was spotted in six replicates onto Aldehyde coated slides (Genetix) using QArray2 arrayer (Genetix). The same sixfold-spot panel was prepared in duplicate as area "up" and "down" on the same slide. The slides were then pre-treated, denatured, and stored in a desiccator until use.

Array validation
The targeted-array validation was performed by array-CGH analysis of 47 cytogenetically known DNA isolated from 27 cultured amniotic fluid samples, chorionic villus samples and peripheral blood samples and 20 commercial cell lines (Table 2). 20 cytogenetically normal DNA were analyzed as control samples. Dye-reversal array-CGH analysis was performed as described below.

Samples collection
20 cell lines amongst ECACC human genetic collection and 27 cell cultures (Table 2) were selected to represent a broad spectrum of cytogenetic abnormalities

including the most common aneuploidies (trisomies of chromosomes 13, 18, and 21, and sex chromosome aneuploidies), with particular emphasis on microdeletion rearrangements and unbalanced structural rearrangements.

DNA was isolated from cultured amniocytes, cultured chorionic villus samples, or postnatal blood specimens for samples previously confirmed by either microscopic karyotype analysis or FISH as carrying chromosomal rearrangements. The results of these investigations were blinded prior to further analysis by array-CGH. Clones exceeding experimental thresholds were identified by Bluefuse Software (BlueGnome).

DNA labelling and array hybridization

Briefly, 600 ng of test DNA were labelled with the cyanine Cy3, and 600 ng of the control DNA with the cyanine Cy5 (CGH 1). In order to conduct a dye-swap experiment, reverse labelling (test DNA with Cy5, and control DNA with Cy3) was also performed (CGH 2). Genomic DNA was labelled with Cy3- or Cy5-dCTP by random prime labelling (BioPrime Genomic labelling System,). After co-precipitation with salmon sperm DNA and human CotI DNA (Roche), labelled probe mixtures of CGH1 and CGH2 were denatured at 72 °C for 10 min, preannealed at 37 °C for 30 min and then simultaneously applied to area 'up' and 'down', respectively. Slides were scanned with ScanArray (Perkin-Elmer) and analyzed with Bluefuse software (Bluegnome).

Image acquisition and data analysis

Arrays were scanned using a ScanArray (Perkin-Elmer) and the acquired images were analyzed using Bluefuse software (Bluegnome). Data analysis was performed setting the treashold level to 0,299. Value 0 means no gain or loss of DNA. Values > 0,299 correspond to a DNA duplication, values < 0,299 to a deletion.

Results

We designed a targeted-array called GOLD (Gain or Loss Detection) Chip consisting of 900 FISH-mapped non-overlapping BAC clones spanning the whole genome to enhance the coverage of 66 unique human genomic regions involved in well known microdeletion/microduplication syndromes (Table 1).

We identified multiple clones for each genomic locus. Loci covered by only a single clone may show dosage variation because of the intrinsic technical variability of the procedure or because of polymorphic repetitive sequences inherent to the specific locus. The use of multiple clones provides confidence in the results. All polymorphic clones identified were discarded from the microarray.

In order to evaluate clinical diagnostic applicability of GOLDChip, analytical validity was carried-out via retrospective analysis of DNA isolated from a series of cytogenetically normal amniocytes and cytogenetically abnormal DNA obtained from cultured amniocytes, peripheral blood or cell lines.

We recruited from different centres DNA samples (n = 27) corresponding to pathologies with significant frequence (>1/1000), clinical relevance (Cri du Chat syndrome, Williams syndrome, Prader Willi/Angelman syndromes, Smith-Magenis syndrome, DiGeorge syndrome, Miller-Diecker syndrome, chromosomes 13, 18 and 21 trisomies), and clear known karyotypes. Some pathologies were evaluated on cellular lines commercially available (n = 20).

Table 2 represents the main information of the 47 DNA analyzed (OMIM, frequencies, gene map locus or Karyotype, gain or loss of DNA). We identified all the chromosomal rearrangements previously characterized and excluded false negative clones through comparison of clinical known samples.

Chromosomal abnormalities included microdeletions [del4p16.3, del7q11.2] and aneuploidies (trisomy 21) respectively associated to Wolf-Hirschhorn, Williams and Down syndrome are shown in Figures 2, 3 and 4.

In the Wolf-Hirschhorn sample (Fig. 2), deletion in 4p16.3 was identified by the loss of copy number of 11 BAC clones on chromosome 4 (C4P-023, C4P-022, C4P-021, C4P-020, C4P-018, C4P-016, C4P-014, C4P-013, C4P-011, C4P-010, C4P-008). Moreover, duplication in 12p13.33 was identified by BAC clones C12P-010 and C12P-009.

Deletion in Williams syndrome (del7q11.2) (Fig. 3) is represented from BAC clones C7Q-015 and C7Q-012, and a-CHG analysis of the sample with trisomy 21 (Fig. 4) had a gain in copy number of clones corresponding to chromosome 21.
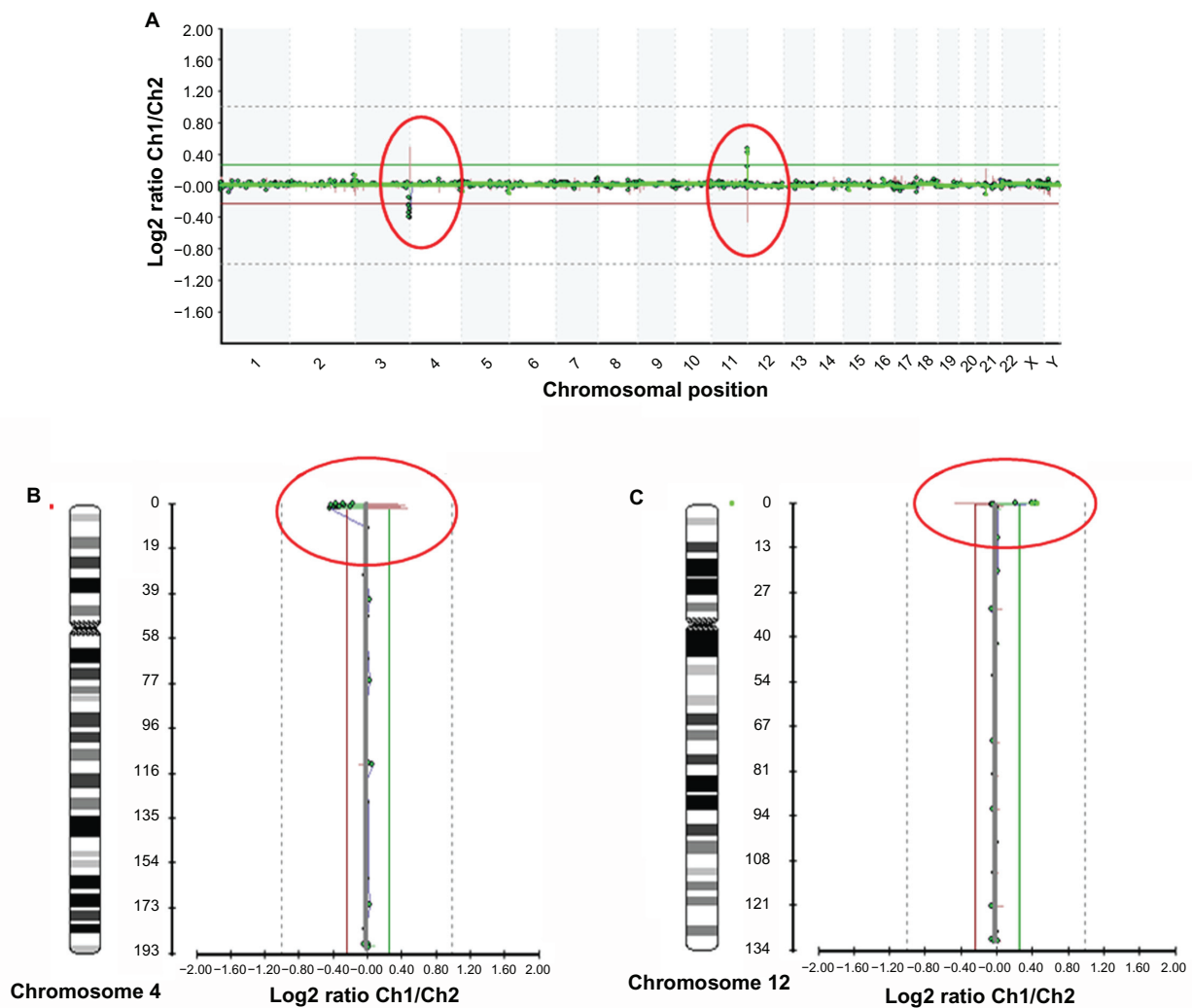
Figure 2. A. Microarray profile of Wolf-Hirschhorn syndrome (del 4p16.3) in a patient also carrying dup12p13.33, identified by microarray-based comparative genomic hybridization (array CGH) using GOLD Chip. Each clone represented on the array is arranged along the x-axis according to its location on the chromosome with the most distal/telomeric p-arm clones on the left and the most distal/telomeric q-arm clones on the right. The green lines represent the log2 ratios from the first experiment (patient Cy3/control Cy5), whereas the red plots represent the log2 ratios obtained from the second experiment in which the dyes have been reversed (patient Cy5/control Cy3) . **B** and **C**. Microarray profiles of chromosomes 4 (B) and 12 (C). The green line represents the patient-to-control fluorescence intensity ratios (gain of DNA); the red line represents dye-reversed control-to-patient fluorescence ratios (loss of DNA). Deletion in 4p16.3 is represented from BAC clones C4P-023, C4P-022, C4P-021, C4P-020, C4P-018, C4P-016, C4P-014, C4P-013, C4P-011, C4P-010, C4P-008; duplication in 12p13.33 is represented from BAC clones C12P-010 and C12P-009.

Discussion

Microarrays have been successfully constructed for all or parts of the human genome. Snijders et al[21] constructed one of the first "whole-genome" arrays using 2,400 BAC clones to scan for genome-wide copy-number alterations. More recently different arrays have been developed, consisting of overlapping clones spanning the entire genome,[22] covering the subtelomeric regions[23] or focusing on specific chromosomes and chromosome regions.[24–29]

These and other arrays constructed for research purposes are designed to screening chromosomal segments or the whole-genome for DNA gains or losses at unprecedented resolution.

Thus, whole genome arrays are likely to generate data that are difficult to be interpreted and are subjected to multiple FISH verifications per patient. Furthermore, alterations in regions of the genome that do not have established clinical relevance may be difficult to interpret in a clinical setting. Moreover, with a whole-genome approach, polymorphisms are expected to be abundant. This assumption is based on the data from subtelomeric FISH analysis revealing many telomeric alterations with no apparent clinical
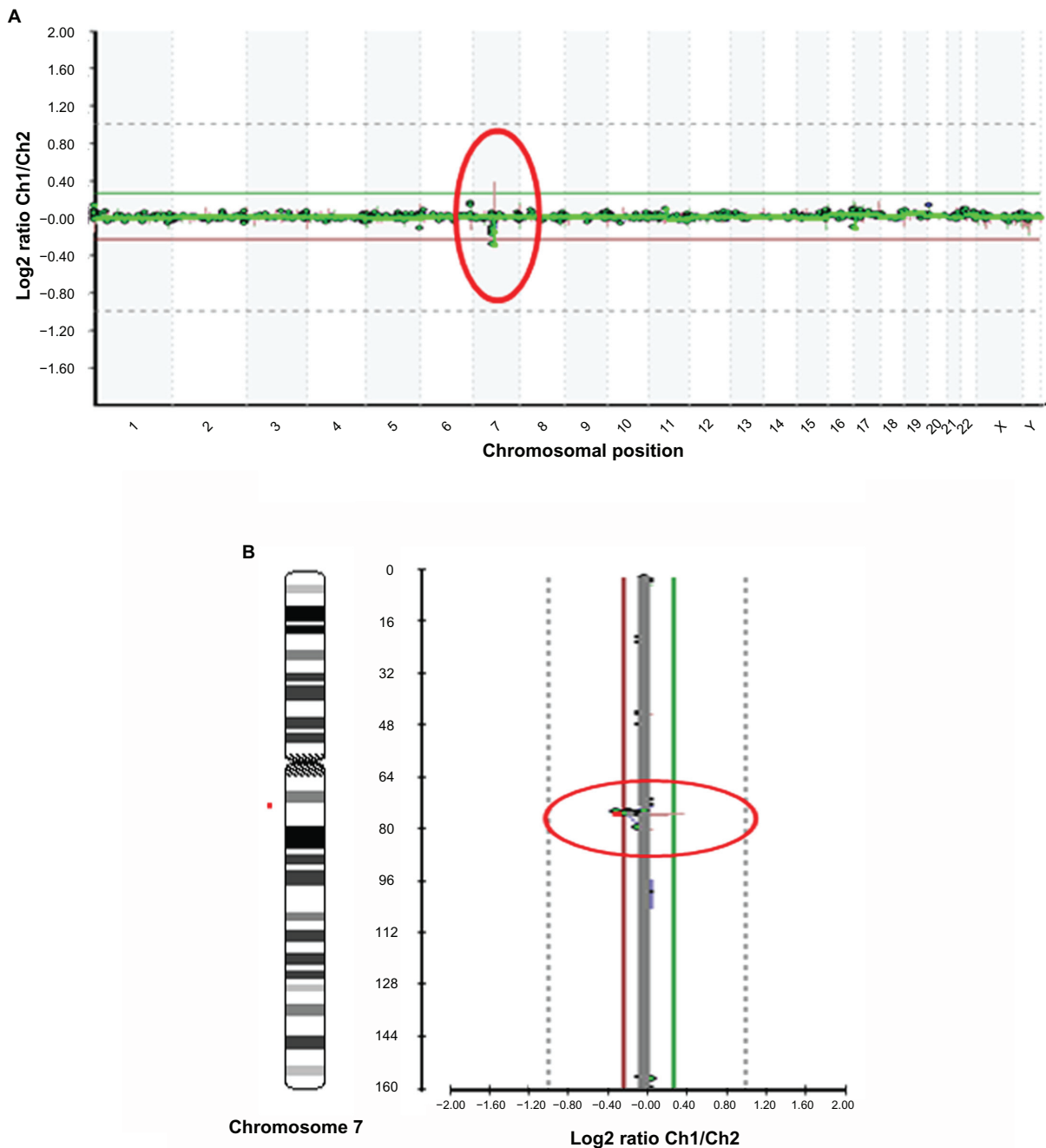
Figure 3. A. Microarray profile for Williams syndrome (7q11.2) identified by microarray-based comparative genomic hybridization (array CGH) using GOLD Chip. Each clone represented on the array is arranged along the x-axis according to its location on the chromosome with the most distal/telomeric p-arm clones on the left and the most distal/telomeric q-arm clones on the right. The green lines represent the log2 ratios from the first experiment (patient Cy3/control Cy5), whereas the red plots represent the log2 ratios obtained from the second experiment in which the dyes have been reversed (patient Cy5/control Cy3). **B.** Microarray profiles of chromosome 7. The green line represents the patient-to-control fluorescence intensity ratios (gain of DNA); the red line represents dye-reversed control-to-patient fluorescence ratios (loss of DNA). Deletion in 7q11.2 is represented from BAC clones C7Q-015 and C7Q-012.

significance.[30] Supporting this, two recent studies have reported the prevalence of large-scale copy-number variations (LCVs) throughout the human genome.[31,32]

The adoption of such arrays into clinical diagnostics is unwise and may lead to many false positive diagnoses that necessitate expensive follow-up confirmatory tests by FISH or other methods, additional blood draws
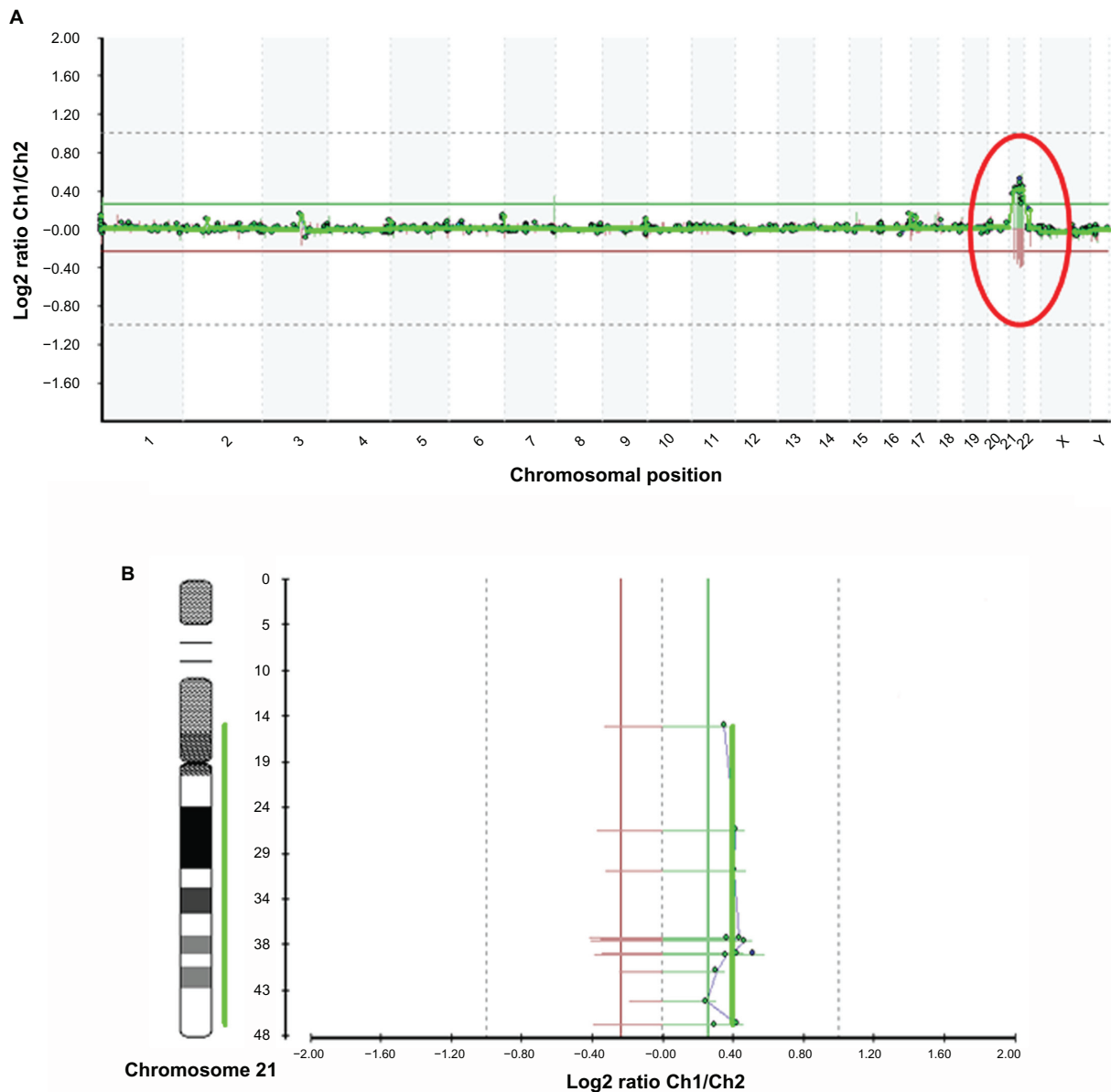
A

B

Chromosome 21

Figure 4. A. Microarray profile for Down syndrome (47,XX+21) identified by microarray-based comparative genomic hybridization (array CGH) using GOLD Chip. Each clone represented on the array is arranged along the x-axis according to its location on the chromosome with the most distal/telomeric p-arm clones on the left and the most distal/telomeric q-arm clones on the right. The green lines represent the log2 ratios from the first experiment (patient Cy3/control Cy5), whereas the red plots represent the log2 ratios obtained from the second experiment in which the dyes have been reversed (patient Cy5/control Cy3). **B.** Microarray profiles of chromosome 21. The green line represents the patient-to-control fluorescence intensity ratios (gain of DNA); the red line represents dye-reversed control-to-patient fluorescence ratios (loss of DNA). Some clones appear in the same horizontal line because they are overlapping. In the image are shown only the 13 clones that, after data analysis and normalization, were selected by Bluefuse considering the parameters of the experiments. Some regions of chromosome 21 (p-arm) are not covered by BAC clones.

from unaffected relatives to determine the segregation of these deletions, duplications, or polymorphisms and unnecessary anxiety for the families.

Array-CGH hybridization results for single clones that show dosage difference would need to be examined and each clinical case may result in a mini-research project. Thus, the genome-wide dense arrays

that are currently available for research use are not appropriate to use in a clinical setting.

A diagnostically useful microarray must accurately detect the chromosome abnormalities assayed, and must provide interpretable results. Additionally, clinical confidence must be established using microarrays that interrogate regions of known clinical relevance.

Respecting the mentioned rules for microarray with diagnostic use, we designed a targeted array called GOLD (Gain or Loss Detection) Chip consisting of 900 FISH mapped non-overlapping BAC clones spanning the whole genome, to enhance the coverage of about 66 unique human genomic regions involved in microdeletion/microduplication syndromes (Table 1).

Targeted-arrays were designed for specific regions of the genome to study specific chromosome or chromosomal segment or to identify and evaluate specific DNA dosage abnormalities in individuals with suspected microdeletion syndromes or unbalanced subtelomeric rearrangements.[24,33–35]

Our targeted microarray has a crucial goal in medical practice, to provide clinically useful results for diagnosis, genetic counselling, prognosis, and clinical management of unbalanced cytogenetic abnormalities. However, it is well know that BAC-based CGH microarray has some drawbacks. Since its resolving power depends on the number of clones printed and the genomic distance between the clones, a microdeletion or microduplication may be overlooked if the clones printed are less dense. Furthermore, CGH microarray cannot detect balanced rearrangements, polyploidies and low mosaics.[36] An alternative is offered by synthetic oligonucleotides microarrays, for which the exact sequence and length for each element on the arrays is known. For PCR amplified BACs, this is not the case since the amplification procedure is not linear and is variable for each amplification round.

The odds are that the array-CGH field is evolving towards high resolution oligonucleotide array-CGH for the measurement of chromosomal copy number changes in human genetics and cancer, analogous to the way cDNA arrays for expression profiling have been replaced by oligonucleotide arrays. For specific applications there will still be a place for BAC arrays, like in the case of methylation studies.[37]

At this time our results provide evidence that our BAC array can be used for the identification of the most commons microdeletion syndromes and common aneuploidies. Probably, it has the potential to replace karyotyping for prenatal cytogenetic analysis, but at the same time a deep clinical trial is strongly required to confirm sensitivity and specificity in clinical operating conditions, to establish guide lines to array-CGH uses in prenatal diagnosis.

Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors report no conflicts of interest.

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