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Original Article

Clinical application of oligo array-CGH for detecting balanced translocations in preimplantation genetic diagnosis

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Abstract: Array comparative genomic hybridization (array-CGH), which facilitates to detect unbalanced reciprocal translocation and allows screening aneuploidy for chromosomes, has been repeatedly verified to be valid for diagnosis of translocations in preimplantation human embryos. Currently, the main microarrays used for CGH are bacterial artificial chromosome (BAC)-based arrays. Compared with the BAC-based arrays, oligonucleotide (oligo)-based arrays have a relatively higher resolution and optimal coverage particularly in the subtelomeric regions. Herein, we described the clinical application of a newly designed oligo-based array by Agilent in preimplantation genetic diagnosis (PGD) and aneuploidy screening for balanced translocations.In the study, a total of 144 embryos from 9 couples carrying Robertsonian translocations and 5 carrying reciprocal translocations were biopsied on day 3 for array-CGH analysis. Overall, 135 (93.8%) embryos were successfully diagnosed to be free of either aneuploidies or unbalanced fragments. However, the remained 9 (6.2%) embryos failed to be amplified due to failed cell lysis, DNA damage or the absence of nuclei in the biopsied cells. Collectively, 23 embryos were identified as "euploid and balanced" and suitable to be transferred. Finally, 9 embryos of satisfactory quality were transferred to 6 women, among which 4 recipients exhibited positive hCG level. Fortunately, one recipient with positive hCG level has delivered one baby, and two pregnancies were continuing. Our study served as the first clinical application of oligo-based array CGH technology in PGD for both reciprocal and Robertsonian translocations concomitant with comprehensive aneuploidy screening.

Keywords: Preimplantation genetic diagnosis, chromosomal translocation, oligo array comparative genomic hybridization

Introduction

It has been reported that 1 out of 625 individuals is a carrier of balanced chromosome translocations. But among subfertile individuals, the incidence of carrying a structural chromosome abnormality, mainly reciprocal translocation and Robertsonian translocation, is as high as 5% [1]. Individuals, who carry a balanced chromosome translocation, are also known to be at high risk of producing chromosomally unbalanced gametes and consequently generating embryos or offspring with the same defects [2, 3]. Theoretically, for individuals with reciprocal translocations, the rate of producing normal or balanced gametes is 4 out of 32. While for individuals with Robertsonian translocations, the rate equals to 4 out of 16. However, the actual percentage varies significantly with the involved abnormal chromosomes and breakpoints in the chromosomes. It has been reported that the sex of the carrier also affected the observed rates. As a consequence of unbalanced segregation, carriers of balanced chromosomal translocations are at increasing risk for suffering from infertility and recurrent miscarriage; and their offspring are apt to suffer from mental retardation together with congenital abnormalities [4].

Since its first successful performance in prevention of X-linked disease in 1990, Preim-

plantation Genetic Diagnosis (PGD) has played an increasingly important role in detection of unbalanced chromosomal translocation [5]. Previous studies have shown that, for carriers of balanced translocations, PGD also provides an alternative option to reduce the chance of negative effects by screening embryos with normal/balanced chromosomes. Through excluding early embryos with unbalanced translocations during in vitro fertilization (IVF), PGD has been proved to have the potential to decrease the rate of recurrent pregnancy loss, minimize the chance for adverse outcomes of bearing chromosomal abnormality and increase pregnancy rates for infertile couples [3, 6, 7].

Fluorescence in-situ hybridization (FISH) is the first and the most widely used method in PGD for detection of structural chromosome abnormalities so far [8]. In order to detect unbalanced chromosomal rearrangements in embryos, various strategies for FISH have been introduced. The preconceptional diagnosis for the first pregnancy was performed by using whole chromosome painting probes on polar bodies after metaphase conversion, which could only detect translocations of maternal origin [9, 10]. Afterwards, probes, which could specifically bind to chromosomal fragments in the interphase nuclei of a blastomeric, were used for cleavage-stage embryos [11-13]. However, as a result of the fact that probes should be changed accordingly to the translocation carriers, it will be expensive and time-consuming to make case-specific probes, which therefore inevitably restrict the clinical application of analyzing interphase chromosomes in blastomere with probes spanning or flanking the specific translocation breakpoints [14-16]. The further developed subtelomere probes together with centromeric probes, which avoid the effort and time to design specific probe for each case, have been commonly used yet, with the disadvantage of inability to discriminate between normal and balanced embryos [4, 17]. In spite of the relatively successful role that FISH played on PGD for balanced translocation in initial researches, its accuracy for interpretation has been compromised due to encounter of technical difficulties as well as drawbacks, such as poor or failed fixation, hybridization failure, and signal splitting or overlapping, which may substantially bring about transferring embryos with abnormality [18]. Surprisingly, considerable studies have reported that error rates of FISH detection for translocation could be as high as 10%, with an average error rate of 6% [15, 19, 20]. Thus, it is of essence to adopt a newfound strategy to minimize the risk of misdiagnosis.

Since it has been suggested that a higher pregnancy rate and improved IVF outcomes could be reached by preimplantation genetic screening (PGS) [21, 22], it is relatively reasonable to detect chromosomal translocations as well as to screen common aneuploidies for patients with advanced maternal age in PGD. Although improvements in technical skills and strategies have been well established to minimize the risk of misdiagnosis of FISH [23-25] and to improve aneuploidy screening in PGD [26, 27], certain general shortcomings remain. The one most difficult to overcome is the inability to simultaneously analyze 24 chromosomes (comprehensive aneuploidy screening) other than those involved in the structural chromosome rearrangement, spontaneous abortions [7] or 12 chromosomes to the maximum extent [27, 28]. The limitations have also compromised the clinical outcome of newly improved PCR-based PGD approach for balanced translocations, even if it overcame several previously described shortages for FISH technique and was regarded as a valuable alternative option to supersede FISH-based PGD protocols [29, 30]. Large number of randomized clinical trials [31] in combination with several subsequent trials have demonstrated that the use of aneuploidy testing for limited numbers of chromosomes on cleavage-stage embryos from patients with advanced maternal age results in either the same or reduced live-birth rate. The possible reason why clinical outcome were negatively affected lies in that the approaches like FISH- or PCR-based PGD method, which merely focused on a limited number of chromosomes, may lead to the transfer of reproductively incompetent embryos with undetectable chromosomes aneuploidy.

Recently, the applications of whole genome amplification approaches on single cell or biopsied embryos have triggered the introductions of a series of comprehensive chromosome screening techniques into routine PGD practices for reciprocal and Robertsonian translocations combined with PGS [32-35], including CGH, array-CGH, and single-nucleotide polymorphism (SNP) array. Actually, compared with SNP array, array-CGH is a costless option with

relatively less labor-intensive for the rapid detection of gain and loss in both whole-chromosome scale and chromosome-segment scale for all 24 chromosomes. Besides, PGD, tested by either blastomere biopsy or polar body, exhibited high accuracy in the corresponding embryos [36].

Currently, array-CGH has been extensively used in PGD for detecting both whole chromosomal aneuploidy and imbalanced segmental chromosome rearrangement in couples carrying balanced reciprocal or Robertsonian translocations [33, 37]. However, the microarrays for CGH are mainly bacterial artificial chromosome (BAC) -based arrays, whose probe density is still seriously influenced by the availability of BAC clones as well as the presence of potentially interfering genomic architectures, such as segmental duplications [38]. Additionally, it has also been reported that translocation of smaller fragments in embryos could not be detected by the BAC-based array CGH [34], indicating the limited resolution of BAC-based array CGH for diagnosis of translocations. Because the oligonucleotide (oligo)-based arrays provide a higher resolution for single chromosome [38], which may be sufficient to accurately identify translocations involving breakpoints near the telomeric ends of chromosomes, it has been presumed that compared with the BAC arrays, the oligo-based arrays would be more suitable for certain applications such as PGD of translocation chromosome imbalance as well as comprehensive detection of aneuploidy. However, to the best of our knowledge, no prospective study of oligo-based array CGH has been carried out to simultaneously screen of aneuploidy together with unbalanced translocations for all 24 chromosomes in preimplantation human embryos.

The present study described the clinical application of a newly designed oligo-based array by Agilent in preimplantation genetic diagnosis (PGD) and aneuploidy screening for balanced translocations. In the study, a total of 144 embryos from 9 couples carrying Robertsonian translocations and 5 carrying reciprocal translocations were biopsied on day 3 for array-CGH analysis. Overall, 135 (93.8%) embryos were successfully diagnosed to be free of either aneuploidies or unbalanced fragments. However, the remained 9 (6.2%) embryos failed to be amplified due to failed cell lysis, DNA damage or the absence of nuclei in the biopsied cells. Collectively, 23 embryos were identified as "euploid and balanced" and suitable to be transferred. Finally, 9 embryos of satisfactory quality were transferred to 6 women, among which 4 recipients exhibited positive hCG level. Fortunately, one recipient with positive hCG level has delivered one baby, and two pregnancies were continuing. In conclusion, we confirmed that oligo array CGH is a relatively low-cost and high-resolution option for the rapid detection of whole-chromosome or chromosome-segment gain and loss for all 24 chromosomes in PGD. Our study served as the first clinical application of oligo-based array CGH technology in PGD for both reciprocal and Robertsonian translocations concomitant with comprehensive aneuploidy screening.

Materials and methods

Clinical cases

A total of 14 couples included in the present study were given genetic counseling, in which clinical geneticists reviewed the couples' genetic histories and requirements for PGD, explained the process of PGD for the specific translocation involved, and then discussed with them the accuracy of the procedure, the related limitations, and possible diagnostic choices. Possible genetic disorders, the success rates, the possible risk of misdiagnosis were further discussed, followed by extra explanation about the possibility of termination of the cycle due to not having suitable embryos to transfer. Written informed consent, concerning the requisition of confirmatory prenatal diagnosis for any ensuing pregnancy, were obtained from all the couples. Afterwards, patients were referred to an IVF clinician and technicians to arrange the clinical treatments and laboratory procedures. Only couples, in which the female partners were younger than 40 years old and had no uterine malformation and other genetic indications, having undergone cryopreserved cycles, were finally included. Also, these couples signed informed consents of donating the discarded embryos for research. This study and the institution involved in have obtained ethical approval from the Institutional Review Board of the Affiliated Hospital of Hainan Medical University.

Collectively, 14 couples, consists of 9 Robertsonian and 5 reciprocal translocation carriers, were included in the present study. The detailed clinical data is listed in **Table 1**.

Table 1. Clinical data of PGD cases

No. of PGD	Karyotype	Biopsy day	Smallest Frament (Mbp)	Maternal age (Years)	No. of embryos		Embryo Transferable/ Transferred	ET results	Fetal karyotype
					Biopsied	Diagnosed			
1	46, XX, t (1; 5) (p32; q35)	3		29	23	19	2/2 (1st)	Non-pregnancy (1st)	-
							1/1 (2nd)	Non-pregnancy (2nd)	-
2	46, XX, t (3; 22) (q11.1; p13)	3		28	9	8	3/2	Non-pregnancy	-
3	45, XX, der (13; 14) (q10; q10)	3	Robertsonian	37	6	5	1/1	Non-pregnancy	-
4	46, XY, t (3; 6) (p23; p23)	3		27	20	20	3/1	Non-pregnancy (1st)	-
							2	Non-pregnancy (2nd)	
5	45, XX, der (13; 22) (q10; q10)	3	Robertsonian	31	7	7	0/0	-	-
6	46, XY, t (1; 4) (q42; q21)	3		30	11	11	1/1	Delivery	46, XY, t (1q42; 4q21)
7	45, XY, der (14; 21) (q10; q10)	3	Robertsonian	30	15	13	4/2	Twin pregnancy	Not yet tested
8	45, XY, der (13; 14) (q10; q10)	3	Robertsonian	27	14	14	2/1	Non-pregnancy (1st)	-
							1 (twice)	Pregnancy (2nd)	Not yet tested
9	45, XY, der (13; 14) (q10; q10)	3	Robertsonian	32	12	12	3/1	Miscarriage	Normal (POC)
10	45, XX, der (13; 14) (q10; q10)	3	Robertsonian	24	6	6	2/0	-	-
11	45, XY, der (14; 22) (q10; q10)	3	Robertsonian	39	6	6	0/0	-	-
12	45, XX, der (14; 15) (q10; q10)	3	Robertsonian	33	5	4	0/0	-	-
13	46, XY, t (6; 8) (q27; q21)	3		36	4	4	0/0	-	-
14	45, XY, der (13; 14) (q10; q10)	3	Robertsonian	32	6	6	1/0 (not survive after thawing)	-	-

Experimental design

Blastomeres were collected from 144 embryos which were produced by intracytoplasmic sperm injection (ICSI) and had undergone Day 3 biopsy. The samples were then amplified with SurePlexDNA Amplification System (BlueGnome, Cambridge, UK), and the products were applied to array-CGH, and for further confirmation by the KaryoLite™ BACs-on-Beads™ (KL-BoBs™) assay, which has been developed to detect arm-specific aneuploidies in all 24 chromosomes. Among 70 embryos diagnosed as unbalanced, 40 embryos were further cultured, among which 11 embryos who developed to blastocyst stage were re-biopsied on Day 6 and investigated by using fluorescence in situ hybridization (FISH).

Embryo handling

As described elsewhere [39], standard IVF procedure was carried out to produce cleavage-stage embryos. Briefly, 72 h after insemination, embryos, with a minimum of 6 cells and maximum of 50% fragmentation, were dealt with Ca^{2+}/Mg^{2+} free buffered media under mineral oil and then subjected to the mechanical partial zona-pellucida dissection (PZD) followed by biopsy. Using micromanipulation, one blastomere was separated from each embryo. Afterwards, all blastomeres were transferred into sterile tubes with 2.5 μL of phosphate-buffered saline (Cell Signaling Technologies, MA, USA). For every three blastomeres, 2.5 μL of the third cell washing drop was used as a negative control, and was placed in a separate tube. After biopsy, all embryos were immediately cryopreserved. According to the testing results, 'normal/balanced' ones were selected for frozen thawed-embryo transfer cycles.

Blastocyst biopsy was also manipulated with micromanipulation, as described [40]. Detailedly, several trophectoderm (TE) cells (2-3), from the same hole in the zona pellucida, were collected on Day 3. The collected cells were then placed on microscope slides, fixed and reanalyzed by FISH.

WGA

Whole genomic DNA amplification (WGA) was carried out according to the manufactures' protocol of SurePlex DNA Amplification System (BlueGnome, Cambridge, UK). Genomic DNA of single blastomeres or blastocyst microdissections, wash droplet negative controls, matched controls like Human Reference DNA Male (Agilent technologies, CA, USA), together with one blank reagent, were all randomly fragmented and subsequently subjected to PCR with universal priming sites.

Array-CGH

WGA products were processed referring to the protocol of Agilent oligonucleotide array-based CGH for single cell (Revision A0, June 2012). These products were fluorescently labelled with matched controls (Human Reference DNA Male) according to the instructions of SureTag Complete DNA Labeling Kit (Agilent technologies, CA, USA), and then competitively hybridized to SurePrint G3 Human CGH 8×60K microarray (G4450A, Agilent technologies, CA, USA). With increased screening resolutions (approximate 100 Kb in genome-wide scale and 50 Kb across sub-telomeric and peri-centromeric regions (UCSC hg18)), these microarrays facilitate a comprehensive identification and characterization of structural chromosome imbalances in single cell. Excitement of the hybridized fluorophores and storage of the data of the hybridization were completed using an Agilent DNA Microarray Scanner (G2565CA). Sequencing data was then extracted using the Feature Extraction Software (Agilent technologies, CA, USA), followed by analysis and quantification using the Agilent Genomic Workbench 7.0 Software (Agilent technologies, CA, USA) with algorithm default settings.

Interpretation of array-CGH results

Using array-CGH approach, abnormalities or imbalances are theoretically identified when copy number for a test sample significantly deviated from that of reference sample. Embryos were determined to be "normal/balanced" if no gain or loss larger than 3×SD of autosome could be observed; otherwise, embryos were diagnosed as "unbalanced". A partial or full trisomy was determined when the clones of data shifted above the baseline depicted with red points (gain). While a partial or full monosomy was detected when a shift below the baseline occurred, which was depicted with green points (loss) in **Figure 1.** Contrast to the phenomenon that no change could be observed for either chromosome X or chromo-

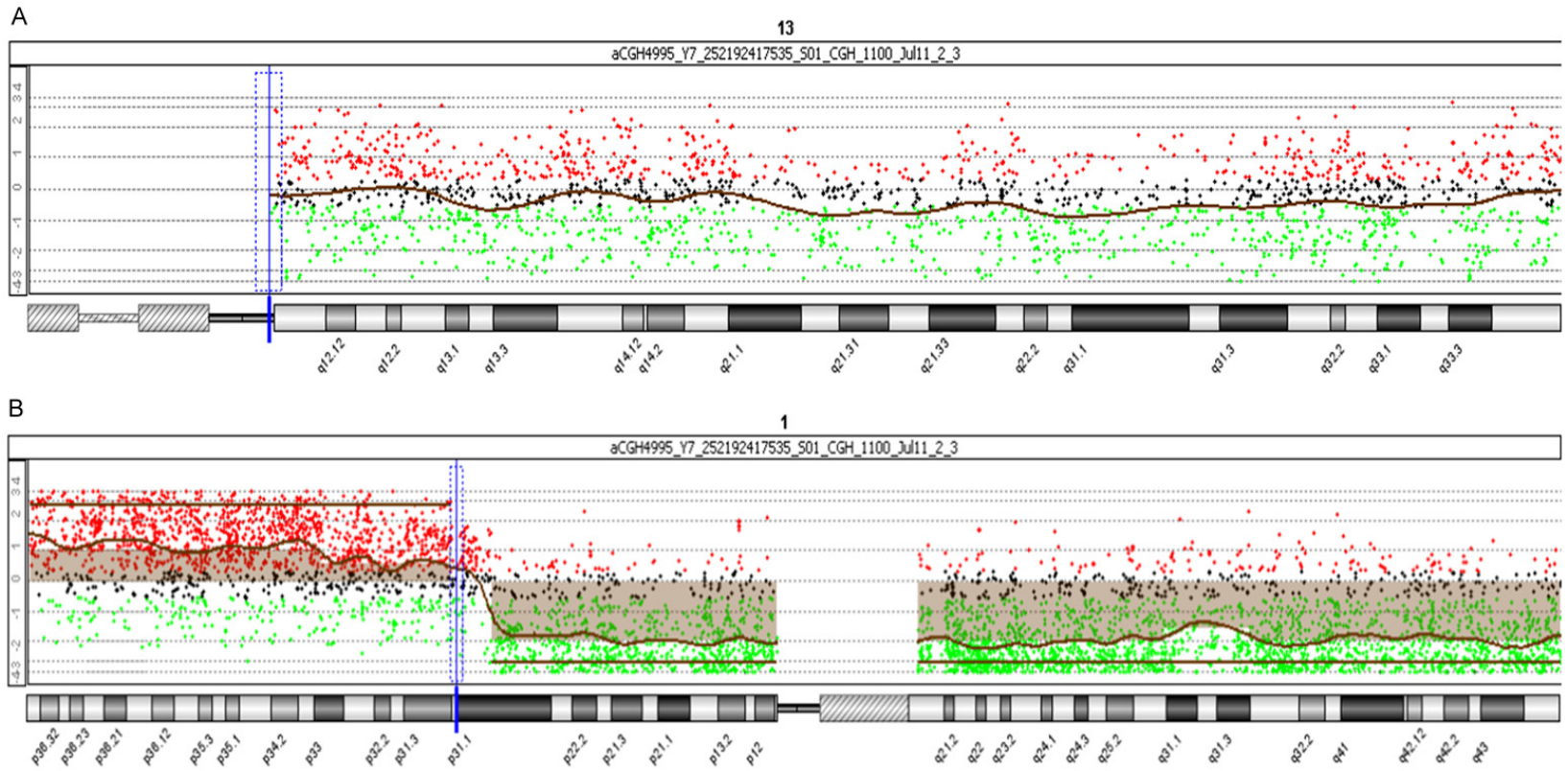

Figure 1. Examples of array CGH results showing balanced or unbalanced chromosomes. A. An euploidy was detected as the clones of data for the specific chromosome around the baseline showing by black points (balanced). B. A trisomy (partial or full) was detected as a shift of the clones of data for the specific chromosome above the baseline showing by red points (gain), while a monosomy (partial or full) was identified as a shift below the baseline showing by green points (loss).

Table 2. Clinical results from 14 PGD cycles for reciprocal and Robertsonian translocations

Clinical data	Couples		Total
	Robertsonian translocation	Reciprocal translocation	
No. of couples treated	9	5	14
Maternal age (average, years)	31.7±4.6	30.0±3.5	31.1±4.2
No. of cycles performed	9	6	15
No. of oocytes retrieved	116	107	223
No. of mature oocytes injected (%)[a]	94 (81.0)	87 (81.3)	181 (81.2)
No. of oocytes fertilized (%)[b]	80 (85.1)	75 (86.2)	155 (85.6)
No. of embryo thawed	9	10	19
No. of embryo surviving after thawing	8	10	18
No. of embryos biopsied	77	67	144
Mean	8.6±3.9	13.4±7.9	10.3±5.9
No. of blastomeresanalysed	77	67	144
No. of blastomeres with a WGA failure	4	5	9
No. of embryos diagnosed (%)	73 (94.8)	62 (92.5)	135 (93.8)
Balanced (%)	13 (17.8)	10 (16.1)	23 (17.0)
Unbalanced (%)	12 (16.4)	14 (22.6)	26 (19.3)
Balanced + aneuploid (%)	29 (39.7)	13 (21.0)	42 (31.1)
Unbalanced + aneuploid (%)	19 (26.1)	25 (40.3)	44 (32.6)
Total no. of balanced embryos (%)	42 (57.5)	23 (37.1)	65 (48.1)
Total no. of unbalanced embryos (%)	31 (42.5)	39 (62.9)	70 (51.9)
Total no. of aneuploid embryos (%)	48 (65.8)	38 (61.3)	86 (63.7)
No. of embryos transferable	13	10	23
No. of embryos transferred	6	9	15
No. of pregnancies still ongoing	2	0	2
No. of babies born	0	1	1

WGA, whole-genome amplification. A inical data of PGD cases. [a]Calculated on No. of mature oocytes injected. [b]Calculated on No. of oocytes fertilized.

some Y for sexmatched male, sex-mismatched female exhibited a consistent gain on chromosome X and a consistent loss on chromosome Y.

KL-BoBs™ assay

For the purpose to verify the PGD results from array based CGH strategy, some amount of WGA product from each blastomere was applied in the KaryoLite™ BACs-on-Beads™ (KL-BoBs™) assay. As described elsewhere [41], aneuploidy detection was carried out based on the comparison of the test sample with four individual reference DNA samples, which came from 2 male and 2 female.

FISH analysis

As previously described [42], the single TEs were spread on slides using HCL/tween 20 and methanol: acetic acid (3:1) fixative. Different combinations of probes were specially designed by and purchased from Abbott Laboratories (Abbott Park, IL, USA) to properly characterize the structural rearrangements in interphase nuclei. Before PGD was carried out, the specificity and sensitivity of the probes were tested on metaphase chromosome slides prepared from cultured peripheral blood cells of the carriers. All FISH results were classified and evaluated referring to the criteria described before [42].

Follow-up analysis

In order to detect mosaicisms, all other embryos diagnosed as 'unbalanced', after gaining the consent of the PGD couples, will be cultured to develop into blastocyst stage, re-biopsied on Day 5 or 6, and be re-analyzed by array-CGH and KL-BoBs™ assay, using the same approaches described above. For cases in which pregnancies were established, couples were

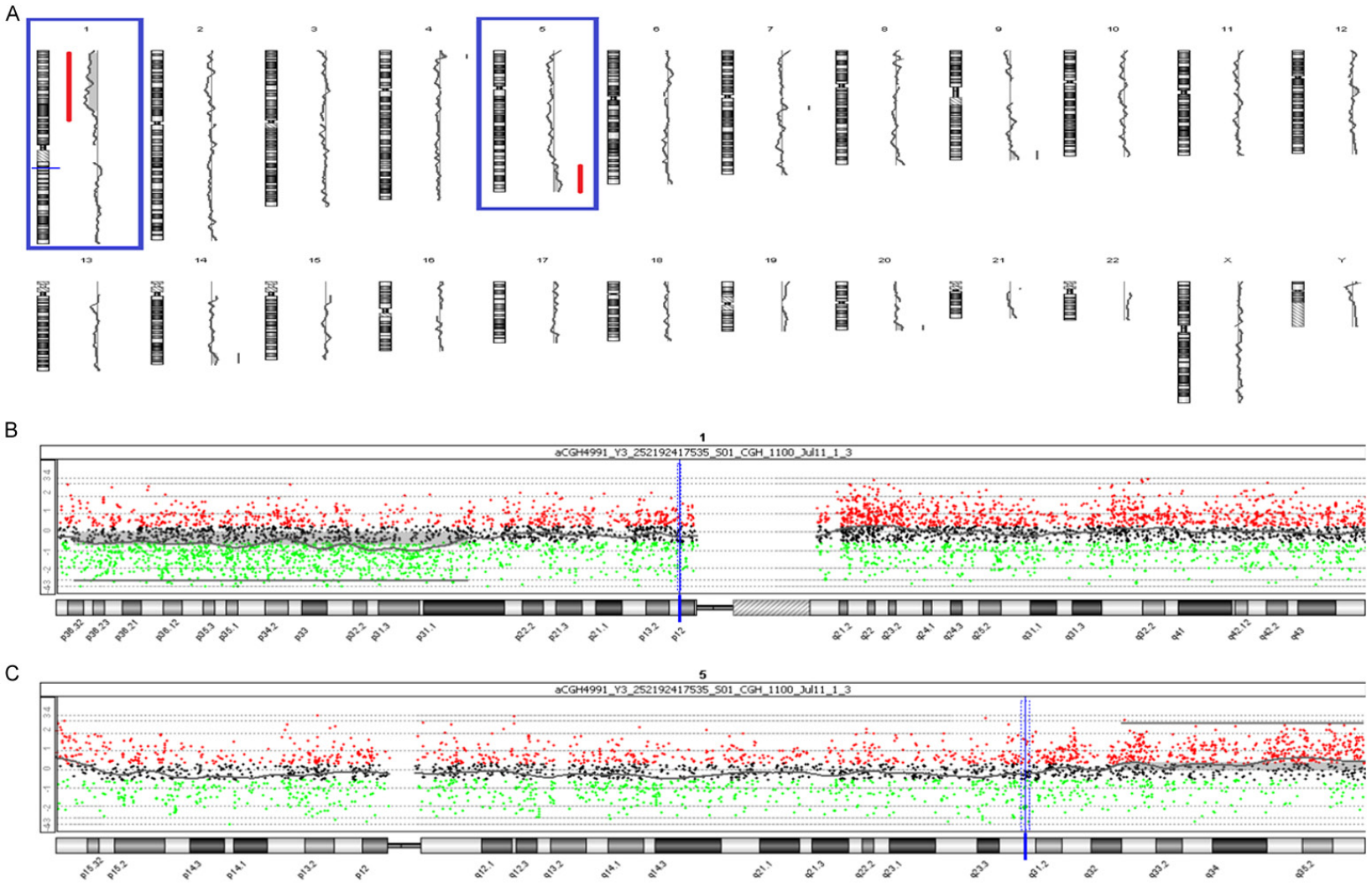

Figure 2. Examples of the array-CGH-based PGD resulted from a chromosomally unbalanced embryo derived from a patient carrying a balanced translocation 46, XX, t (1; 5) (p32; q35). A. Red lines in the blue frames indicated chromosomal imbalances inherited from the reciprocal translocation. B. Embryo with a partial monosomy 1p32-pter, detected as a shift of the clones below the baseline, was shown by green points (loss). C. A partial trisomy 5q33-qter, identified with a shift of the specific clones above the baseline, was shown by red points (gain).

strongly encouraged to take a conventional prenatal diagnosis to confirm the karyotype of the fetus. Whenever possible, buccal cells from newborns would be used to extract DNA, which would facilitate array-CGH analysis.

Results

Array CGH resolution

As illustrated in **Table 1**, 14 couples, carrying 4 different Robertsonian translocations and 5 different reciprocal translocations, were detected with 15 cycles of PGD (**Table 1**). A total of 223 oocytes were collected, among which, 181 (81.2%) were at mature metaphase II stage. Moreover, 155 oocytes were normally fertilized by ICSI, and we got 144 embryos (92.9%, mean number 9.6±4.6 per cycle; ranging from 4 to 20), which were subsequently biopsied on Day 3 (**Table 2**).

During WGA, no amplification could be detected among all of the negative controls, including washing solution and reagent negative controls, which suggested the accuracy of the tests. Credible data of WGA were obtained from 135 blastomeres among the 144 ones (93.8%), while 9 blastomeres failed to be amplified due to failed cell lysis, DNA damage or the absence of nuclei in the biopsied cells.

Overall, a clear and well-defined array-CGH profile was obtained from the 135 blastomeres with positive WGA results, meaning that the success rate of the detection of qualified WGA products were 100%. Whereas, only 23 out of 135 detected blastomeres were determined to have normal and balanced chromosomes, while great majority (112) were either unbalanced or with aneuploidy. In detail, 44 were both aneuploid and unbalanced, and 42 had balanced but aneuploid chromosomes. The remaining 26 blastomeres were determined to have unbalanced translocation but normal ploidy. These results illustrated that large proportion of blastomeres with positive WGA results could still be abnormal, which might lead to reproductively incompetent embryos. Since the resolution of microarray is sufficient to detect the gain and loss of small fragments, we detected the gain and loss events on all fragments from all the embryos. **Figures 2** and **3** showed examples of the results of array-CGH, which illustrated the feability of the approach for identifying various structural chromosome imbalances, inheritance of unbalanced translocation products (**Figure 2**), as well as aneuploidies of chromosomes unrelated to the translocation (**Figure 3**).

Error rate of translocations

In order to verify the accuracy of the array CGH, WGA products from 135 analyzed embryos were validated by the recently developed KL-BoBs™ assay. The paired comparison between the two assays showed highly concordant results for aneuploidy screening in the embryos of both reciprocal and Robertsonian translocation groups. Specifically, among the 86 embryos diagnosed as aneuploidy by array CGH, 86 (100%) got the same diagnosis in the KL-BoBs™ assay. Particularly, all the 23 embryos (100%) identified to have normal or balanced chromosomes by array CGH, were repeatedly diagnosed as normal or balanced by KL-BoBs™ assay. These results revealed that the accuracy of CGH is highly comparable with the popular assay kits in the screening of aneuploidy. However, 3 out of 26 embryos, which were determined to be unbalanced in the translocation detection but normal in the aneuploidy screening by array CGH, were diagnosed as normal or balanced by KL-BoBs™ assay.

To further confirm the chromosomal rearrangements involved in the translocations, a total of 40 embryos diagnosed as unbalanced were further cultured, among which the 11 ones who developed into blastocyst stage were re-biopsied on Day 6 and reanalyzed by the classic PGD approach FISH. 10 out of the 11 embryos, which were diagnosed as unbalanced in the translocation detection by array CGH, were repeatedly verified to be unbalanced by the FISH method. Our results illustrated that diagnosis by array CGH were highly repeatable by both the popular assay kits and the classical FISH approach.

Pregnancy outcome for transferred embryos

Pregnancy outcome for these diagnosed embryos could further verify the efficiency of array CGH. Overall, no embryo was transferred in 5 cycles, and 4 cycles were canceled due to lack of normal or balanced embryos. Among the

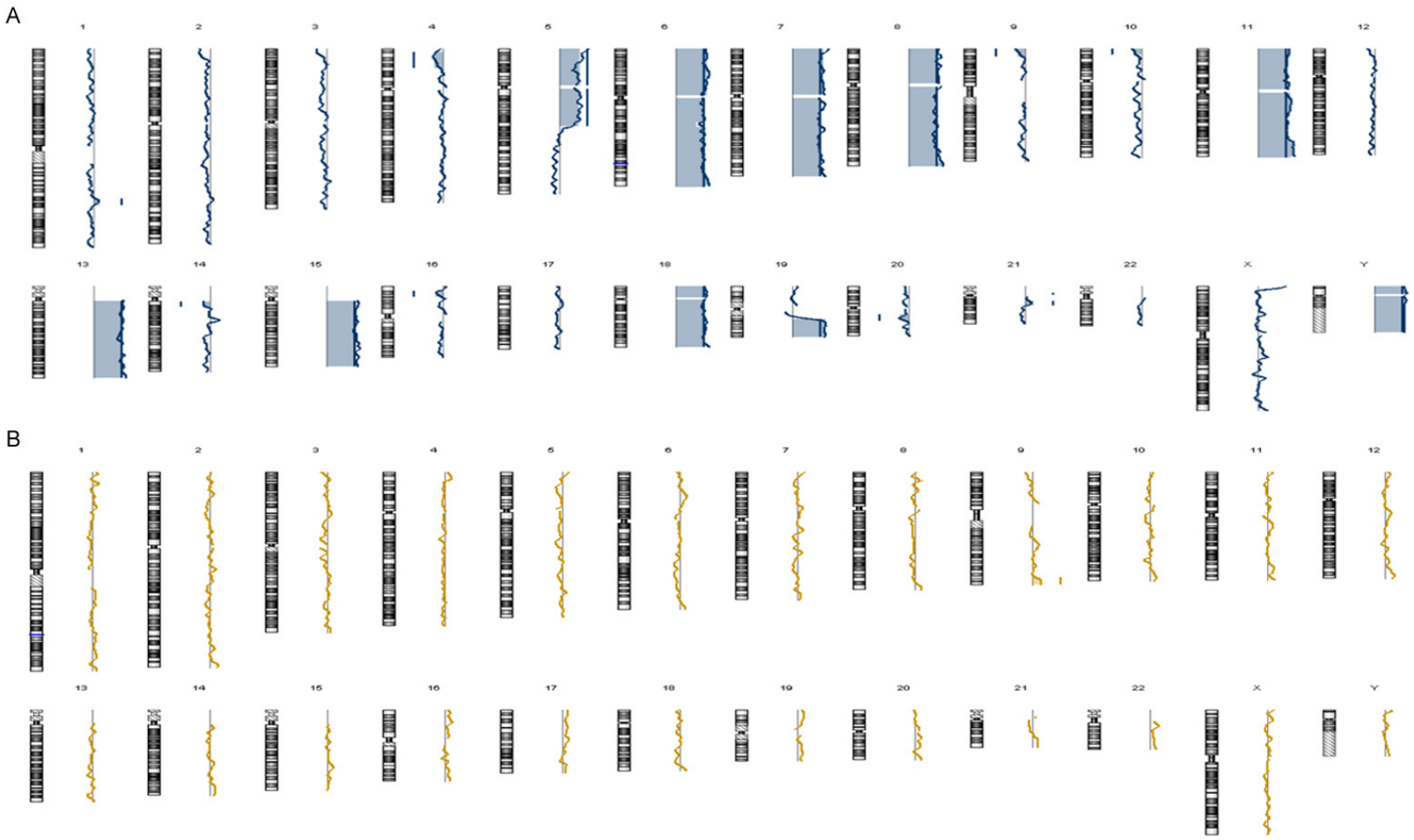

Figure 3. Examples of the array-CGH-based PGD resulted from a chromosomally unbalanced embryo derived from a patient carrying a balanced translocation 46, XX, t (1; 5) (p32; q35). A. Embryo with aneuploidies of chromosomes was unrelated to the translocation. B. Embryo without chromosomal segmental imbalances showed euploidy.

cases carrying Robertsonian translocation, there was only one transferable embryo (1/6) in one case. But it didn't survive after thawing, leading to no transfer in that cycle. Among the 15 transferred embryos, 9 were of satisfactory quality, which were subsequently transferred to 6 women. 4 recipients exhibited positive hCG level. However, although the other 2 received 4 embryos of good quality, they unfortunately have not had a pregnancy until recently (**Table 1**). Fortunately, one recipient with positive hCG level has delivered one baby; and microarray analysis of the newborn buccal cells has confirmed that the diagnosis by array CGH for the translocation and euploidy was credible. The other two pregnancies were continuing, and were confirmed to be normal by detecting fetal sac and heartbeat. The remaining one turned out to be a biochemical pregnancy only (**Table 1**).

Discussion

It has been well documented that array-CGH is a valuable alternative tool for PGD, since it not only successfully overcomes most of the limitations of the FISH-based protocols, but also avoids the time-consuming work to prepare for different PGD cases due to structural rearrangements. Therefore, array-CGH has become routine in many PGD centers [32-34, 43]. The present study described the first clinical application of oligo-based array CGH in 15 PGD cycles for the identification of structural chromosome imbalances in embryos derived from both reciprocal and Robertsonian translocation carriers.

In order to minimize the risk of transferring reproductively incompetent embryos, it is critical to assess the diagnostic accuracy of different methods in PGD. In our study, high percentage of cleavage embryos (93.8%) were successfully detected by array CGH, among which 23 embryos were suitable for transfer, which was in agreement with previous studies [32-34, 43]. Moreover, the reanalysis of non-transferred embryos in blastocyst stage by FISH with probes specific for the structural chromosome abnormality indicated an error rate of 9.1% (1/11), which may be due to high rate of mosaicism common observed in cleavage-stage embryos or errors that occurred infrequently in the array CGH technique.

In the present study, 31.1% embryos were determined to be normal or balanced during the detection of structural rearrangement, but with aneuploidy, which were unable to be detected by regular FISH-based PGD approach. According to previous study [43], even PGS utilizing standard 12-probe FISH approach detected approximately 42% less abnormalities and 13% less abnormal embryos than the array CGH approach. Ours results indicated that array CGH, in comparison with FISH, provides the obvious advantage for screening of aneuploidy for all 24 chromosomes simultaneously.

Additionally, another extra benefit of array-based CGH strategy lies in that it does not require any preclinical validation before each IVF cycle, which is critical for FISH. This avoids postponement of the start of IVF treatment and also saves the cost of work-up testing for the couples. In fact, unlike FISH based ones, array based CGH PGD cycles can be scheduled, based on the number of embryos available for biopsy, even at the day for biopsy.

As for the aneuploidy screening, the other form of validation was to obtain the relatively large quantity of amplified whole genome DNA from the same DNA sample in different platforms and compare the results. Our approach was to apply those samples to the KL-BoBs™ assay, a relatively lower resolution analysis compared with chromosomal microarray, but with a very high sensitivity for aneuploidy detection [41]. And for the aneuploidy detection, the results we got from the KL-BoBs™ assay were almost identical to those obtained from array CGH. Data from comprehensive aneuploidy screening in this study and some other reported before [35, 43] showed that aneuploidies may occur in any of the 24 chromosomes of pre-implantation embryos, indicating that aneuploidy screening of all chromosomes is necessary to determine whether an embryo is chromosomally normal. We also noticed that the results from array CGH were disaccord with those from KL-BoBs in the reciprocal translocation group, in which 11.5% (3/26) embryos were classified as unbalanced for the translocation but normal for aneuploidy by array CGH, but were diagnosed as normal and balanced by KL-BoBs™ assay. While none from the Robertsonian translocation group showed inconsistence. It is because that KL-BoBs™ assay has a

much lower resolution and cannot detect small segmental chromosome imbalances derived from the reciprocal translocations; While as an effective method for detecting arm-specific aneuploidies in all 24 chromosomes, KL-BoBs™ assay is capable of detecting partial or full trisomy or monosomy derived from Robertsonian translocations.

Currently, we achieved 4 pregnancies (including one twin pregnancy) after transferring 15 embryos. In the present study, the implantation rate was 33.3% (5/15) per embryo and pregnancy rate was 36.4% (4/11) per cycle. The reason why implantation and pregnancy rates were not so satisfactory may lie in that 6 out of 15 (40%) embryos were of low quality. Additional factors other than tubal factors, such as diminished ovarian reserve (DOR) or immune infertility factors, may also lead to the sterility of the couples. For example, one couple carrying 46, XX, t (1; 5) (p32; q35) received 3 normal but of low quality embryos in 2 PGD cycles and none were implanted. However, the same recipient achieved clinical pregnancy later in a donor egg IVF cycle. Additionally, the other two recipients received 4 satisfying embryos but achieved no pregnancy. It is pity to found that one recipient had suffered miscarriage 4 times for unknown reason and the other recipient's husband had teratozoospermia. Therefore, despite of quality of embryos, the implantation and pregnancy rates could also be affected by numerous factors.

For diagnosis of translocations with array CGH, the only limiting factor is the resolution of the array. In current days, the relatively popular platform for array CGH is the BAC-based platform. It has been reported that even the highest-density BAC-based array was unable to detect fragments smaller than 2.5 Mbp in single blastomeres [32, 33]. In some cases of reciprocal translocations, the fragments are either too small to be detected by the BAC array or fall within gaps in the BAC array coverage, but they are not below the resolution of the oligonucleotide platform [38]. Current study carried out the first comprehensive aneuploidy screening in parallel with translocation-related chromosome screening using oligo-array CGH in embryos derived from patients carrying balanced reciprocal or Robertsonian translocations. The resolution of the oligo array we used is about 100 K. At such a high resolution, the results of our array CGH were still robust and simple to interpret (**Figure 1**). However, it is important to note that, oligo array CGH cannot detect some polyploidies and uniparental disomy, which is the common shortcoming of array-CGH intrinsic to this technique, regardless of whether the array is based on BAC or on oligonucleotide.

These shortcomings can be overcome by SNP array which is also used to detect translocations and aneuploidy in PGD with the added ability to distinguish between normal and balanced embryos by identifying the normal and derivative chromosomes at the translocation breakpoints, which array CGH cannot achieve. Regardless of its extra resolution and parent-of-origin information, SNP arrays and data analysis is laborintensive and costly, and it requires optimized protocols before the IVF cycle and needs specialized algorithms for identifying chromosome copy number following whole-genome amplification from single cells [36]. Compared with SNP arrays, array-CGH does not require the testing of DNA samples from the couple before the IVF cycle, and it can be scheduled on the day of biopsy based on the number of embryos available for biopsy [36]. Advantageously, the analysis of array CGH is fully automated, the whole procedure can be completed within 24 hours and it is possible to perform the embryo transfer in a fresh cycle on Day 5 of embryo development following the cleavage biopsy on Day 3.

In conclusion, oligo array CGH is a relatively low-cost and high-resolution option for the rapid detection of whole-chromosome or chromosome-segment gain and loss for all 24 chromosomes in PGD.

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Disclosure of conflict of interest

None.

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