Diagnostic accuracy and value of chromosomal microarray analysis for chromosomal abnormalities in prenatal detection

A prospective clinical study

Hailong Huang, PhD, Yan Wang, MM, Min Zhang, MM, Na Lin, MM, Gang An, PhD, Deqin He, PhD, Meihuan Chen, MM, Lingji Chen, BS, Liangpu Xu, BS

Abstract
Chromosomal microarray analysis (CMA) has emerged as a primary diagnostic tool for the evaluation of developmental delay and structural malformations in children. The aim of this study was to compare the accuracy and value of CMA and karyotyping on diagnosis of chromosomal abnormalities in Fujian province of South China.

In the study, 410 clinical samples were collected from pregnant women between March 2015 and December 2016, including 3 villus (0.73%, 3/410), 296 amniotic fluid (72.20%, 296/410), and 111 umbilical cord blood (27.07%, 111/410). All samples were screening for chromosomal abnormalities by both using CMA and karyotyping.

The success rate of CMA and karyotyping was 100% (410/410) and 99.27% (407/410), respectively. Sixty-one (14.88%, 61/410) samples were presented with chromosomal abnormalities by using CMA, whereas 47 (11.55%, 47/407) samples were shown with chromosomal abnormalities by using karyotyping. Thirty-one (8.61%, 31/360) samples with normal karyotypes were found to exist chromosomal abnormalities by using CMA. Receiver operating characteristic analysis showed that the area under the curve of karyotyping on the diagnosis of chromosomal abnormalities was 0.90 (95% confidence interval: 0.87 to 0.93), the sensitivity and specificity was 87.56% and 91.22%, respectively. The area under the curve of CMA on the diagnosis of chromosomal abnormalities was 0.93 (95% confidence interval: 0.90 to 0.95), with 90.68% sensitivity and 94.40% specificity. Notably, the combination of CMA and karyotyping could improve the diagnosis of chromosomal abnormalities.

CMA has a better diagnostic value for screening chromosomal abnormalities, especially for those pregnant women with normal karyotypes. This study has guiding value for prenatal diagnosis in Fujian province of South China.

Abbreviations: AUC = area under the curve, CMA = chromosomal microarray analysis, NIPT = noninvasive prenatal testing, PND = prenatal diagnosis, ROC = receiver operating characteristic.

Keywords: chromosomal abnormalities, chromosomal microarray analysis, diagnosis, karyotyping, value

1. Introduction
Currently, the mainly means of prenatal diagnosis (PND) are to apply a combination of diagnostic procedures in the 1 and 2-trimester based on concentrations of serum analytes, genetic history, maternal age, and ultrasound-detected data from pregnant women.1-3 Chromosomal abnormalities in PND have focused commonly on detection of human aneuploidy including trisomy 21 and 18.4,5 Karyotyping is commonly technique in screening chromosome abnormalities from fetuses with congenital malformations, including deletion, inversion, duplication,
translocation, aneuploidy, and polyploidy.\cite{6} Due to the reliable detection of aneuploidy and large rearrangement, karyotyping is the preferred method for PND for a long time in the past. However, recently many studies have found karyotyping exhibits considerable limitations, especially for the lack of detection of unbalanced structural abnormalities from submicroscopic chromosomal aberrations. At present, molecular cytogenetic techniques including multiplex ligation-dependent probe amplification, quantitative fluorescence polymerase chain reaction, and fluorescence in situ hybridization, are gradually applied to detect submicroscopic chromosomal aberrations in clinical practices.\cite{7,8} However, these methods are not feasible to detect all possible chromosome deletion and duplication.

Chromosomal microarray analysis (CMA) is known as array-based comparative genomic hybridization, which is a detection technology that screens for abnormalities in the number and structure of chromosomes by scanning the whole genome of chromosomes.\cite{9,10} At present, the advantages of CMA in PND are gradually presented with the rapid development of gene chip technology. CMA has the ability to disclose a wide range of chromosomal abnormalities with length from 50 kb to 100 kb, which can produce 100 times better resolution than karyotyping.\cite{11} More and more evidence has shown that CMA improves the diagnostic accuracy by approximately 15% to 20% over that of karyotyping when applied for the evaluation of fetuses with unexplained developmental delay, mental retardation, and autism.\cite{12} According to epidemiological statistics, CMA can raise the diagnostic rate from 0.5% to 16% for screening commonly chromosomal abnormalities in PND.\cite{13} Simultaneously, CMA can obviously increase the success rate for diagnosing fetuses with chromosomal structural anomalies compared with karyotyping.\cite{14,15} Due to the diversity of regions in China, the use of CMA in PND has great differences. Up to now, there is still no systematic study on the diagnostic accuracy and value of CMA for chromosomal abnormalities in Fujian province of South China.

In the present study, we aimed to investigate the diagnostic accuracy and value of CMA for screening chromosomal abnormalities in PND. First, 3 villus, 296 amniotic fluid, and 11 umbilical cord blood samples were collected from pregnant women in Fujian province of South China. Second, CMA and karyotyping methods were performed to determine chromosomal abnormalities from all samples. Finally, the sensitivity and specificity of CMA in the diagnosis of chromosomal abnormalities were calculated by using receiver operating characteristic (ROC) analysis.

### 2. Materials and methods

#### 2.1. Patient samples collection

This research was a prospective clinical study conducted in the Fujian Provincial Maternity and Children’s Hospital (Fujian, China). A total of 410 samples were collected from pregnant women between March 2015 and December 2016 in Fujian province of South China. Inclusion criteria: normal pregnant women. Exclusion criteria: history of chronic diseases and family history of genetic diseases. The detailed clinicopathological parameters of each pregnant woman were presented in Table 1. Ethics approval (No. 00157) was acquired from the Medical Research Ethics Committee of Fujian Provincial Maternity and Children’s Hospital in compliance with ethics of the World Medical Association (version 1991) Declaration of Helsinki, and written informed consent was provided by each patients.

#### 2.2. Karyotyping analysis

Karyotyping was performed by using G-banding analysis as previous literatures.\cite{16,17} G-banding was conducted according to the manufacturer operational protocols. Each sample was digested mechanically with collagenase II (TIANGEN, Beijing, China) at 37°C for 20 minutes. After that, the metaphases were analyzed with the CytoVision computer assisted karyotyping system version 2.7 (Santa Clara, CA). Five metaphase cells were checked carefully by 3 experienced diagnostic specialists to determine chromosomal structural abnormalities, and at least 15 metaphase cells were used to define chromosomal numerical abnormalities. Results were shown based on the criterion of the International System for Human Cytogenetics Nomenclature in 1995.

#### 2.3. Chromosomal microarray analysis

CMA was carried out as previous literatures.\cite{18,19} Briefly, genomic DNA from each sample was isolated by using a commonly DNA extraction kit (Qiagen, Hilden, Germany), according to the manufacturer’s procedures. Then the isolated DNA was quantified using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). When the concentration of DNA from sample was >100ng/µL and optical densities were 1.8 to 2.0 at 260/280nm, the qualified DNA samples were selected and stored at −20°C. Finally, all DNA samples were loaded with Affymetrix CytoScan HD/750k array (Affymetrix, CA), hybridized, and scanned with DNA MicroArray SureScan scanner (Affymetrix), according to the manufacturer’s procedures. Data were analyzed with the Affymetrix Chromosome Analysis Suite software (ChAS v1.1.1, Affymetrix). All chromosomal abnormalities were checked and compared with carefully the well-known databases, including the DECIPHER v9.30 (https://decipher.sanger.ac.uk/), Online Mendelian Inheritance in Man (http://omim.org/), and Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home/). According to the deletion and duplication in chromosome location, the clinical significances of chromosomal abnormalities were evaluated and defined as 5 types of properties, including pathogenicity, possible pathogenicity, benign, possible benign, and unclear.

| Variables | Number of cases | Percentage (/n, %) |
|-----------|----------------|-------------------|
| High age | 69/410, 16.83% |
| Abnormal ultrasound | 182/410, 44.39% |
| High risk of serological screening in early or middle pregnancy | 25/410, 6.10% |
| Fetuses with abnormal karyotypes | 13/410, 3.17% |
| Patients with abnormal karyotypes | 12/410, 2.93% |
| Adverse pregnancy history | 23/410, 5.61% |
| High risk of NIPT | 5/410, 1.22% |
| Two kinds of abnormal indications | 70/410, 17.07% |
| Three kinds of abnormal indications | 3/410, 0.73% |
| Others | 8/410, 1.95% |

NIP = noninvasive prenatal testing.
2.4. Statistical analysis

The statistical analyses were conducted with the SPSS version 18.0 (SPSS Inc., Chicago, IL). Data were shown as mean ± standard deviation from 3 independent assays with each measured in triplicate. Difference between the 2 groups was assessed by using Chi-square test. ROC curves were drawn, and the area under curves (AUC) were analyzed to determine the specificity and sensitivity of CMA, karyotyping, and their combination in the diagnosis of chromosomal abnormalities. A value of P < .05 was considered to be a statistically significant difference.

3. Results

3.1. General clinical information

A total number of 410 samples from pregnant women was recruited in this study, including 3 villus (0.73%, 3/410), 296 amniotic fluid (72.20%, 296/410), and 111 umbilical cord blood (27.07%, 111/410). The age of pregnant women varied from 19 to 42 years (mean at 24.33 ± 2.74 years), and gestational ages from 11 to 31 weeks (mean at 17.54 ± 3.17 weeks). The gestational ages in villus, amniotic fluid, and umbilical cord blood groups were 11′ to 13′, 18′ to 24′, and 24′ to 31′ weeks, respectively. As shown in Table 1, the prenatal samples are classified into 10 subgroups according to clinical indications, including high age (16.83%, 69/410), abnormal ultrasound (44.39%, 182/410), high risk of serological screening in early or middle pregnancy (6.10%, 25/410), fetuses with abnormal karyotypes (3.17%, 13/410), patients with abnormal karyotypes (2.93%, 12/410), adverse pregnancy history (5.61%, 23/410), high risk of noninvasive prenatal testing (NIPT) (1.22%, 5/410), 2 kinds of abnormal indications (17.07%, 70/410), 3 kinds of abnormal indications (0.73%, 3/410), and others (1.95%, 8/410).

3.2. Diagnostic efficacy of karyotyping for chromosomal abnormalities

Study flow chart was shown in Figure 1. In the 410 samples, only 3 cases of samples were not successful detected with karyotyping, including 1 villus, 1 amniotic fluid, and 1 umbilical cord blood, the success rate of karyotyping was 99.27% (407/410). Forty-seven samples (11.53%, 47/407) were found to exist chromosomal abnormalities by karyotyping, including 13 cases of numerical abnormalities (27.66%, 13/47), 26 cases of structural abnormalities (55.32%, 26/47), and 8 cases of chimeras (17.02%, 8/47). The representative images of chromosomal abnormalities by karyotyping were presented in Figures S1 to S3, Supplemental Digital Contents, http://links.lww.com/MD2/A163, http://links.lww.com/MD2/A164, and http://links.lww.com/MD2/A165, respectively. Among the 13 cases of numerical abnormalities, including 5 cases of trisomy 21 (38.46%, 5/13), 2 cases of trisomy 18 (15.38%, 2/13), 1 case of trisomy 18 (7.69%, 1/13), and 4 cases of extra small marker chromosome (30.77%, 4/13), and 1 case of triloid (7.69%, 1/13).

3.3. Diagnostic efficacy of CMA for chromosomal abnormalities

In the 410 samples, the success rate of CMA was 100% (410/410). Sixty-one (14.88%, 61/410) samples were found to have chromosomal abnormalities, including 10 cases of copy number variations (16.39%, 10/61), 9 cases of large fragment abnormality (≥10 Mb) (14.75%, 9/61), 38 cases of small fragment abnormality (<10 Mb) (62.30%, 38/61), and 4 cases of heterozygous abnormality (6.56%, 4/61). Among the 9 cases of large fragment abnormality, including 4 cases of deletion (44.44%, 4/9), 3 cases of duplication (33.33%, 3/9), and 2 cases of deletion and duplication (22.22%, 2/9). Among the 38 cases of small fragment abnormality, including 14 cases of microdeletion (36.84%, 14/38) and 24 cases of microduplication (63.16%, 24/38). In addition, 31 (8.61%, 31/360) samples with normal karyotypes by using karyotyping were found to have chromosomal abnormalities. A Venn diagram was used to generalize chromosomal abnormalities from 410 samples by karyotyping and CMA (Fig. 2).

3.4. Diagnostic value of CMA and karyotyping for chromosomal abnormalities

To investigate the potential diagnostic value of CMA and karyotyping for diagnosing chromosomal abnormalities, ROC curves were plotted on data from 410 samples. As presented in Figure 3, representation of the data revealed the AUC of karyotyping was 0.90 (95% confidence interval [CI]: 0.87–0.93), the sensitivity and specificity were 87.56% and 91.22%, respectively. The AUC of CMA was 0.93 (95% CI: 0.90–0.95) with 90.68% sensitivity and 94.40% specificity. Compared with karyotyping, the diagnostic value of CMA was remarkable for chromosomal abnormalities. When combination of CMA and karyotyping, the AUC for chromosomal abnormalities was 0.98 (95% CI: 0.96–0.99), the sensitivity and specificity was 96.04% and 97.59%, respectively.

3.5. Analysis of the relationship between the chromosomal abnormalities and clinical indications

As shown in Table 2, the rate of chromosomal abnormalities by karyotyping in high age group was 5.8%, in abnormal ultrasound group was 8.24%, in fetuses with abnormal karyotypes group was 76.92%, in patients with abnormal karyotypes group was 41.67%, in adverse pregnancy history group was 8.70%, in high risk of NIPT group was 20%, in 2 kinds of abnormal indications group was 11.43%, and in 3 kinds of abnormal indications group was 66.67%. The rate of chromosomal abnormalities by CMA in high age group was 1.45%, in abnormal ultrasound group was 14.84%, in high risk of serological screening in early or middle pregnancy group was 8.00%, in fetuses with abnormal karyotypes group was 61.54%, in patients with abnormal karyotypes group was 16.67%, in adverse pregnancy history group was 17.39%, in high risk of NIPT group was 20%, and in 2 kinds of abnormal indications group was 14.29%, in 3 kinds of abnormal indications group was 16.67%, in others group was 50.00%. Comparing with karyotyping, the rate of chromosomal abnormalities of abnormal ultrasound group by CMA was increased. However, there was no significant difference in chromosomal abnormalities of other clinical indication groups by CMA and karyotyping.

4. Discussion

At present, CMA utilizes various array techniques to determine molecular karyotype including oligonucleotide array, bacterial artificial chromosome array, and single nucleotide polymorphism
Increasing evidence has shown that a mostly proportion of chromosomal abnormalities has been verified with the use of CMA in PND, in addition to some balanced rearrangements, triploidies, and uniparental disomy. By using CMA, the resolution of detectable chromosomal abnormalities has heightened from 10 kb or larger-sized rearrangements to a few Mb in size, thus significantly improves the application in PND.

Here, the purpose of our study was to assess the accuracy of CMA and karyotyping on diagnosing chromosomal abnormalities, and to analyze its diagnostic value as a routine inspection in PND. The 410 samples of villus, amniotic fluid, and umbilical cord blood from pregnant women in Fujian province of South China were collected and cultured, and all samples were screening for chromosomal abnormalities by both using CMA and karyotyping. In the 410 samples, the success rate of CMA was 100%, and 61 samples were found to show chromosomal abnormalities by CMA. Furthermore, 31 samples with normal karyotypes by using karyotyping were presented with chromosomal abnormalities. The possible practical reasons that generated the accuracy difference in CMA and karyotyping were as following:

1. karyotyping can only detect larger-sized rearrangements in chromosomal abnormalities,
2. heterogeneity of samples,
3. unqualified sample quality,
4. deviations in the data analysis process,
5. other reasons.

In our study, the overall abnormal rate of chromosomal abnormalities by CMA (14.88%) was higher than several reports at recent studies (2%–7.1%). The causes might focus on larger proportion of women with high risk of serological
Figure 2. A Venn diagram for generalizing chromosomal abnormalities from 410 samples by karyotyping and CMA. CMA = chromosomal microarray analysis.

Figure 3. The ROC curves of karyotyping and CMA for screening chromosomal abnormalities. (A) The AUC of karyotyping was 0.90 (95% CI: 0.87–0.93), the sensitivity and specificity was 87.56% and 91.22%, respectively. (B) The AUC of CMA was 0.93 (95% CI: 0.90–0.95), with 90.68% sensitivity and 94.40% specificity. (C) The AUC of the combination of CMA and karyotyping was 0.98 (95% CI: 0.96–0.99), the sensitivity and specificity was 96.04% and 97.59%, respectively. AUC = area under curve, CI = confidence interval, CMA = chromosomal microarray analysis, ROC = receiver operating characteristic.
screening in early or middle pregnancy accounted for 6.10% of our cohort.

Simultaneously, 47 samples were shown with chromosomal abnormalities by karyotyping, including 13 cases of numerical abnormalities, 26 cases of structural abnormalities, and 8 cases of chimeras. Sixty-one samples were presented with chromosomal abnormalities by CMA, including 10 cases of copy number variations, 9 cases of large fragment abnormality, 38 cases of small fragment abnormality, and 4 cases of heterozygous abnormality. The overall abnormal rate of chromosomal abnormalities by CMA was 14.88%, which reflected 14 more cases than identified by karyotyping (11.55%), for an additional diagnostic yield of 3.33%. In addition, the samples with chromosomal abnormalities by CMA were exhibited as small fragment abnormality, deletion, and duplication. These results were consistent with a recent meta-analysis (3%–5.2%) by Hillman et al. The diagnostic yield of CMA is related to the particular population, clinical indications, fetuses from selective terminations, and spontaneous miscarriages. Moreover, the ROC curve of CMA showed 90.68% sensitivity and 94.40% specificity. The AUC of CMA was significantly larger than that of karyotyping, indicating that CMA may display excellent diagnostic value for chromosomal abnormalities in PND. Notably, the combination of CMA and karyotyping could improve the diagnosis of chromosomal abnormalities.

Besides, we also analyzed the relationship between chromosomal abnormalities and clinical indications. In terms of single clinical indication, the rate of chromosomal abnormalities has no obvious difference by CMA and karyotyping in high age, abnormal ultrasound, fetuses and patients with abnormal karyotypes, adverse pregnancy history, and high risk of NIPT groups. However, the rate of chromosomal abnormalities by CMA had an increased tendency in abnormal ultrasound group. The possible explanation might be caused by CMA detection of wide range of chromosomal abnormalities.

Finally, there are still some limitations in our study. First, the clinical samples were relative small. Second, the heterogeneity of samples might have an impact on the test results. Third, limited number of clinical indicators. Therefore, further researches with larger population and more clinical indicators should be conducted to confirm support our findings.

5. Conclusions

CMA is efficient to improve diagnostic accuracy of chromosomal abnormalities in PND. CMA has a higher diagnostic value for chromosomal abnormalities, especially for those pregnant women with normal karyotypes.

Acknowledgments

The authors appreciate all the colleagues who offered assistance to their project. The authors also thank all families for participating in this study.

Author contributions

Conceptualization: Liangpu Xu.
Data curation: Hailong Huang.
Formal analysis: Deqin He, Meihuan Chen, Lingji Chen.
Investigation: Hailong Huang, Yan Wang.
Methodology: Min Zhang, Na Lin, Gang An.
Supervision: Liangpu Xu.
Validation: Hailong Huang.
Writing – original draft: Hailong Huang.
Writing – review & editing: Hailong Huang, Liangpu Xu.

References

[1] Levy B, Stosic M. Traditional prenatal diagnosis: past to present. Methods Mol Biol 2019;1883:3–22.
[2] Cheng WL, Hsiao CH, Tseng HW, et al. Noninvasive prenatal diagnosis. Taiwan J Obstet Gynecol 2015;54:343–9.
[3] Vermeesch JR, Voet T, Devriendt K. Prenatal and pre-implantation genetic diagnosis. Nat Rev Genet 2016;17:643–56.
[4] Dugoff L, Norton ME, Kuller JA. The use of chromosomal microarray for prenatal diagnosis. Am J Obstet Gynecol 2016;215:B2–9.
[5] Zhang L, Ren M, Song G, et al. Prenatal diagnosis of sex chromosomal inversion, translocation and deletion. Mol Med Rep 2018;17:2811–6.
[6] Hay SB, Sahoo T, Travis MK, et al. ACOG and SMFM guidelines for prenatal diagnosis: is karyotyping really sufficient? Prenat Diagn 2018;38:184–9.
[7] Shah MS, Cinnioglu C, Mainsenbacher M, Comstock I, Kort J, Lathi RB. Comparison of cytogenetics and molecular karyotyping for chromosome testing of miscarriage specimens. Fertil Steril 2017;107:1028–33.
[8] Lovrecic L, Perez M, Jaklic H, Ostojić S, Peterlin B. Combination of QF-PCR and aCGH is an efficient diagnostic strategy for the detection of chromosome aberrations in recurrent miscarriage. Mol Genet Genomic Med 2019;7:e980.
[9] Oneda B, Rauch A. Microarrays in prenatal diagnosis. Best Prac Res Clin Obstet Gynaecol 2017;42:53–63.
[10] Stosic M, Levy B, Wapner R. The use of chromosomal microarray analysis in prenatal diagnosis. Obstet Gynecol Clin North Am 2018;45:55–68.
[11] Pauta M, Grande M, Rodriguez-Reyenga I, Kolomietz E, Borrell A. Added value of chromosomal microarray analysis over karyotyping in early pregnancy loss: systematic review and meta-analysis. Ultrasound Obstet Gynecol 2018;51:453–62.
[12] Wapner RJ, Martin CL, Levy B, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med 2012;367:2175–84.
[13] Reddy UM, Page GP, Saade GR, et al. Karyotype versus microarray testing for genetic abnormalities after stillbirth. N Engl J Med 2012;367:2185–93.
[14] Wu Y, Wang Y, Tao J, et al. The clinical use of chromosomal microarray analysis in detection of fetal chromosomal rearrangements: a study from China Mainland. Eur J Obstet Gynecol Reprod Biol 2017;212:44–50.
[15] Deng Q, Huang L, Liu J, et al. Prenatal diagnosis of submicroscopic chromosomal aberrations in fetuses with congenital cystic adenomatoid malformation by chromosomal microarray analysis. J Matern Fetal Neonatal Med 2019;34:1–7.
[16] Bayani J, Squire JA. Traditional banding of chromosomes for cytogenetic analysis. Curr Protoc Cell Biol 2004;Chapter 22:Unit 22.3.
[17] Martin CL, Warburton D. Detection of chromosomal aberrations in clinical practice: from karyotype to genome sequence. Annu Rev Genomics Hum Genet 2015;16:309–26.
[18] Resta N, Memo L. Chromosomal microarray (CMA) analysis in infants with congenital anomalies: when is it really helpful? J Matern Fetal Neonatal Med 2012;25(Suppl 4):124–6.
[19] Faucett WA, Savage M. Chromosomal microarray testing. JAAPA 2012;25:65–6.
[20] Levy B, Wapner R. Prenatal diagnosis by chromosomal microarray analysis. Fertil Steril 2018;109:201–12.
[21] Rajcan-Separovic E. Chromosome microarrays in human reproduction. Hum Reprod Update 2012;18:555–67.
[22] Ouahchi I, Zhang L, Benitez Brito R, et al. Microarray-based comparative genomic hybridisation reveals additional recurrent aberrations in adult patients evaluated for myelodysplastic syndrome with normal karyotype. Br J Haematol 2019;184:282–7.
[23] Zhang C, Cervera E, Romanovitch M, et al. Array-based comparative genomic hybridization (aCGH). Methods Mol Biol 2017;1541:167–79.
[24] Malan V, Lapierre JM, Egloff M, et al. A French approach to test fetuses with ultrasound abnormalities using a customized microarray as first-tier genetic test. Cytogenet Genome Res 2015;147:103–10.
[25] Papoulidis I, Sotiriadis A, Siomou E, et al. Routine use of array comparative genomic hybridization (aCGH) as standard approach for prenatal diagnosis of chromosomal abnormalities. Clinical experience of 1763 prenatal cases. Prenat Diagn 2015;35:1269–77.
[26] Hillman SC, Pretlove S, Coomarasamy A, et al. Additional information from array comparative genomic hybridization technology over conventional karyotyping in prenatal diagnosis: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 2011;37:6–14.
[27] Sagi-Dain L, Maya I, Reches A, et al. Chromosomal microarray analysis results from pregnancies with various ultrasonographic anomalies. Obstet Gynecol 2018;132:1368–75.
[28] Karim S, Jamal HS, Rouzi A, et al. Genomic answers for recurrent spontaneous abortion in Saudi Arabia: an array comparative genomic hybridization approach. Reprod Biol 2017;17:153–43.