Integrated CNV-seq, Karyotyping and SNP-array Analyses for Effective Prenatal Diagnosis of Chromosomal Mosaicism

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Abstract

**Background:** Emerging studies suggest that low-coverage massively parallel copy number variation sequencing (CNV-seq) provide advantage in detecting low-level mosaicism compared with chromosomal microarray analysis (CMA). However, a prospective back-to-back comparison evaluating accuracy, efficacy, and incremental yield of CNV-seq compared with CMA is warranted.

**Methods:** A total of 72 mosaicism cases identified by karyotyping or CMA were recruited in this study, and 67 samples (40 sex chromosome aneuploidy, 22 autosomal aneuploidy and 5 submicroscopic CNVs) were eventually analyzed by CNV-seq.

**Results:** CNV-seq not only identified all 43 chromosomal aneuploidies or submicroscopic CNVs detected by CMA, but also provided a 34.88% (15/43) increased yield compared with CMA. Besides, the level of mosaicism defined by CNV-seq range from 6% to 92%.

**Conclusion:** In the context of prenatal diagnostic, CNV-seq identified additional and clinically significant information with enhanced resolution and increased sensitivity of detecting mosaicism as compared with the CMA platform we used. This study provides strong evidence for applying CNV-seq as an alternative prenatal diagnostic test.

**Background**

Chromosomal mosaicism is defined by the presence of two or more cell populations within the body and results from either gamete meiotic or mitotic cleavage-stage errors in the early preimplantation embryo[1]. Depending on the differentiation stages when the mosaicism occurred, the abnormal cells can reside only in extra-fetal tissues (e.g. the placenta), only in the fetus, or in both. Therefore, it has an important impact on the phenotype variability in first generation carriers but also on the recurrence risk and thus prenatal counselling[2].

Karyotyping, with a maximum resolution of 5 Mb, is referred as golden standard for identifying chromosomal abnormality in prenatal diagnosis for more than 50 years, the lower mosaicism detection limit of karyotype analysis has been reported as 19%[3] (conventional G-banded karyotype; 15 cells examined) and the mosaic pattern maybe caused by culture artifact. Chromosomal microarray (CMA) on uncultured cells from chorionic villus sampling or amniocentesis has gradually replaced conventional karyotyping for all prenatal diagnosis indications owing to a higher diagnostic yield, quicker turnaround time and elimination of cultural artifacts (pseudo mosaicism)[4]. Although it has been demonstrated to be a powerful tool to detect mosaicism at levels as low as 5%[5], but it is difficult to detect mosaicism in clinical research when the ratio is below 20% due to platform differences and specimen quality. Besides, the efficiency to detect the mosaicism of submicroscopic copy number variations (CNVs) is defined by the design and upgrade of probes which was based on the chromosomal abnormality and clinical information from public databases.
More recently, low-coverage massively parallel copy number variation sequencing (CNV-seq) is emerging as a higher-resolution and lower-costs technology in clinical research for detecting CNVs[6]. The CNV-seq is reported to detect structural abnormalities larger than 100 bp and aneuploid chimerism lower to 5%, which is more effective than CMA[7, 8]. However, no prospective back-to-back comparison study evaluating accuracy and efficacy of CNV-seq compared with CMA has been reported in routine prenatal diagnosis. Herein, we conducted a study to evaluate the diagnostic outcome and technical limitations of CMA and CNV-seq in detecting mosaicism.

**Methods**

**Study subjects**

A total of 72 mosaicism cases identified by karyotyping or CMA were recruited in this study, all samples were collected in the department of medical genetics of Hunan Provincial Maternal and Child Health Care Hospital from May 2018 to November 2019. The ages of the pregnant women range from 22 to 43. The application of prenatal diagnosis involves a range of fetus analyses, including screening tests, such as: advanced maternal age (>35 years)(AMA) (21/72, 29.17%), abnormal ultrasound structure (aUS)(13/72, 18.06 %); high-risk of maternal serum screening (hMSS) (19/72, 26.39%); high-risk of T21/T18/T13 in noninvasive prenatal screening (NIPS) (54/72, 68.91%); poor fertility histories (3/72, 4.17%). Notably, 38 cases were mixed indications, so the sum of aforementioned percentage was higher than 100%.

**Karyotyping**

The amniotic fluid and fetal cord blood were obtained under sterile conditions. G-banded (320-400 bands) karyotyping analyses were performed on 20 independent metaphases cells according to standard protocols. Karyotyping of at least 50 independent metaphases cells diagnosed the sample as mosaicism.

**CMA analysis**

Genomic DNA was extracted from amniocytes or fetal cord blood by using DNA Extraction Kit (Tissue and cells) and QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) separately. SNP array was performed using Affymetrix CytoScan®750 K Array (Affymetrix Inc, CA, USA), according to the manufacturers protocol. The theoretical values for the detection of a single copy gain or loss are described as previously reported [9, 10].

**CNV-seq analysis**

The DNA library was constructed as previously described[7]. Multiple libraries were indexed and pooled into a single lane and sequenced to 45 bp (with 8 bp being the index sequence) on the Nextseq CN500
instrument (Illumina, Inc.). Data analysis pipeline of sequencing reads and the percentage of chromosomal mosaicism were described as previously published [7, 8].

Results

A total of 72 subjects were initially enrolled in the study, which were generally categorized into three groups: autosomal aneuploidy (n=22, 30.55%); sex chromosome aneuploidy (n=40, 55.56%); submicroscopic CNVs (n=10, 13.89%). But five samples were excluded due to lack of enough DNA after CMA testing (Table S1). Therefore, 67 samples (40 sex chromosome aneuploidy, 22 autosomal aneuploidy and 5 submicroscopic CNVs) were eventually analyzed (Fig. 1).

Diagnostic concordance of CNV-seq and CMA vs karyotyping

Positive result of CMA was obtained on 63.08 % (41/65) samples which showed a mosaic pattern identified by karyotyping, of which, 37 samples showed a mosaic pattern with the level as low as 20% (Fig. 1). Besides, CMA detected additional mosaic trisomy 8 and mosaic partial trisomy 8 which was not detected by karyotyping in the cultured AF sample. In comparison, CNV-Seq not only identified all 43 chromosomal aneuploidies or submicroscopic CNVs detected by CMA, but also provided a 34.88% (15/43) increased yield compared with CMA(Figure 1). Besides, the level of mosaicism defined by CNV-seq range from 6% to 92%. The chromosomal map intervals, size, and copy number of the reportable mosaicism detected by both DNA-based techniques were almost identical. In addition, there was a 100% negative concordance of CNV-Seq for the 9 remaining samples diagnosed as normal by CMA.

Chromosomal mosaicism for autosomal aneuploidy

As showed in Figure 1, a total of 22 subjects were enrolled in the group, which involving 21 cases identified by karyotyping and 1 case identified by CMA. High-risk noninvasive prenatal screening (16/22) were the most prevalent diagnosis indications (Table 1). The relative frequencies of mosaic aneuploidies showed 54.54 % (12/22) for trisomy 21, 9.09 % (2/22) for trisomy 18, 9.09 % (2/22) for trisomy 15 and 27.27 % (6/22) for other autosomal aneuploidies.

13 of 22 cases were confirmed by CMA with the level as low as 20%, while consistent CNV-seq and chromosome results were noted in 19 cases with the identified mosaicism at levels as low as 5 % (Fig. 2). Of the 19 cases confirmed by CNV-seq, the percentages of cells for trisomy 21, 18 and 13 were in good agreement when CNV-seq and cytogenetics were compared. While in Case 16, Case 17, Case 18, Case 19, Case 22, the proportion of abnormal chromosome was much lower in culture samples compared with uncultured. Notably, in Case 18, the mosaic trisomy 8 was not detected in the cultured AF sample by metaphase analysis of 100 G-banded cells, while CMA and CNV-seq showed 24 % and 18 % trisomy 8 mosaicism, respectively (Supplemental Fig. S1). Of the remaining three cases (case 11, 20, 21), both CNV-
seq and CMA showed a normal result in uncultured amniotic fluid cells but karyotype showed a mosaic pattern of trisomy 21, trisomy 9 and trisomy 20 in cultured amniotic fluid cells, respectively.

**Chromosomal mosaicism for sex chromosome aneuploidy**

The 40 cases with sex chromosome aneuploidies accounted for 59.70 % (40/67) of total cases with a mosaic pattern identified by karyotyping. Clinical indications for these cases included abnormal ultrasound structure (aUS, n=6), advanced maternal age (AMA, n=11), high-risk of maternal serum screening (hMSS, n=12) or NIPS (n=33), and poor maternal history (n=2). Details of the chromosomes involved and the clinical course of the 40 pregnancies are presented in Table 1. The mosaic findings including twenty-five cases for monosomy X(45, X/46, XX), seven cases for monosomy X and trisomy X(45, X /47, XXX) or monosomy X and disomy X(45, X /47, XXY), five cases for disomy X(47, XXY /46, XY), one case for disomy Y(47, XYY /46, XY), one case for trisomy X(47, XXX /46, XX), and one case for both monosomy X, disomy X, and trisomy X(45, X /46, XX/47, XXX) are listed in Table 1.

Based on the karyotyping data, the percentage of monosomic or trisomic cells varied from 3.8 % to 92 % (Table 1). Of the 40 sex chromosome aneuploidies identified by karyotyping, 25 cases including mosaic pattern in 23 cases were confirmed by CMA with the mosaicism level as low as 20 %, while consistent CNV-seq and chromosome results were noted in 34 cases with the level as low as 8 %. Namely, the incremental yield of mosaicism less than 20% achieved by CNV-Seq was 22.5% (9/40). There was a 100% positive concordance between CMA and CNV-Seq for 23 samples. It should be noted that the proportion of monosomy X or disomy Y was more than 30% differences in culture samples compared with uncultured samples in 5 samples (Case 38, Case 47, Case 50, Case 54 and Case 55).

In addition, for the 9 incremental cases of mosaicism identified by CNV-seq, CNV-Seq showed the level of mosaicism was range from 8 % to 23 %. Besides, for Case 36 and Case 41, which showed 1.92 and 1.90 haploid equivalents of chromosome X for the amniotic fluid sample, CNV-seq of induced fetal placenta confirmed the placental mosaicism with chromosome X of 1.17-1.87 and 1.3-1.85 haploid equivalents (Supplemental Fig.S2).

In Case 23, 26, 27, 30, 32 and 61, both CNV-seq and CMA showed a normal result in uncultured amniotic fluid cells but karyotype showed a mosaic pattern of monosomy X or disomy X in cultured amniotic fluid cells. Among these cases, karyotype detected a mosaic pattern of monosomy X or disomy X less than 10% in 5 cases. The negative results of CMA and CNV-seq were most likely due to technical limitations.

**Chromosomal mosaicism for submicroscopic CNVs**

A total of 5 cases with a mosaic submicroscopic CNVs pattern were enrolled in the group. 4 out of 5 had a mosaic pattern with small supernumerary marker chromosome (sSMC) or unclarified derived chromosome identified by karyotyping were clarified the character, origin and pathogenicity of sSMC with
the help of CMA (Number 64, 65, 66, 67)(Supplemental Fig. S3-S7). Details of the chromosomes involved and the clinical course of the 5 pregnancies are presented in Table 1.

**Discussion**

Current prenatal practice showed mosaicism can involve in any chromosome and be presented as many types of chromosome abnormalities including trisomy, monosomy, triploidy, deletions, duplications, rings and other types of structural rearrangements. A clinical cytogenetics laboratory performing prenatal diagnosis should understand the limitations of cell-based chromosome analyses and DNA-based CNV-seq and CMA analysis on detecting mosaic aneuploidies and other submicroscopic CNVs.

To our knowledge, this is the first prospective back-to-back study evaluating the efficacy of CNV-seq in detecting mosaicism by using CMA and karyotyping as a reference. In our study, 72 of 5367 cases showed a mosaic pattern in prenatal diagnosis with 1.39% (67/4825) detection rates among AF samples and 0.92% (5/542) detection rates among CB samples. This rate is higher than previously reported chromosomal mosaicism rates of 1%-2% in CVS[11, 12] and 0.1%-0.5% in AF samples[13, 14]. The high overall prevalence of 1.34%(72/5367) could be explained by the use of CMA which has a higher resolution than conventional karyotyping and therefore detects the additional mosaicism for CNVs. Besides, among the 72 mosaic pattern fetuses, high-risk of NIPS (53/72, 68.91%) are the most common prenatal diagnosis indications, NIPS provides an important clinically indications that an increased number of cells should be examined and these extra cells need to be available with analysis often targeted to the chromosome/region of interest, which is also the reason for what most of the mosaicism identified by karyotyping rather than CMA. Thus, doctors should be aware of the possibility of low levels of mosaicism or confined placental mosaicism (CPM) when performing prenatal diagnosis to whom with a positive result of NIPS[15].

The current study demonstrated that CNV-seq provide an additionally diagnostic yield of 34.88% (15/43) compared to CMA, and can detect the level of mosaicism down to 5%. This is equal to the level reported by DNA models mimicking XXX and XO mosaicism[8] and supports the contention that CNV-seq is able to resolve lower levels of mosaicism than CMA. Although SNP arrays has been demonstrated to be a powerful tool to detect mosaicism at levels as low as 5% by using IlluminaQuad610 array[5], the detected rate is still variable among different CMA platform (9%-20% for array CGH[13, 16] and 30%-70% for Affymetrix arrays[17, 18]). Besides, it perform poorly on array CGH platforms with poor-quality, contaminated or fragmented DNA when compared with CNV-seq[19]. In addition, for submicroscopic CNVs mosaicism, the detected rate is not only due to size but also due to nonuniformly distributed probes of the CMA platform which was used[20]. Wang et al[21] had showed a variable probe density in the targeted region among different CMA platforms, and identified a pathogenic 298.7-kb deletion (affecting FBN2) by low-pass GS which was missed by CMA. This is indicating the advantages of applying low-pass GS for CNV analysis which is a technology relying on genome-wide uniformly distributed reads/windows.
Variable proliferation of cells with different karyotype under in vitro cell culture may also contribute to the inconsistent results between CNV-seq/CMA (uncultured samples) and karyotyping (cultured samples). Cell culture may promote the in vivo selection of euploid over aneuploid cells, which has been reported to increase with age of the culture[22]. In our study, the percentages of cells for trisomy 21, 18 and 13 were in good agreement when DNA-based CNV-seq or CMA and cell-based chromosome analyses were compared. While mosaic trisomy 15 (Case 16 and Case 17), trisomy 8 (Case 18), trisomy 2 (Case 22) and trisomy 22 (Case 19) were detected in the direct AF sample by CNV-Seq and CMA, however obviously lower levels of mosaicism was detected by karyotyping on cultured amniocytes which is consistent with the research reported previously[23–26]. It is feasible that the normal cells may have had a growth advantage in culture or the abnormal cell line may have a culturing disadvantage[13]. While increased proportions of monosomy X in karyotyping compared to CNV-seq, it is seems that 7 of 30 the monosomy X cell line have growth advantage than the normal cells or this deviations is caused by artificial counts. This highlights the advantage of using direct uncultured samples where a quicker result is possible and which can avoid artifact of culture and promote the accuracy of fetal outcome.

There are nine inconsistent results between CNV-seq (uncultured sample) and karyotype. In Case 27, the mosaic finding of monosomy X was confirmed by two independent prenatal clinics with the karyotype of 45,X[1]/46,XY[49] and 45,X[4]/46,XY[69], respectively. Thus, the negative results of CMA and CNV-seq were most likely due to technical limitations. Besides, a healthy 2870-g female baby was delivered with no phenotypic abnormality at 39 weeks of gestation. The postnatal blood karyotype was 46, XX. The abnormal chromosome confirmed by karyotyping in 5 cases (case 11, 23, 26, 30, 61) is consistent with results of NIPS. In case 20, 21 and 32, the levels of mosaicism detected by karyotyping were higher than 10%, which were less likely to be caused by culture artifact. Besides, studies[27, 28] has showed the discrepancy in the trisomy mosaicism level between cultured amniocytes and uncultured amniocytes in prenatally detected mosaic trisomy 20 and trisomy 9. Comprehensive evaluation of prevalent diagnosis indications and karyotype, it implies that the negative results of CNV-seq is more likely limited by technology with the chimerism level less than 5% in uncultured samples. In Case 23, the mosaic pattern of monosomy X detected in cultured amniocytes would not rule out a pseudo-mosaicism caused by culture artifact.

CNV-Seq is a low-read depth platform and unable to detect balanced translocations and inversions. Clarifying mosaic pattern result from structural chromosomal rearrangements should combine with chromosome analysis. A clinical cytogenetics laboratory performing prenatal diagnosis should understand the technical limitations of cell-based chromosome analyses and DNA-based CNV-seq and CMA analysis on detecting mosaic aneuploidies and other submicroscopic CNVs. Thus, the analysis and the interpretation of a mosaic pattern should be cautious in the prenatal diagnosis. Collectively, these validation studies confirm that CNV-seq is a quantitative NGS protocol with high sensitivity and reproducibility for also detecting chromosome mosaicism in prenatal diagnosis.

Conclusions
This study evaluated the effectiveness of CNV-Seq for detecting low-level mosaicism in prenatal diagnosis. The retrospective analysis found that CNV-seq identified additional and clinically significant information with enhanced resolution and increased sensitivity of detecting mosaicism (34.88%) as compared with the CMA platform we used. So it provides strong evidence for applying CNV-seq as an alternative prenatal diagnostic test for detecting mosaicism. Whilst the diagnosis of mosaicism in a prenatal setting remains challenging for scientists, we believe that combined use of cell-based chromosome analyses and DNA-based CNV-seq and CMA analysis would provide help in prenatal genetic diagnosis and counseling for mosaicism.

**Abbreviations**

CNV-seq: Copy Number Variation Sequencing; CMA: Chromosomal Microarray Analysis; CNV: Copy Number Variation; AMA: Advanced Maternal Age; aUS: Abnormal Ultrasound Structure; hMSS: High-risk of Maternal Serum Screening; NIPS: Noninvasive Prenatal Screening; sSMC: Supernumerary Marker Chromosome; CPM: Confined Placental Mosaicism.

**Declarations**

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**Authors’ contributions**

NM, HX, HW and JL had major roles in the design of the study. NM and JL drafted the manuscript. JP and YZ performed the molecular genetic experiments and analyzed the data. JH and RH performed the cytogenetic experiments and analyzed the data. JC, ZJ, YP, SY and HX analyzed the clinical data. HW and JL are corresponding authors of this manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**
The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

Written informed consent for prenatal genetic investigation was obtained from each study participant. For this retrospective study, there were no pre-study requirements on the patient’s specimens and clinical indications and there were no post-study interaction and intervention with the patients. This project was categorized as a chart review retrospective study and approved by the Ethics Committee of Hunan Provincial Maternal and Child Health Care Hospital (approved number 20180920-5).

**Consent for publication**

Written informed consent for publication of clinical and genetic data was obtained from all participants.

**Competing interests**

The authors declare no conflict of interest.

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**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**References**

1. A Kuliev, Y Verlinsky: Meiotic and mitotic nondisjunction: lessons from preimplantation genetic diagnosis. *Hum Reprod Update* 2004, 10:401-7.

2. L Castera, M Gauthier-Villars, C Dehainault, D Michaux, A Benachi, RL Lumbroso-Le, D Stoppa-Lyonnet, C Houdayer: Mosaicism in clinical practice exemplified by prenatal diagnosis in retinoblastoma. *Prenat Diagn* 2011, 31:1106-8.
3. EB Hook: Exclusion of chromosomal mosaicism: tables of 90%, 95% and 99% confidence limits and comments on use. *Am J Hum Genet* 1977, 29:94-7.

4. H Wang, Z Dong, R Zhang, M Chau, Z Yang, K Tsang, HK Wong, B Gui, Z Meng, K Xiao, et al: Low-pass genome sequencing versus chromosomal microarray analysis: implementation in prenatal diagnosis. *Genet Med* 2020, 22:500-510.

5. LK Conlin, BD Thiel, CG Bonnemann, L Medne, LM Ernst, EH Zackai, MA Deardorff, ID Krantz, H Hakonarson, NB Spinner: Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. *Hum Mol Genet* 2010, 19:1263-75.

6. H Wang, Z Dong, R Zhang, M Chau, Z Yang, K Tsang, HK Wong, B Gui, Z Meng, K Xiao, et al: Low-pass genome sequencing versus chromosomal microarray analysis: implementation in prenatal diagnosis. *Genet Med* 2020, 22:500-510.

7. D Liang, Y Peng, W Lv, L Deng, Y Zhang, H Li, P Yang, J Zhang, Z Song, G Xu, et al: Copy number variation sequencing for comprehensive diagnosis of chromosome disease syndromes. *J Mol Diagn* 2014, 16:519-526.

8. Y Wang, Y Chen, F Tian, J Zhang, Z Song, Y Wu, X Han, W Hu, D Ma, D Cram, et al: Maternal mosaicism is a significant contributor to discordant sex chromosomal aneuploidies associated with noninvasive prenatal testing. *Clin Chem* 2014, 60:251-9.

9. J Liu, H Hu, N Ma, Z Jia, Y Zhou, J Hu, H Wang: A de novo duplication of chromosome 9q34.13-qter in a fetus with Tetralogy of Fallot Syndrome. *Mol Cytogenet* 2016, 9:54.

10. X Zhu, J Li, T Ru, Y Wang, Y Xu, Y Yang, X Wu, DS Cram, Y Hu: Identification of copy number variations associated with congenital heart disease by chromosomal microarray analysis and next-generation sequencing. *Prenat Diagn* 2016, 36:321-7.

11. T Eggermann, L Soellner, K Buiting, D Kotzot: Mosaicism and uniparental disomy in prenatal diagnosis. *Trends Mol Med* 2015, 21:77-87.

12. TH Taylor, SA Gitlin, JL Patrick, JL Crain, JM Wilson, DK Griffin: The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans. *Hum Reprod Update* 2014, 20:571-81.

13. L Carey, F Scott, K Murphy, N Mansfield, P Barahona, D Leigh, R Robertson, A McLennan: Prenatal diagnosis of chromosomal mosaicism in over 1600 cases using array comparative genomic hybridization as a first line test. *Prenat Diagn* 2014, 34:478-86.

14. LY Hsu, MT Yu, KE Richkind, DL Van Dyke, BF Crandall, DF Saxe, GS Khodr, M Mennuti, G Stetten, WA Miller, et al: Incidence and significance of chromosome mosaicism involving an autosomal structural abnormality diagnosed prenatally through amniocentesis: a collaborative study. *Prenat Diagn* 1996, 16:1-28.

15. RV Lebo, RW Novak, K Wolfe, M Michelson, H Robinson, MS Mancuso: Discordant circulating fetal DNA and subsequent cytogenetics reveal false negative, placental mosaic, and fetal mosaic cfDNA genotypes. *J Transl Med* 2015, 13:260.
16. BC Ballif, EA Rorem, K Sundin, M Lincicum, S Gaskin, J Coppinger, CD Kashork, LG Shaffer, BA Bejjani: Detection of low-level mosaicism by array CGH in routine diagnostic specimens. *Am J Med Genet A* 2006, 140:2757-67.

17. IP Pinto, LB Minasi, R Steckelberg, SC Da, CA Da: Mosaic Tetrasomy of 9p24.3q21.11 postnatally identified in an infant born with multiple congenital malformations: a case report. *Bmc Pediatr* 2018, 18:298.

18. FR Zahir, MA Marra: Use of Affymetrix Arrays in the Diagnosis of Gene Copy-Number Variation. *Curr Protoc Hum Genet* 2015, 85:8.13.1-8.13.13.

19. K Cohen, A Tzika, H Wood, S Berri, P Roberts, G Mason, E Sheridan: Diagnosis of fetal submicroscopic chromosomal abnormalities in failed array CGH samples: copy number by sequencing as an alternative to microarrays for invasive fetal testing. *Ultrasound Obstet Gynecol* 2015, 45:394-401.

20. JC Wang, J Radcliff, SJ Coe, LW Mahon: Effects of platforms, size filter cutoffs, and targeted regions of cytogenomic microarray on detection of copy number variants and uniparental disomy in prenatal diagnosis: Results from 5026 pregnancies. *Prenat Diagn* 2019, 39:137-156.

21. JC Wang, J Radcliff, SJ Coe, LW Mahon: Effects of platforms, size filter cutoffs, and targeted regions of cytogenomic microarray on detection of copy number variants and uniparental disomy in prenatal diagnosis: Results from 5026 pregnancies. *Prenat Diagn* 2019, 39:137-156.

22. GP Nowinski, DL Van Dyke, BC Tilley, G Jacobsen, VR Babu, MJ Worsham, GN Wilson, L Weiss: The frequency of aneuploidy in cultured lymphocytes is correlated with age and gender but not with reproductive history. *Am J Hum Genet* 1990, 46:1101-11.

23. CP Chen, SR Chern, YN Chen, PS Wu, CW Yang, LF Chen, W Wang: Mosaic trisomy 15 at amniocentesis: Prenatal diagnosis, molecular genetic analysis and literature review. *Taiwan J Obstet Gynecol* 2015, 54:426-31.

24. CP Chen, CY Hsu, SR Chern, PS Wu, SW Chen, W Wang: Prenatal diagnosis of mosaic trisomy 8 by amniocentesis in a fetus with ventriculomegaly and dysgenesis of the corpus callosum. *Taiwan J Obstet Gynecol* 2020, 59:127-129.

25. CP Chen, YN Su, SR Chern, YT Chen, PS Wu, JW Su, CW Pan, W Wang: Mosaic trisomy 2 at amniocentesis: prenatal diagnosis and molecular genetic analysis. *Taiwan J Obstet Gynecol* 2012, 51:603-11.

26. CP Chen, MC Huang, SR Chern, PS Wu, SW Chen, TY Chuang, DD Town, W Wang: Mosaic trisomy 22 at amniocentesis: Prenatal diagnosis and literature review. *Taiwan J Obstet Gynecol* 2019, 58:692-697.

27. CP Chen, SD Chang, HY Chueh, YN Su, SR Chern, JW Su, YT Chen, WL Chen, MS Lee, W Wang: Discrepancy in the trisomy mosaicism level between cultured amniocytes and uncultured amniocytes in prenatally detected mosaic trisomy 20. *Taiwan J Obstet Gynecol* 2013, 52:145-6.

28. CP Chen, HM Lin, YN Su, SR Chern, FJ Tsai, PC Wu, CC Lee, YT Chen, MS Lee, CW Pan, et al: Mosaic trisomy 9 at amniocentesis: prenatal diagnosis and molecular genetic analyses. *Taiwan J Obstet Gynecol* 2019, 58:692-697.
Table

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.