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# Identification of minor chromosomal defects causing abnormal foetus and spontaneous abortions

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**ABSTRACT**

**Background**: Chromosomal abnormalities are the most common cause of recurrent abortions and miscarriages (RAM), but micro-variations on chromosomes causing RAM have never been previously studied. Single nucleotide polymorphisms (SNPs) are the single nucleotide variations frequently present at genome with the density of at least one common (>20% allele frequency) SNP per kilobase pair. It has already been reported that SNP array examination for chromosomal abnormalities has better performance than the conventional cytogenetic karyotyping.

**Methods:** We applied SNP array to detect the chromosomal defects in 80 placental villi and foetal tissues of abnormal foetus and spontaneous abortions.

**Results:** The analyses of data revealed that total 52.5% (42/80) cases were found to have chromosomal abnormalities. The trisomies were most commonly found 26/42 (61.9%) in current samples. Total 8/42 (19.1%) cases were found to have other structural aberrations including translocations in 2/8 (25%), duplications and deletions in 3/8 (37.5%) cases, respectively. SNP analysis also successfully detected triploidy 69,XXX and tetraploidy 92,XXXY. Total 12/80 cases were performed by cytogenetic karyotyping and results were compared with SNP data. Total 5/12 (41.7%) cases were found to have same findings with SNP data while results of 2/12 (16.7%) cases had partial similarity between both techniques. Four cases were declared as karyotypically normal (46,XY or 46,XX) by cytogenetic examination, but later on these four cases were found to have small chromosomal variation which could be the cause of RAM in women.

**Conclusion:** Therefore, we conclude that use of a high-density SNP platform in diagnosis can give better understanding of molecular causes of pregnancy loss and foetal abnormalities.

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Pregnancy loss; chromosomal abnormalities; SNP array; karyotyping; foetal diagnosis

## Introduction

The complications of pregnancy include recurrent and sporadic miscarriages, and it has been estimated that about 15% of all clinically recognised pregnancies terminated in spontaneous loss, as pregnancy failures occur prior to their clinical recognition, only 30% of all pregnancies end in a live birth.[1,2] Recurrent miscarriages are defined as the occurrence of three consecutive pregnancy losses within 20 weeks after conception and its occurrence is 2–5% worldwide.[3] However, the causes of recurrent miscarriage are still not well understood. Several contributing factors have been identified previously such as genetic anomalies, pathological placental conditions, maternal thrombophilia, and infections. Although there are multiple contributing factors of recurrent miscarriage, chromosomal abnormalities contribute more than 50% in first trimester and one third in second trimester's miscarriages.[4,5]

Cytogenetic examination of the pregnancy loss completely relies on the conventional chromosomal karyotyping. However, this technique has some limitations such as time consuming, failure of cell culture, maternal contamination and ambiguity in the results.[6] Other molecular cytogenetic methods can avoid some of these pitfalls. Fluorescence *in situ* hybridisation (FISH), quantitative fluorescence polymerase chain reaction and multiplex ligation-dependent probe amplification act as rapid techniques which do not require cell culturing and are capable of reducing experimental time.[7–10] Moreover, these methods still have some disadvantages, such as being unable to obtain information about the whole genome because used probes and primers can only target a selected region of chromosomes or only specific sub-telomeric loci.

In the last few years, array-based techniques have been introduced for genome-wide scan for the detection of unbalanced genomic aberrations at higher resolution without cell culturing. Among these array-based platforms, single nucleotide polymorphism arrays (SNP arrays) have highest resolution (5–10 kb) as well as are

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able to acquire further information.[11] Although the advantages of SNP array on conventional cytogenetic karyotyping have already been well explained, there is no previous data available regarding micro-variation on chromosomes causing recurrent abortions and miscarriages in women. We therefore applied SNP array technology to identify potential mico-defects on chromosomes associated with pregnancy loss and spontaneous abortion.

## Methods

A total of 80 placental villi and foetal tissue samples were collected from miscarried and abnormal foetuses, during September 2013 to June 2015 at Maternal and Child Health Hospital Hefei, Anhui, PR China. G banded karyotyping was performed for 12 cases and then compared with SNP array data of same samples. DNA was extracted from tissue samples by DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). The SNP array analysis was performed by using HumanCytoSNP-12, Illumina – HCS (San Diego, CA, USA) platform which includes ~300000 markers genome-wide tag SNPs and markers targeting all regions of known cytogenetic abnormalities. This included dense coverage of around 250 genomic regions commonly screened in cytogenetic laboratories, including sub-telomeric regions, peri-centromeric regions, sex chromosomes and targeted coverage in around 400 additional disease-related genes (http://www.illumina.com). We used 200ng of DNA as an input for a single array. DNA amplification, tagging and hybridisation assays were performed according to the manufacturer's protocols. The array slides were scanned on HiscanSQ (Illumina, USA), and the analyses of data were performed by using GenomeStudio version 2010.1. (Illumina, standard settings). The HapMap control data of Han Chinese was used as control of this study. Samples were assessed for genotype and copy number using the B allele frequency (BAF) which was examined by Illumina GenomeStudio software. The BAF is a value between 0 and 1, and represents the proportion contributed by one SNP allele (B) to the total copy number. A BAF value of 0.5 indicates a heterozygous genotype (AB), whereas 0 and 1 indicate homozygous genotypes (AA and BB, respectively). For example, a region with a deletion in all cells will show homozygous bands at 1 and 0. A region of single-copy-number gain in all cells, in addition to the two bands of homozygous SNPs at BAF¼0 (AAA) and BAF¼1 (BBB), also show two bands: one at BAF¼0.33 with SNPs having genotype AAB and one at BAF¼0.67 with SNPs having genotype ABB.

Informed written consents were taken from all participants for the use of their placental villi and foetal tissue samples for current analyses. All the experimental and sampling procedures were approved by Ethical review Committee of Maternal and Children Health Hospital Hefei and Anhui Medical University, Anhui, PR China. All the procedures were performed in accordance with the ethical standard of 1964 Helsinki declaration and its later amendments or comparable ethical standards.

## Results

Initially, we performed cytogenetic karyotyping for 12 cases and found 4 cases with normal karyotype 46,XY ($n$ = 2) and 46,XX ($n$ = 2) which were later identified as partial deletions or partial duplications by SNP array (Table 1), which can be considered as causes of abnormal foetus and pregnancy loss in these cases. Trisomy 8 (47,XX,+8) was identified in one case but partial duplication was also on chromosome X in addition to (47,XX,+8) by SNP assay. One case has shown partial duplication on chromosome 16 [46,XY, dup (16)(q23)] and subsequently found partial deletion on chromosome 15 by SNP array. Cell culturing of one case failed, but later SNP array identified it as tetraploid 92,XXXY (Figure 1). Both techniques have shown same results for remaining 3 cases (69,XXX, trisomy 9 and trisomy 21) (Figure 2, 69,XXX Karyotype and BAF of Chr.1 and X). Interestingly, two cases have shown similar chromosomal abnormalities by both techniques, but SNP also provided more detailed information of the chromosomal abnormalities than the cytogenetic karyotype. Cytogenetic karyotyping identified 46,XY,dup(16)(q23) and 46,XX,der(5)t(5;7)(p15;q34) while more precise information of defect was obtained by SNP (Table 2). Overall, both techniques showed 5/12 (41.7%) similarity while 2/12 (16.7%) cases had partial similarity between both techniques.

The SNP analyses of 80 placental villi and foetal tissues of abnormal foetus and spontaneous abortions revealed that 38/80 (47.5%) had normal chromosomal configuration while various chromosomal abnormalities

**Table 1.** SNP array data revealed micro-variations on chromosomes causing abnormal foetus and pregnancy loss in Han Chinese women.

| Serial No | SNP array studies | | Karyotype studies |
|---|---|---|---|
| | Results | The size of abnormal fragment | |
| 1 | arr8p23.3p23.1(176,818–6,974,050)×1 | 6.79 Mbp | 46,XY |
| 2 | arr6q11.1(61,891,118–62,965,057)×3 | 1.07 Mbp | 46,XX |
| 3 | arr17p12(14,101,029–15,449,627)×1 | 1.34 Mbp | 46,XY |
| 4 | arr4q35.1q35.2(183,935,289–190,880,409)×1 | 6.94 Mbp | 46,XX |

*Note.* p, the short arm of a chromosome; q, the long arm of a chromosome; the number on the right of 'arr' represent the location of the fragment; ×1, ×3 and ×4 represent copy number.

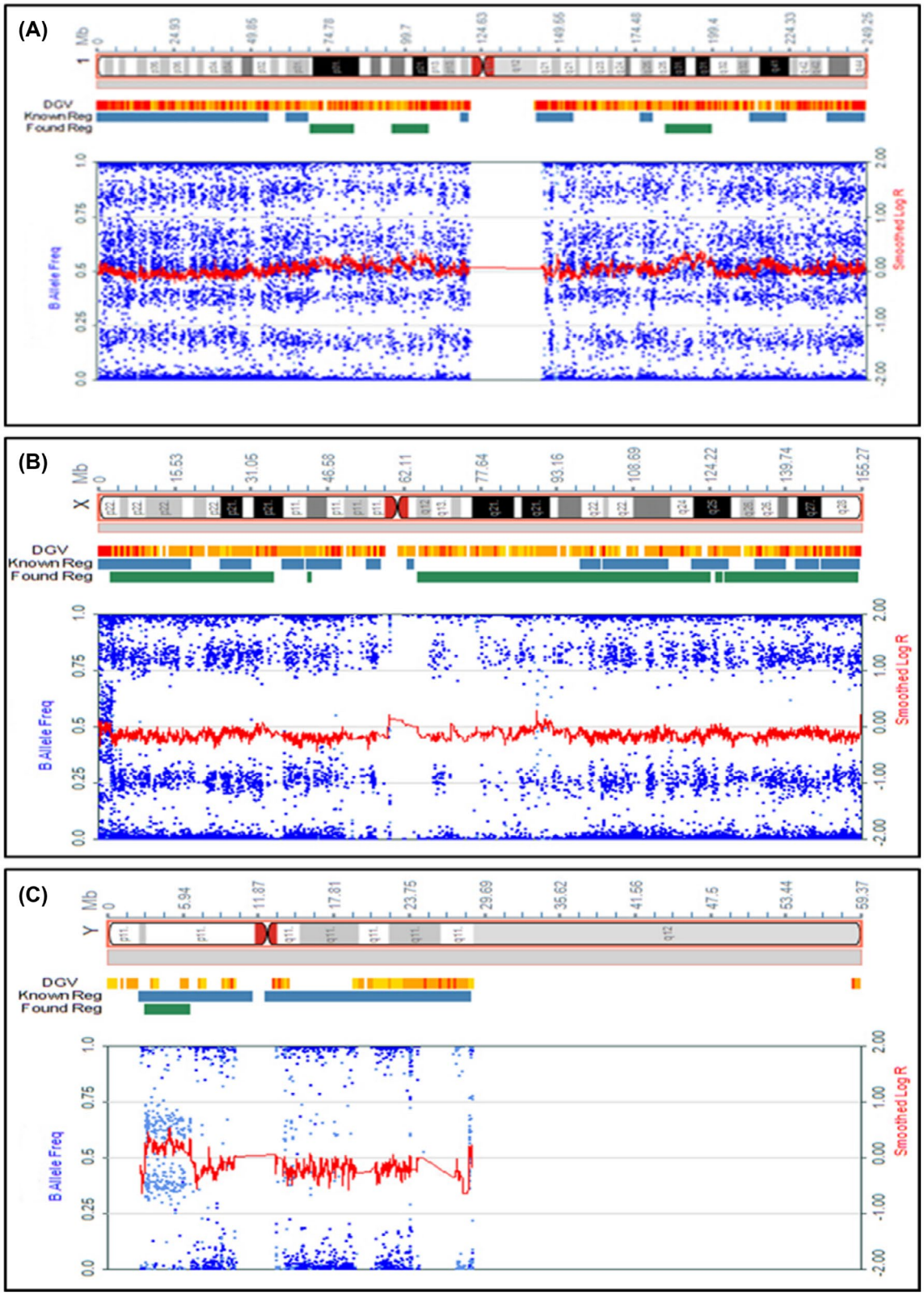


Figure 1. Tetraploid 92,XXXY Foetus Diagnosed by SNP array. (A) Indicating abnormal BAF of the chromosome 1 (B) BAF of chromosome X and (C) showing BAF of Y chromosome of the Tetraploid 92,XXXY Foetus. Red lines show logR ratio and blue data points represent the BAF of each individual SNP. All BAF showing signals for two extra set of the chromosome.

were observed in 52.5% (42/80) cases. The trisomies were found (61.9%) as a most common aneuploidies in the studied cases, which were identified on different chromosomes, including 7/26 (26.9%) cases of trisomy (4,8,9,12,14,15,X), 2/26 (7.7%) cases of trisomy 13, 3/26(11.5%) cases of trisomy 18, 4/26 (15.4%) cases of trisomy 16 and 7/26 (26.9%) cases of trisomy 21, while monosomy X was found in 4/42 (9.5%) samples (Table 3). Total 8/42 (19.1.%) cases were found to have other structural aberrations including translocations in 2/8 (25%), duplications and deletions in 3/8 (37.5%) cases, respectively (Figure 3, selected BAF of

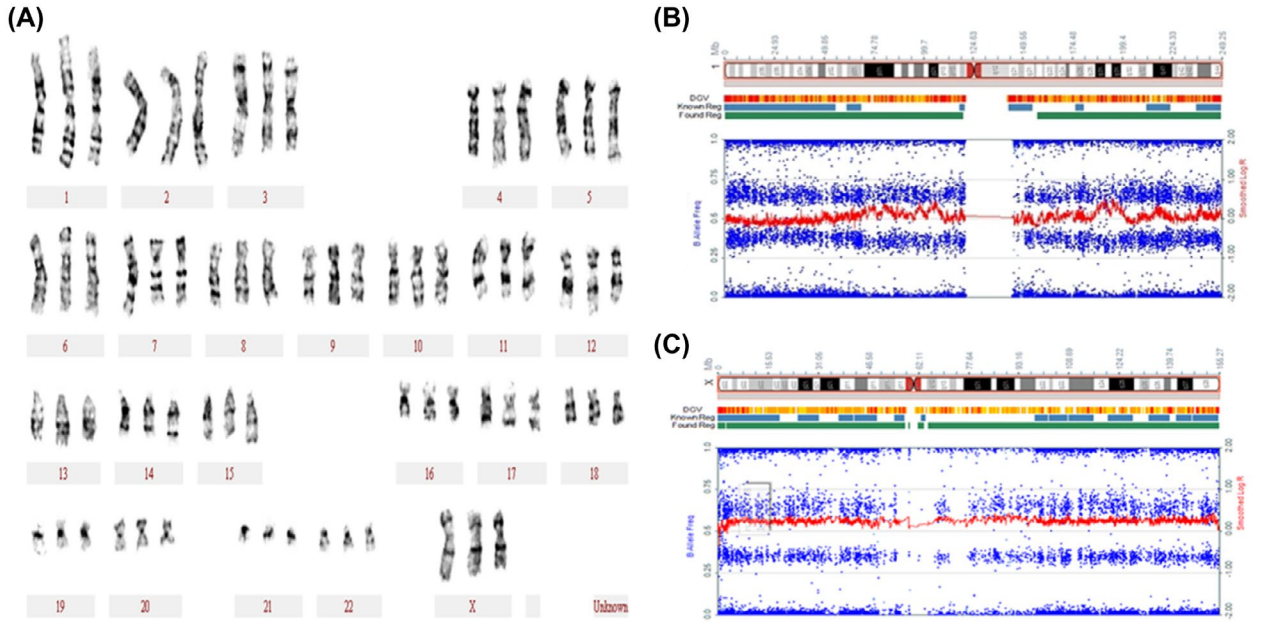


Figure 2. Triploid 69,XXX Foetus Detected by Cytogenetic Karyotyping and SNP array. (A) Karyotype picture of cytogenetic analysis showing triploidy 69,XXX (B) BAF of the chromosome 1 and (C) chromosome X of the triploid foetus detected by SNP array. Red lines show logR ratio and blue data points represent the BAF of each individual SNP.

Table 2. Comparative SNP array and conventional cytogenetic analyses for chromosomal abnormalities in cases with spontaneous abortions.

| | SNP array studies | | |
|---|---|---|---|
| Serial No | Results | The size of abnormal fragment | Karyotype studies |
| 1 | arr13q31.3q34(91,007,492–115,106,996)×3 | 24.1Mbp | 46,XX,dup(13)(q31q34) |
| 2 | arr5p15.33p15.32(38,139–5,723,290)×1,7q34q36.3(139,695,316–159,119,486)×3 | 5.68Mbp/19.42Mbp | 46,XX,der(5)t(5;7)(p15;q34) |
| 3 | arr8p23q24(176,818–146,293,086)×3,Xp22.31(6,516,735–8,131,442)×3 | Trisomy 8/1.61Mbp | 47,XX,+8 |
| 4 | arr15q26.3(99,168,770–102,397,836)×1,16q23.1q24.3(78,033,371–90,148,796)×3 | 3.22Mbp/12.1Mbp | 46,XY,dup(16)(q23) |
| 5 | arr(1–22,X)×3 | Triploid | 69,XXX |
| 6 | arr(1–22)×4,(XXXY)×1 | Tetraploid | Culture failure |
| 7 | arr(9)×3 | Trisomy 9 | 47,XX,+9 |
| 8 | arr(21)×3 | Trisomy 21 | 47,XX,+21 |

p, the short arm of a chromosome; q, the long arm of a chromosome; the number on the right of 'arr' represent the location of the fragment; ×1, ×3 and ×4 represent copy number.

Table 3. Abnormal molecular karyotype detected by SNP array in 80 placental villi and foetal tissues.

| Abnormalities detected | *N* (%) |
|---|---|
| Trisomies | 26 (61.90%) |
| Monosomy X | 4 (9.54%) |
| Triploidy | 2 (4.76%) |
| Tetraploidy | 2 (4.76%) |
| Structural aberrations | 8 (19.05%) |
| Total | 42 |

monosomy X, Trisomy 16, deletion at Chr.15, duplication at Chr. 16). Chromosomal structural aberrations were found in the form of deletion on single chromosome or duplication on one or two chromosomes. The gestational ages of the foetuses ranged from 6 to 31 weeks and the ages of the women whose pregnancies failed range from 21 to 39 years (average age 27.8 years).

## Discussion

Genetic analyses of foetuses are important for the determination of pathogenic abnormalities of the chromosomes. Several technologies were tested to obtain the best performance and better results, but an SNP array is considered as reliable and affordable.

In a previous study, researchers compared the findings of array-based comparative genomic hybridisation (aCGH) analyses of 100 miscarriages with both G-banding karyotyping and FISH results. Although a CGH achieved a highest detection rate, it still missed triploid cases which were further detected by other techniques.[12] Array-based CGH analysis was applied to the affected infant whose parents were normal; the analysis identified a combination of 18p deletion and 7q duplication.[13]

The SNP array analysis in current study identified two cases with triploid 69,XXX, but we did not know the exact inheritance pattern of extra haploid set of chromosome. The current analysis of SNP array also identified a case of tetraploidy but we were unable to confirm its exact type. There are two types of tetraploidy; one is 2:2 tetraploidy, which normally occur due to the failure of cytokinesis at all the chromosomes and both parents contribute equally in inheritance of full extra set of chromosome to foetus,

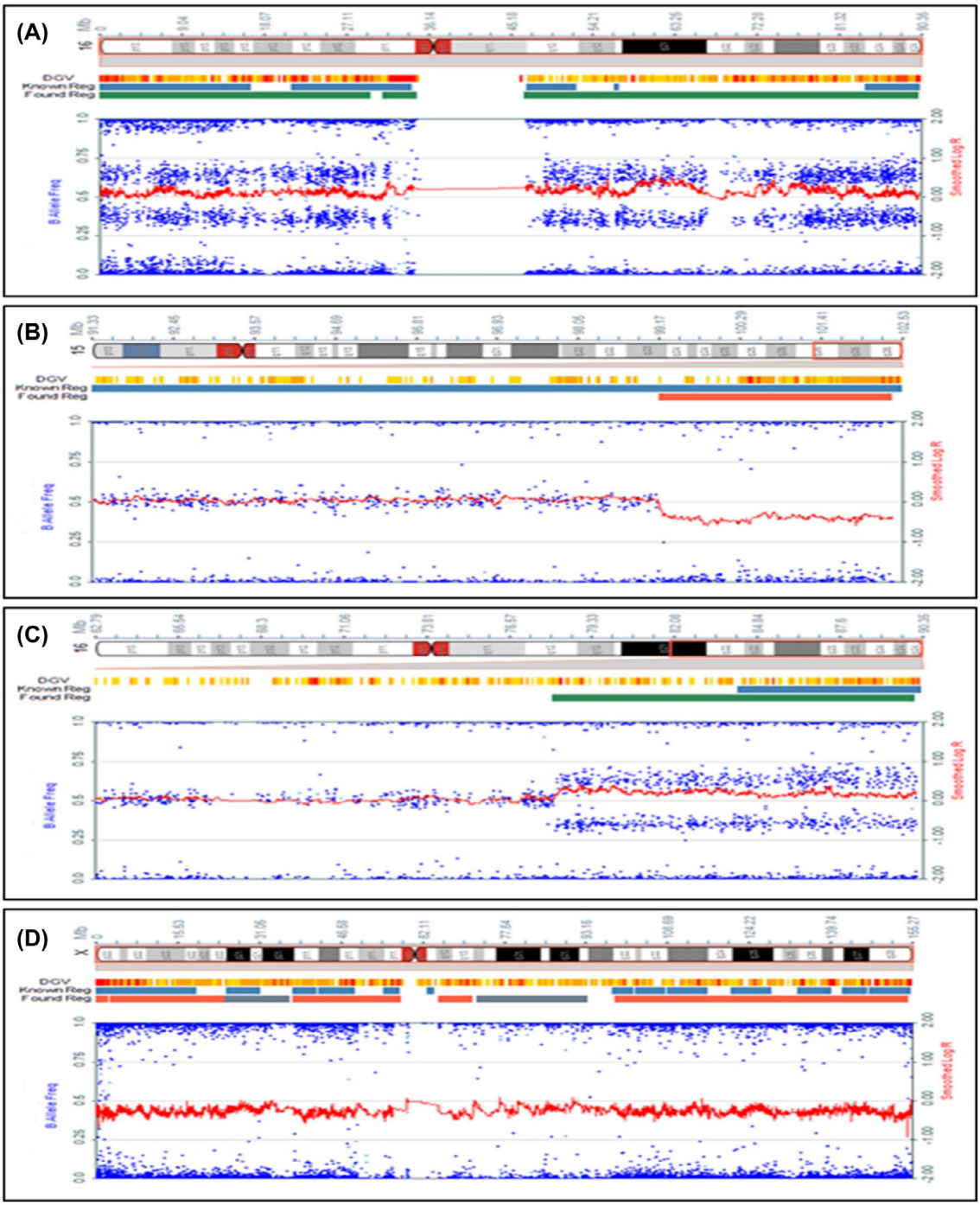


Figure 3. BAF of different chromosomal abnormalities detected in the placental villi and foetal tissues of abnormal foetus and abortion. SNP array successfully detected (A) monosomy X, (B) Trisomy 16 (C) deletion of q26.3 at chr 16 and (D) duplication q23.1q24.3 at chromosome 16. Red lines show logR ratio and blue data points represent the BAF of each individual SNP.

while the other type of tertraploidy is that in which 3 sets of chromosome are contributed from one parent while one chromosome from other parental counterpart.[14] We did not use parents' samples for SNP array analysis because our main aim was to detect the chromosomal defects in the material of pregnancy loss and abnormal foetus. G banding analysis revealed trisomy 18, trisomy 21 and 45,X were the most common aneuploidies identified in CHD foetuses, while Affymetrix SNPArray 6.0 was used to detect copy number variation.[15] The karyotype 46,XY,der (18) t (18;21)(q10; q10) was found in cultured amniotic cells which was compatible to a male foetus with trisomy of long arm of chromosome 18.[16] After the identification common trisomies in 29 cases, authors concluded that low-coverage whole-genome sequencing of maternal plasma DNA is highly accurate for the detection of common trisomies.[17] Array CGH confirmed the partial trisomy on short arm of chromosome 2 and partial monosomy of the long arm of chromosome 13 in abnormal foetus.[18] SNP array analysis was use to analyse the copy number variation in abnormal foetus.[19]

A similar study has been conducted previously which reported several chromosomal abnormalities in aborted

**Table 4.** Summary.

| |
|---|
| What is known about this subject: |
| • Traditional cytogenetic techniques has several limitations therefore are only partially useful in identifying chromosomal abnormalities |
| • Single nucleotide polymorphism microarray (SNP array) found less time consuming and efficient than other conventional techniques used for identification of chromosomal abnormalities |
| What this paper adds: |
| • SNP array identified micro variations on chromosomes causing of RAM in women |
| • SNP analysis provided more precise information about exact location, size of the breakpoint on the chromosome |
| • Current SNP analysis identified chromosomal rearrangements such as translocations, insertion and inversions which were not detected previous array analyses |

tissues.[20] Most common aneuploidies were monosomies, trisomies and triploidy. In current study, we performed 12 karyotyped samples by SNP to find accuracy difference. Both techniques have 5/12 (41.7%) of similarity in results while (16.7%) of the cases show partial similarity. SNP analysis gave more precise information of the chromosomal defects, like exact location, size of the breakpoint and exact location on the chromosome, and even minor changes on chromosomes leading to abnormal foetus and pregnancy loss. Interestingly, our SNP analysis identified chromosomal rearrangements such as translocations, insertion and inversions which were not detected previously[20]; this could be due to the difference of SNP chip density or analysis errors. Lathi et al.[13] studied time difference between SNP analysis and Karyotyping and found SNP is quicker than the Karyotyping. In current study, we successfully identified Tatraploidy 92,XXXY by SNP analysis while five cases identified as normal by cytogenetic karyotype were found to have deletion or duplication at chromosomes. These deletions and duplications can be associated with the pregnancy loss in the studied patients. Secondly, for two cases SNP and cytogenetic identified same major defect of chromosomes but SNP data also provide extra information of the minor defects present on the other chromosomes (see Table 4). As it is earlier known that trisomies have high frequency in aborted tissues, similar finding are generated by our SNP analysis, and chromosomal structural aberrations are second leading cause of recurrent abortion in our tested samples.

Current analysis was done on only 80 cases which are not enough for the generalisation of results. Therefore, we suggest a large-scale studies would give more precise information about the minor chromosomal defects leading to pregnancy loss.

Short-CGH technique detected chromosomal abnormalities, among these aneuploidy and structural chromosomal errors were found more frequently in Robertsonian than in reciprocal translocation carriers.[21] Combined analysis by Array-based CGH and karyotyping techniques revealed that unbalanced chromosomal translocations are inherited from balanced translocation carrier parents.[22] PGD FISH detected unbalanced chromosomal translocations cleavage-stage embryos, further SNP CN analysis of embryos identified same unbalanced translocations in blastomeres.[23] The results of our current study are consistent with previous studies; we identified translocations on various chromosomes in samples obtained from abnormal foetal tissues and pregnancy loss. This study represents an advance in biomedical science because it highlights new aspect of using SNP data in routine diagnosis of abnormal foetus, pregnancy loss and other genetic diseases.

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## Disclosure statement

All contributing authors declare that they have no conflict of interest.

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