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Original Article

Retrospectively investigating the 12-year experience of prenatal diagnosis of small supernumerary marker chromosomes through array comparative genomic hybridization

Min-Hui Huang [a, b, c], Cagge Lee [b, c], Jia-Shyuhn Chang [b, c], Han-Chow Wang [b, c], Hui-Ling Lai [b, c], Chu-Chu Chang [b, c], Tzu-Wang Chen [b, c], Yu-Fen Li [b, c], Ting-Tse Lin [b, c], Chih-Yun Yang [b, c], Shu-Peng Ho [a, *]

[a] Department of Veterinary Medicine, National Chung Hsing University, Taichung, Taiwan
[b] Youthgene Medical Laboratory, Taipei, Taiwan
[c] Dr. Lee Woman Clinic, Taipei, Taiwan

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ABSTRACT

Objective: This study retrospectively evaluated the incidences of small supernumerary marker chromosomes (sSMCs) in prenatal diagnoses and detected with gain of pathogenic copy number variation through array comparative genomic hybridization (CGH) in a laboratory in Taiwan.
Materials and methods: We retrospectively searched and reviewed the sSMC cases detected during prenatal diagnoses in the Youthgene medical laboratory, between 2004 and 2015 and used array CGH to successfully analyze 45 of 47,XN,+mar or 47,XN + mar/46,XN.
Results: A total of 68,087 cases of amniocentesis were analyzed, of which 59 were identified as sSMCs. The overall frequency of sSMCs was 0.087%, and 7 of 45 sSMCs were identified with gain of pathogenic copy number variation (CNV).
Conclusion: Array CGH offers useful tools that can be used to detect small fragments of chromosomal abnormalities and sSMC origins in prenatal diagnosis. In this study, we successfully used array CGH to detect 7 out of 45 sSMCs, which were identified with gain in pathogenic CNV.

Introduction

The small supernumerary marker chromosome (sSMC) is a structurally abnormal chromosome that cannot be uniquely identified or characterized using conventional banding methods alone [1]. The sSMC is generally equal in size to or smaller than chromosome 20 in the same metaphase spread with highly variable cytogenetic morphology; elucidating the chromosomal origin and phenotype implications is difficult [2,3].

Studies have reported detection of sSMCs in 0.075% of unselected prenatal cases and 0.044% of postnatal cases [4]; 0.043% of unselected amniocentesis cases have *de novo* sSMCs [5]. When the sSMC is present in prenatal cases, it suggests that the fetus might be born with clinical phenotype. sSMCs including euchromatin are generally associated with abnormal phenotypes, whereas sSMC without euchromatin tends to be correlated with normal phenotypes [6–8]. However, the existence of euchromatin cannot be characterized through conventional cytogenetic testing.

Therefore, in prenatal diagnosis, identifying the genotype–phenotype correlations is challenging, especially in fetuses with *de novo* sSMC. Recently, array comparative genomic hybridization (CGH) has been considered a powerful diagnostic tool and has been frequently applied in prenatal sSMC diagnoses to help define the origin of sSMCs [9–11].

Here, we report a 12-year survey of prenatal sSMCs on the basis of 68,087 diagnoses collected from a single cytogenetic laboratory in Taiwan. We analyzed the overall frequency of sSMCs in amniotic fluid and investigated the correlation between the incidence of sSMCs, indications for invasive prenatal diagnosis, and chromosome origin of sSMCs. Our experience suggests that applying array CGH is valuable for improving prenatal genetic

* Corresponding author. Department of Veterinary Medicine, National Chung Hsing University, 250 KuoKuang Road, Taichung 402, Taiwan.
E-mail address: spho@dragon.nchu.edu.tw (S.-P. Ho).

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Table 1
Incidences of sSMCs with different indications for amniocentesis.

sSMC	No. of cases/total cases	AMA (%)	Abnormal biomedical markers (%)	Abnormal ultrasound findings (%)	Family history (%)	Others (%)	Frequencies %
All	59/68,087	42/47,942 (0.088)	6/8533 (0.070)	2/4182 (0.048)	2/652 (0.307)	7/6778 (0.103)	0.087
Inherited	8	4	1	0	1	2	0.012
De novo	40	29	5	2	1	3	0.059
NA[a]	11	9	0	0	0	2	0.016

AMA, advanced maternal age (≧35 years old); abnormal biomedical markers, increased-risk maternal triple-marker Down screening test (≧1/270); family history, family history of chromosomal abnormality.
[a] Inheritance not available.

Table 2
Percentages of cells with sSMCs and inheritance in 59 detected cases.

Case No.	aCGH No.	Karyotype	Inheritance	[a]Percentage of cells with sSMC
1	1	47,XY,+mar	*de novo*	100
2	2	47,XX,+mar	paternal	100
3	3	47,XX,+mar	maternal	100
4	4	47,XY,+mar	*de novo*	100
5	5	47,XX,+mar[25]/46,XX[17]	*de novo*	60
6	6	47,XY,+mar[45]/46,XY[35]	*de novo*	56
7	7	47,XX,+mar[15]/46,XX[29]	*de novo*	34
8	8	47,XY,+mar[33]/46,XY[150]	*de novo*	18
9	9	47,XY,+mar	maternal	100
10	10	47,XY,+mar	paternal	100
11	11	47,XX,+mar[29]/46,XX[18]	*de novo*	62
12	12	47,XY,+mar[27]/46,XY[38]	*de novo*	42
13	13	47,XX,+mar[32]/46,XX[23]	*de novo*	58
14	14	47,XX,+mar[28]/46,XX[23]	*de novo*	55
15	15	47,XX,+mar[6]/46,XX[58]	unknown[b]	9
16	16	47,XX,+mar[73]/46,XX[12]	*de novo*	86
17	17	47,XY,+mar	unknown	100
18	18	47,XY,+mar[16]/46,XY[27]	unknown	37
19	–	46,X,+mar[3]/46,XY[19]	*de novo*	14
20	–	47,XY,+mar	*de novo*	100
21	–	47,XY,+mar[28]/46,XY[8]	*de novo*	78
22	19	47,XY,+mar	*de novo*	100
23	20	47,XY,+mar[30]/46,XY[28]	*de novo*	52
24	21	47,XY,+mar[85]/46,XY[6]	*de novo*	93
25	22	47,XX,+mar[44]/46,XX[28]	*de novo*	61
26	23	47,XX,+mar[11]/46,XX[47]	*de novo*	19
27	24	47,XX,+mar[52]/46,XX[95]	*de novo*	35
28	25	47,XX,+mar[17]/46,XX[63]	unknown	21
29	–	47,XX,+mar[39]/46,XX[17]	*de novo*	70
30	–	47,XX,+mar[50]/46,XX[28]	*de novo*	64
31	–	47,XX,+mar	maternal	100
32	–	47,XX,+mar	maternal	100
33	26	47,XX,+mar[16]/46,XX[45]	*de novo*	26
34	27	47,XY,+mar	maternal	100
35	28	47,XX,+mar[61]/46,XX[14]	*de novo*	81
36	29	47,XY,+mar[46]/46,XY[25]	*de novo*	65
37	–	47,XX,+mar[20]/46,XX[45]	unknown	31
38	30	47,XX,+mar[13]/46,XX[59]	*de novo*	18
39	–	47,XY,+mar[32]/46,XY[17]	*de novo*	65
40	31	47,XY,+mar[37]/46,XY[39]	*de novo*	49
41	32	47,XY,+mar	*de novo*	100
42	33	47,XY,+mar	*de novo*	100
43	34	47,XX,+mar	unknown	100
44	–	47,XY,+mar	maternal	100
45	35	47,XY,+mar[16]/46,XY[80]	unknown	17
46	36	47,XY,+mar[82]/46,XY[12]	*de novo*	87
47	37	47,XY,+mar[51]/46,XY[58]	unknown	47
48	–	47,XY,+mar[41]/46,XY[31]	*de novo*	57
49	–	47,XY,+mar[54]/46,XY[16]	*de novo*	77
50	–	46,X,+mar	*de novo*	100
51	38	47,XY,+mar[23]/46,XY[30]	*de novo*	43
52	39	47,XY,+mar[36]/46,XY[46]	*de novo*	44
53	40	47,XY,+mar[14]/46,XY[78]	unknown	15
54	–	45,X[31]/46,X + mar[13]	unknown	30
55	41	47,XX,+mar[12]/46,XX[48]	unknown	20
56	42	47,XY,+mar1[36]/48,XY,+mar1,+mar2[18]/47,XY,+mar2[7]/46,XY[20]	*de novo*	44/22/9
57	43	47,XY,+mar[31]/46,XY[7]	*de novo*	82
58	44	47,XY,+mar[46]/46,XY[5]	*de novo*	90
59	45	47,XX,+mar	*de novo*	100

[a] 100: define as nonmosaic; < 100: define as mosaic.
[b] Inheritance is not determined because their parents refused to provide peripheral blood samples.

Table 3
Distribution of sSMCs by their morphologic classification and aCGH assay.

Morphology	Detected on karyotyping		Detected on aCGH assay	
	N (total 59)	Proportion (%)	N (total 45)	Diagnosis yield (%)[a]
Inverted duplicated/isochromosome /isodicentric	28	47%	25	20% (5/25)
Ring chromosomes	3	6%	3	0% (0/3)
Minute	28	47%	17	12% (2/17)

N: number of cases.
[a] Copy number variation indicates fetus with clinical phenotypes.

diagnosis for cases with sSMCs. Array CGH is considered a powerful diagnostic tool.

Methods

Data were obtained from amniocentesis records of the cytogenetic laboratory at Taipei Lee Women's Clinic, Youthgene Medical Laboratory between 2004 and 2015. Detailed information on the indications for prenatal diagnosis of chromosomal abnormality through cytogenetic analysis was obtained, which included (1) an advanced maternal age (AMA, i.e., if the mother was ≧35 years at the expected date of confinement), (2) abnormal biochemical markers in the maternal serum, such as screening maternal blood for Down syndrome (higher than 1/270), (3) abnormal ultrasound findings, (4) family history of chromosomal abnormalities, (5) and other nonspecific indications, such as anxiety.

Cytogenetic testing

Cytogenetic testing was performed on G-banded metaphase chromosomes of cultured amniotic fluid cells.

Array CGH

Array CGH was performed on 45 cases. The DNA of cultured amniocytes was extracted using the SurePrint G3 Human CGH Microarray kit 60 K (Agilent Technologies, Santa Clara, CA, USA). Array CGH analysis was performed in accordance with the Agilent protocol. The microarray kit has 60,000 probes and a median 400–500 kb resolution across the entire genome and a median 25–50 kb resolution at 500 known common chromosomal anomalous areas, pericentromeric areas, and subtelomeric areas.

Results

Frequency, distribution, and incidence of nonmosaic and mosaic sSMCs in amniotic fluid (AF)

In this study, 68,087 cases of amniocentesis were analyzed, of which 59 were identified as sSMCs. The overall frequency of sSMCs was 0.087% (59/68,087 cases), of which 11/59 (18.64%) of the sSMCs were of unknown inheritance because their parents refused to provide peripheral blood samples. Of the 48 sSMCs cases that were available for inheritance analysis, 40 (83.33%) cases were *de novo* and 8 (16.67%) cases were inherited. The highest proportion of sSMC was identified in cases with indications of advanced maternal age (AMA; 42/59), followed by nonspecific indications (7/59), detection of abnormal biochemical markers in maternal serum (6/59), abnormal ultrasound findings (2/59), and family history (2/59) (Table 1). Additionally, the 59 sSMC cases were refined to 18 nonmosaic (30%) and 41 mosaic markers (70%) (Table 2).

Characterization of sSMC by chromosome morphology

According to cytogenetic banding and to the database published by Liehr et al. (available at http://ssmc-tl.com/Start.html), 59 sSMCs were morphologically divided into groups (inverted duplicated/isochromosome/isodicentric chromosome, ring chromosome, and centric minute marker chromosome), as shown in Table 3. In this

Table 4
Summary of 7 cases presenting *de novo* sSMC characterized through array CGH.

aCGH No.	Indication	Karyotype	sSMC Morphology	Percentage of Cells with sSMC	aCGH analysis	Diagnosis[a]
1	AMA	47,XY,+mar *de novo*	inv-dup	100	1.26Mb duplication; arr 22q11.1q11.21(17,397,528 −18,661,749)x4	Cat eye syndrome
16	AMA	47,XX,+mar[73]/46,XX [12] *de novo*	min	86	11.40Mb duplication; arr 11q12.1q13.2(55,084,040 −66,490,712)x3	ASD/DD/ID
20	AMA	47,XY,+mar[30]/46,XY [28] *de novo*	iso	52	34.53Mb duplication; arr 12p13.33p11.1(230,451 −34,756,180)x4	Pallister-Killian syndrome
21	AMA	47,XY,+mar[85]/46,XY [6] *de novo*	min	93	1.43Mb duplication; arr 12p11.21p11.1(32,665,780 −34,091,133)x3	ASD/DD/ID
33	AMA	47,XY,+mar *de novo*	inv-dup	100	1.23Mb duplication; arr 22q11.1q11.21(17,397,528 −18,628,049)x4	Cat eye syndrome
37	AMA	47,XY,+mar[51]/46,XY [58] *de novo*	inv-dup	47	11.82Mb duplication; arr 15q11.1q13.3(20,686,219 −32,509,897)x4	ASD/DD/ID
41	AMA	47,XX,+mar[12]/46,XX [48]	inv-dup	20	1.23Mb duplication; arr 22q11.1q11.21(17,397,528 −18,628,049) x4	Cat eye syndrome

sSMC, small supernumerary marker chromosome; aray CGH, array comparative genomic hybridization; AMA, advanced maternal age (≧35 years old); inv-dup, inverted-duplicated; iso, iso-chromosome; min, centric minute; ASD, autism spectrum disorders; DD, developmental delay; ID, intellectual disability.
[a] Copy number variation indicates fetus with clinical phenotypes.

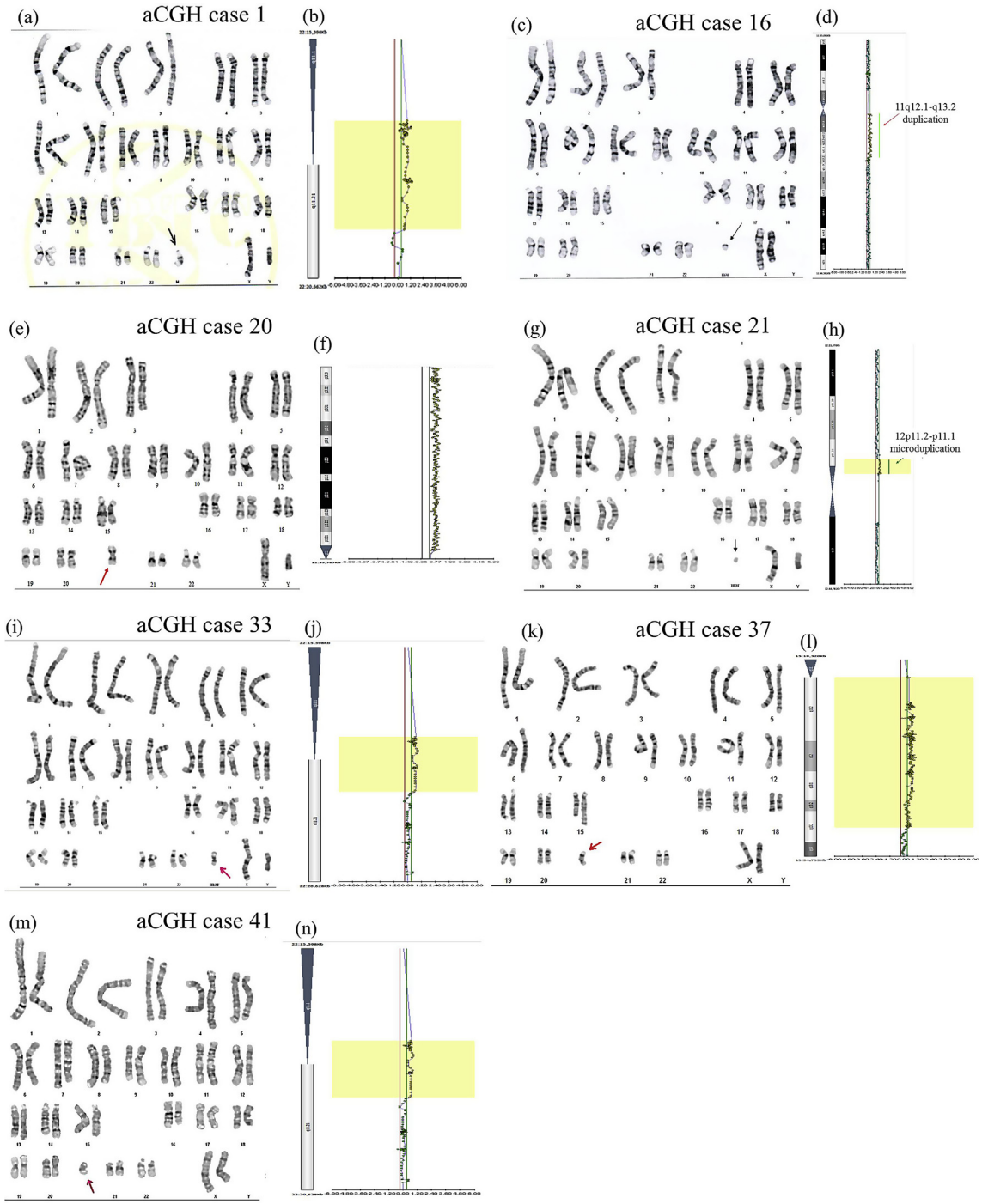

Fig. 1. Chromosome abnormalities detected in amniotic fluid samples with *de novo* sSMCs.(a) The fetal karyotype was 47,XY,+mar and (b) array CGH analysis indicates a 1.26 Mb duplication at 22q11.1-q11.21 in case 1. (c) The fetal karyotype was 47,XX,+mar[73]/46,XX[12]and (d) array CGH analysis indicated a 11.40 Mb duplication at 11q12.1-q13.2 in case 16. (e) The fetal karyotype was 47,XY,+mar[30]/46,XY[28] and (f) array CGH analysis indicated a 34.53 Mb duplication at 12p13.33-p11.1 in case 20. (g) The fetal karyotype was 47,XY,+mar[85]/46,XY[6] and (h) array CGH analysis indicated a 1.43 Mb duplication at 12p11.21-p11.1 in case 21. (i) The fetal karyotype was 47,XY,+mar and (j) array CGH analysis indicated a 1.23 Mb duplication at 22q11.1-q11.21 in case 33. (k) The fetal karyotype was 47,XY,+mar[51]/46,XY[58] and (l) array CGH analysis indicated a 11.82 Mb duplication at 15q11.1-q13.3 in case 37. (m) The fetal karyotype was 47,XX,+mar[12]/46,XX[48] and (n) array CGH analysis indicated a 1.23 Mb duplication at 22q11.1-q11.21 in case 41. The karyotype of cells was examined through G-banding, and the arrow indicates the sSMC. The chromosome gain or loss was analyzed in accordance with NCBI build 37 version.

study, 47% (28/59) of the characterized sSMCs were inverted duplicated/isochromosome/isodicentric chromosomes, 47% (28/59) were centric minute marker chromosomes, and the remaining three cases were ring chromosomes (3/59; 6%). Additionally, one case contained two minute chromosomes and was grouped as minute.

Usefulness of applications of new molecular cytogenetic techniques

In 2012, array CGH was introduced for characterizing sSMCs in our laboratory. A total of 45 cases, including 13 nonmosaic and 32 mosaic sSMCs, were investigated through array CGH, and the

percentages of cells with sSMCs in mosaic cases ranged from 9% to 93% (Table 2). The origins of marker chromosomes in seven cases are presented in Table 4. The pathogenic detection rate in sSMCs was 15.6% (7/45) defined through array CGH (Fig. 1). In 38 out of 45 cases, array CGH showed no pathogenic copy gain, allowing for a reduction in the residual risk of euchromatic sequences within the *de novo* sSMC.

A total of 25 markers derived from inverted duplicated/isochromosome/isodicentric chromosomes were further analyzed through array CGH (Table 3). Five cases (5/25; 20%) involved a gain in pathogenic copy number variation (CNV) (Table 4). Array CGH Nos. 1, 33, and 41 were associated with cat eye syndrome, array CGH No. 20 was associated with Pallister–Killian syndrome, which had been confirmed at age of 2 years 3 months with skin fibroblasts of the child by fluorescent in situ hybridization (FISH) and array CGH [16], array CGH No. 37 was correlated with autism spectrum disorders (ASD), developmental delay (DD), and intellectual disability (ID). Additionally, 17 minute markers were examined through array CGH; two cases (2/17; 12%) contained a gain of pathogenic CNV. Array CGH Nos. 16 and 21 were characterized as related to ASD, DD, and ID. A higher diagnosis yield was present in the inverted duplicated, isochromosome, and isodicentric category (20%), according to the data shown in Table 3.

Discussion

Marker chromosome cases are rarely observed through conventional laboratory cytogenetic analysis. However, when marker chromosomes were found through prenatal amniocentesis chromosomal analysis, the clinical outcomes varied greatly. A more thorough understanding of the karyotype–phenotype correlation of different sSMCs is vital for genetic counseling. Chromosomal microarray–based comparative genomic hybridization is increasingly utilized for genetic testing of individuals using a new diagnostic technique that has been widely used in pediatric genetics for accurate and fast detection of chromosomal abnormalities in patients with multiple congenital anomalies (MCA), DD, ID, ASD and complex genetic disorder [12].

In our laboratory, array CGH was used to analyze the origins of marker chromosome fragments, and it proved useful for learning whether marker chromosomes containing a gain of pathogenic CNV could provide more information for prenatal genetic counseling to reduce maternal anxiety and reduce the number of terminations.

According to Liehr and Weise [4], the chance of marker chromosome preference was investigated in different ethnic groups. The marker chromosome detection rate in healthy adults was 0.071%, and the detection rate in prenatal cases without special screening was 0.075%. Furthermore, Huang reported [13] that sSMCs are frequently encountered during prenatal diagnoses, occurring in 0.8–1.5 per thousand pregnancies. In our study, the detection rate of marker chromosomes in prenatal cytogenetic analysis was 0.087% (59/68,087).

De novo mutation of sSMC was lower in our study (83.33%) than it was in the Liehr study (70%) of 2007 [4]. In addition, according to Dalpra et al. [14], in a joint study featuring 19 laboratories in Italy, 241 marker chromosome cases were subjected to cytogenetic and molecular genetics analysis. The results indicated that the maternal inheritance rate was twice as high as the paternal inheritance rate, and our results indicated that the frequency of maternal inheritance of sSMCs was higher than that of paternal inheritance (maternal, 6; paternal, 2) (Table 2).

Although array CGH can significantly improve the detected resolution compared with karyotype in examining chromosome microdeletions or microduplications, it still has limitations in mosaicism diagnoses. According to Hodge et al. [15], the mosaicisms were not detected through array CGH when mosaic cells were less than 10%. However, most marker chromosomes were present in mosaic cells at varying proportions, which resulted in an increased risk of false-negative detection through array CGH analysis. In this study, 59 marker chromosomes were identified, and 41 of them were mosaic cells (69.49%); the mosaic cells percentage ranged from 9% to 93% (Table 2). To provide excellence in a medical laboratory, providing precise diagnosis reports to doctors and patient is necessary. Therefore a stable and reliable testing method is necessary for prenatal diagnosis. Array CGH must be used carefully to diagnose mosaicism. When low-percentage mosaicism is observed through a karyotype or when a significant decrease in mosaic level is observed after subculture or prolonged amniocyte culture [16], another molecular tool should be considered to support the prenatal diagnosis.

Conclusion

Array CGH offers useful tools for detecting small fragments of chromosomal abnormalities and sSMC origins in prenatal diagnosis. In this study, we successfully used array CGH to detect 7 out of 45 sSMCs, which were identified with gain in pathogenic CNV.

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Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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