Molecular delineation of de novo Small Supernumerary Marker Chromosomes in prenatal diagnosis, a retrospective study

Shuang Hu

the First Affiliated Hospital of Zhengzhou University, Center for Genetics and Prenatal Diagnosis

https://orcid.org/0000-0003-0310-9418

Xiangdong Kong (kongxd@263.net)

The First Affiliated Hospital of Zhengzhou University

Research

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Abstract

Objective To define the genotype-phenotype correlation of small supernumerary marker chromosomes (sSMC) and conduct precise genetic counseling.

Methods We retrospectively searched and reviewed the de novo sSMC cases detected during prenatal diagnosis in The First Affiliated Hospital of Zhengzhou University. Chromosome karyotypes of 20314 cases of amniotic fluid from pregnant women were performed. For 17 samples with de novo sSMC, 11 of them were subjected to single nucleotide polymorphism (SNP) array or low-coverage massively parallel copy number variation sequencing (CNV-seq) analysis.

Results Among the 11 sSMC cases, two sSMC were derived from chromosome 9, four sSMC were derived from chromosome 12, 18, 22 and X , separately. For the remaining 5 cases, they were not identified by SNP array or CNV-seq because they lacked euchromatin or had low proportion mosaicism. Four of them with the karyotype of 47,XN,+mar presented normal molecular cytogenetic results (seq[hg19] 46,XN) , the left one with the karyotype of 46,XN,+mar was Turner syndrome (seq[hg19] 45,XO). Five sSMC samples were mosaics of all these 17 cases.

Conclusion Considering the variable origins of sSMC, further genetics testing of sSMC should be performed by SNP array or CNV-seq. The detailed molecular characterization would allow precise genetic counseling for prenatal diagnosis.

Introduction

Small supernumerary marker chromosomes (sSMC) are abnormal chromosome fragments smaller than chromosome 20 in size, which was reported to be occurred in about 0.043% of live births and 0.075% of prenatal cases[1; 2]. Small supernumerary marker chromosomes can be identified but not be characterized by routine G-banding karyotypes alone. They can derive from any chromosome and sometimes present with mosaic condition, and have morphology like isodicentric bisatellited, ring, isodicentric or centric minute, and inverted duplicated shapes[3]. Earlier study showed that 70% of sSMC are *de novo* and 30% are inherited either from mather(20%) or father(10%) in 2015[4]. Most sSMC are derived from the short arms and the pericentromeric regions of acrocentric chromosomes and a small fraction of them are derived from nonacrocentric autosomes[5]. Owing to the diverse origins of chromosome and various ratios of mosaicism or other concominant chromosome imbalances, the phenotype of patients with sSMC varies significantly from normal to serious illness, including mental retardation, craniofacial abnormalities, autistic behaviors and growth retardation[6].

Specific sSMC have been revealed to be associated with certain clinical syndromes, for example, isochromosome 12p is associated with Pallister-Killian syndrome(OMIM 601803), sSMC(15) and Prader-Willi Syndrome(OMIM176270)/Angelman Syndrome(OMIM105830)(PWS/AS), isochromosome 18p syndrome, one sSMC containing the cat eye critical region (CECR) on chromosome 22q11.21,would cause cat eye syndrome(OMIM 115470). However, most of the sSMC carriers are normal with development, thus the

complexity of the correlation of phenotype-genotype makes prenatal diagnosis challenging. Thus molecular delineation of sSMC is very meaningful for genetic counseling in clinic.

Nowdays, with the development of science and technology, more and more molecular cytogenetic techniques are analyzed to address the characterization of sSMC. Including fluorescence in situ hybridization (FISH), a single nucleotide polymorphism (SNP) array, array-based comparative genomic hybridization (CGH) and High-throughput sequencing for copy number variation (CNV-seq).

Here in the present study, we retrospectively collected 17 sSMC karyotype cases from 20413 pregnant women by amniocentesis from our prenatal and diagnosis center from January 2017 to January 2020. Their molecular cytogenetic characterizations were analyzed by conventional G-banding and SNP or CNV-seq and then appropriate genotype-phenotype correlations for prenatal genetic counseling were provided by our genetic counselors. The primary aim of our research was to provide genetic-based prenatal diagnosis of sSMC in China.

This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Zhengzhou University. All analysed samples were obtained after signed informed consent was provided.

Materials And Methods

Subjects

Our study recruited 20314 pregnant women with the need of prenatal cytogenetic diagnosis who came to the clinic of the prenatal diagnosis center of the First Affiliated Hospital of Zhengzhou University between January 2017 and January 2020. The indications of prenatal diagnosis included the high risk pregnancy by NIPT,the high risk of trisomy 21/18/13 chromosome by serum screening, abnormal ultrasound, the advanced maternal age(age ≥ 35), and the histories of abnormal pregnancy. Conventional G-banding karyotyping were performed for every amniotic fluid samples. sSMC were detected in 17 pregnant women. The pregnant women were at 17–24 weeks' gestation undergoing amniocentesis to get amniotic fluid samples. Abnormal cell lines with sSMC were present in two independent cultures.

Methods

Karyotyping analysis

We retrospectively searched and reviewed the sSMC cases detected during prenatal diagnosis in The First Affiliated Hospital of Zhengzhou University. Amniotic fluid specimens (10 ml) was collected by amniocentesis under the guidance of ultrasonography, and then centrifuged at 1500r/min for 8 minutes. Then discarding the supernatant, the remaining liquid was mixed and incubated with two 5 ml of amniocyte culture medium (Gibco,USA and Isreal). The cells were cultured in an incubator at 37°C and 5% CO_2 for about 9 days. When cell colonies reached more than 15, these cells were collected followed by making sections and classical G-banding karyotyping were performed for every sample. And then the banding sections were scanned by Zeiss Automated Nuclear Scanning System. For each sample, 5 mitotic figures were analyzed with 30 counts

(abnormal karyotype with 100 counts). For these abnormal karyotypes, the second line cells were also managed as the first line.

SNP microarray.

The SNP-array analysis was conducted on the Affymetrix CytoScan platform (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's protocol, in order to perform a whole genome scan on the amniotic cell DNA of Case 16 and 17. Whileas molecular karyotype analysis was conducted using KaryoStudio version 1.4.3.0 (Illumina, San Diego, CA).

Cnv-seq

Low-coverage massively parallel copy number variation sequencing (CNV-seq) was conducted on the illumina platform for patient samples of Case 7–15. A total of 10 ng of input DNA was sufficient for accurate CNV-seq diagnosis, although 50 ng was optimal. Genomic DNA (10 ng) was fragmented and DNA library was constructed as previously described [7]. Multiple libraries were indexed and pooled into a single lane and sequenced on theNextseq CN500 instrument (Illumina, Inc.) to produce approximately 5 million single-end reads of 45 bp (including the 8 bp index sequence). Database of Genomic Variants (http://projects.tcag.ca/variation), DECIPHER database (http://decipher.sanger.ac.uk/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), OMIM (http://www.ncbi.nlm.nih.gov/omim) and ClinGen (https://www.clinicalgenome.org/) were used for interpretation and classification of the clinical significance of candidate CNVs according to previously reported guidelines

Results

The whole distribution of sSMC and mosaicism

In our survey, a total of 17 de novo sSMC cases were detected in 20314 amniotic fluid samples from pregnant women by conventional G-banding karyotyping and among the 17 sSMC, 5 cases demostrated mosaicism. And 11 of 17 samples with de novo sSMC were successfully subjected to single nucleotide polymorphism (SNP) array or low-coverage massively parallel copy number variation sequencing (CNV-seq) analysis. The remaining 6 were not characterized further.

Out of the 11 sSMC only 6 were endowed with detectable euchromatin. For the left 5 sSMC being derived from an acrocentric chromosome, their CNV-seq results were negative, the reason for the negative results was uncertain but it may be due to the sSMC being derived from an acrocentric chromosome and containing only centromere heterochromatin without euchromatic regions. Of these, the CNV-seq results of four were seq[hg19] 46,XN. (Case 8,9,10,11) One sSMC with the karyotype of 46,X,+mar, which turned out to be a Turner Syndrome with the CNV-seq result of 45,XO.

The remaining 6 sSMC with chromosome loss or gain were derived from each of chromosome 12, chromosome 18, chromosome 22, chromosome X and the left two sSMC were originated from chromosome

9.

Cytogenetic analysis displayed mosaic sSMC in 5 of 17 cases.(Case 1,3,10,12 and 16.) Comprehensive analysis of two cell line revealed that their mosaic level ranged from 4–74%.

Chromosome Origins And Genotype-phenotype Ananlysis

For case 1 to 6, they did not perform any subsequent examination to elucidate the origins of sSMC except the G-banding karyotyping. For case 7 to 11, the CNV-seq analysis reavealed that the sSMC did not contain any genomic components. To case 12 to 17, the sSMC contained significant chromosome fragments (Table 1). The following would delineate the correlation between genotype and phenotype in detail.

Table 1
Summary of Karyotypes, CNV-seq or SNP-array results and follow-ups

NO.	Karyotypes	CNV-seq or SNP-array	Significance	Follow-ups
1	47,XN□+mar[74]/46□XN[26]	N.A	N.A	abort
2	47,XN□+mar	N.A	N.A	lose contact
3	47,XN□+mar[8]/46□XN[92]	N.A	N.A	lose contact
4	46,X□+mar	N.A	N.A	abort
5	47,XN□+mar	N.A	N.A	lose contact
6	47,XN□rob(21;21)(q10;q10)□+21□+mar	N.A	N.A	abort
7	46,X□+mar	seq[hg19]45,XO	negative	abort
8	47,XN□+mar	seq[hg19] 46,XN	negative	continue
9	47,XN□+mar	seq[hg19] 46,XN	negative	continue
10	47,XN□+mar[4]/46□XN[96]	seq[hg19] 46,XN	negative	continue
11	47,XN□+mar	seq[hg19] 46,XN	negative	continue
12	47,XN□+mar[70]/46□XN[30]	seq[hg19] 9p12p21(28600000–38780000)×3(40%)	variant of unknown significance	continue,2 years old
13	46,X□+mar	seq[hg19] 46,XX; Xp22.33p11.1(2700000–58560000)x1;Xq25q28(125100000–154940000)x1	pathogenic	abort
14	47,XN□+mar	seq[hg19] 18p11.32p11.21(120000-14980000) x4	likely pathogenic	abort
15	47,XN□+mar	seq[hg19] 12p13.33p13.31(160000–9420000)x3	variant of unknown significance	abort
16	47,XN□+mar[26]/46□XN[74]	arr[hg19]9p21.1p13.1(30498773–39411673)X(2–3)	likely benign	continue,3.5 years old
17	47,XN□+mar)	arr[hg19]22q11.1q11.21(16878002–18656495)×(4–5)	pathogenic	abort

For case 12, the sSMC was derived from chromosome 9, with a gain of about 10Mb of the 9p12p21 region (seq[hg19] 9p12p21(28600000–38780000)×3(40%))(**Figure.1**-A2). The karyotype analyzing showed that there were about 70% of analyzed metaphases with sSMC, thus the karyotype was defined as 47,XN,+mar[70]/46,XN[30] (**Figure.1**-A1). There were no ultrasound anomalies, meanwhile the family desired for a baby, so our genetic counselor advised them to closely monitor the baby after birth. The baby grew up and was a 2 years old girl with normal development. For case 13, 46,X,+mar was the fetus' karyotype result(**Figure.1**-B1). Then CNV-seq revealed a partial loss of X chromosome, (seq[hg19] 46,XX;

Xp22.33p11.1 (2700000–58560000)x1, Xq25q28 (125100000–154940000)x1) (**Figure.1**-B2). The first part Xp22.33p11.1 has a heterozygous loss of about 55.86Mb. In the meantime, the second part Xq25q28 has a heterozygous loss of about 29.84Mb. The pregnancy was terminated. For case 14, the sSMC is derived from chromosome 18 with the karyotype of 47,XN,+mar(**Figure.2**-A1). However, the CNV-seq result showed a triple-dosage increase on 18p11.32p11.21.(seq[hg19] 18p11.32p11.21(120000-14980000) x4) (**Figure.2**-A2).

The G-banding analysis described the fetus' karyotype as 47,XN,+mar without mosaicism in case 15(**Figure.2**-B1). There was a duplication of 9.26Mb at seq[hg19] 12p13.33p13.31(160000–9420000)x3 (spanning 100 OMIM genes) (**Figure.2**-B2). Thus the sSMC originated from chromosome 12. For case 16, the traditional karyotype analysis showed 47,XN,+mar accounting for 32% of all cultured amniocytes and 68% of cultured cells was 46,XN, that is the karyotype was mosaicism 47,XN,+mar[26]/46,XN[74] (**Figure.3**-A1). SNP array revealed that there was a duplication of 8.9 Mb at arr[hg19]9p21.1p13.1(30498773–39411673) in 40% of the fetal cells(**Figure.3**-A2). There were no abnormal ultrasonic manifestations throughout the whole pregnancy. For case 17, the standard karyotype analysis displayed the fetus with abnormal karyotype of 47,XN,+mar(**Figure.3**-B1). The SNP array reported that there was a duplication of 1.8Mb at arr[hg19]22q11.1q11.21(16878002–18656495) in the fetus cells(**Figure.3**-B2).

Pregnancy Outcome And Follow Ups

Pregnancy outcome and follow ups
In our survey, among the 17 sSMC cases, 3 lost contact, 8 families choose abortion(8/17), 6 cases opted to continue their pregnancy(6/17). The detailed information was showed in Table 1.

To be specific, the fetus of case 1 was from a pedigree with hemophilia, and unfortunately the fetus was diagnosed with hemophilia A by prenatal genetic diagnosis. The karyotype of case 4 was 46, X,+mar, the fetus would have Turner syndrome in all probability even the marker contained some genetic content. The fetus of case 6 detected to be Robertsonian translocation type trisomy 21 by chromosomal analysis. Couples of cases 1, 4 and 6 terminated their pregnancies. Cases 2, 3 and 5 lost contact. The sSMC of case 8–11 is completely absent of euchromatin, the babies were born normally and were 18 to 30 months-old.

It was worth emphasizing that for case 16 and case 12, the baby were both born healthy. The two children were about 2(case 12) or 3.5 years old (case 16) respectively, and no clinical phenotypes were observed. As the sSMC of case 13, 14, 15 and 17 were all predicted to be pathogenic or unknown significance, so they all decided to have an abortion.

Discussion

In the current survey, we collected 17 sSMC prenatal samples, and the overall frequency was 0.0836%, which was close to that of other's research[1; 3] Herein, the origins of the sSMC included acrocentric chromosome (1/17), non-acrocentric chromosome (5/17) and no Y chromosome, which was significantly different from others' research observed by Leda Dalpra et al and Bing Huang et al[7; 8]. This may due to the small sample size.

Reportedly, sSMC derived from acrocentric chromosome with Chromosome 15 was the most common type[9], however, there was only one acrocentric chromosome with Chromosome 22 in our data. This may due to the limited amounts of sSMC cases in our report, and six of them lack of SNP or CNV-seq test.

For case 12, the gain in 9p12p21 region was evaluated as a variant of unknown significance. Whilst supersonic ultrasound examination displayed the fetus was normal throughout the whole pregnancy. The baby grew up and was a 2 years old girl without any abnormalities. So we could declare that the sSMC with a gain of about 10Mb of the 9p12p21 region was benign.

For case 13, the CNV-seq revealed a partial loss of X chromosome, (seq[hg19] 46,XX;

Xp22.33p11.1 (2700000–58560000)x1, Xq25q28 (125100000–154940000)x1). The first part Xp22.33p11.1 had a heterozygous loss of about 55.86Mb. The deleted fragment contains 316 protein-coding genes, including 263 OMIM genes. This deletion covers steroid sulfatase deficiency (STS), which manifests with ichthyose changes and was X-linked dominant inherited. It also contains multiple different dominant inherited genes, among which *BCOR* gene is related to microphthalmia syndrome OMIM[300485], *CDKL5* gene is related to epileptic encephalopathy OMIM[300203]. DECIPHER database collected the female cases with similar Xp22.33p11.1 deletion, with DECIPGE ID [284529] (pathogenic), and it showed abnormal heart morphology, mitral valve malformation, heart disease, unilateral ventricle, etc.

In the meantime, the second part Xq25q28 had a heterozygous loss of about 29.84Mb. The deleted fragment encompasses 207 protein-coding genes and 169 OMIM genes. Likewisely, it contains multiple dominant inherited genes. Among them, *FMR1* gene is related to Fragile X syndrome, with the OMIM ID[309550]. *FHL1* is related to reducing body myopathy, with the OMIM ID [300163]. To sum up, the comprehensive analysis revealed that Xp22.33p11.1 deletion and Xq25q28 deletion were evaluated as pathogenic. The pregnancy was terminated.

For case 14, the CNV-seq result showed a triple-dosage increase on 18p11.32p11.21. (seq[hg19] 18p11.32p11.21(120000-14980000) x4). This repetitive fragment encompasses 64 coding genes, of which 56 are OMIM genes, and there is insufficient evidence for Triplosensitivity. The case ID [323729] included in the DECIPHER database with a repetitive fragment slightly smaller than 18p11.32p11.21, the main clinical symptom is central hypotonia. And in case ID [356973], the main clinical symptom is Pathogenic. The DECIPHER database contains many cases that are slightly larger than the repeated fragment in case 14. The clinical manifestations includes abnormal auricle, abnormal EEG, arched eyebrows, mental retardation and other multiple malformations [394100][394870][394877](likely pathogenic). The DGV database does not include 18p11.32p11.21 duplicate reports. Comprehensive analysis suggested that 18p11.32p11.21 was likely pathogenic.

The G-banding analysis described the fetus' karyotype as 47,XN,+mar without mosaicism in case 15. There was a duplication of 9.26Mb at seq[hg19] 12p13.33p13.31(160000–9420000)x3 (spanning 100 OMIM genes). Thus the sSMC originated from chromosome 12. Case ID [339519] of similar repeated fragments is included in DECIPHER database, the phenotype is likely pathogenic (De novo constitutive). For case ID [364080], their clinic manifestation includes EEG abnormality, intellectual disability, prominent fingertip pads,

ptosis, short nose, type I diabetes mellitus. The DGV database does not include 12p13.33p13.31 duplicate reports. According to the comprehensive analysis, the clinical significance of 12p13.33p13.31 gain was evaluated as a variant of unknown significance. However, the woman choosed an abortion in case the baby has any abnormality. We could not get the definite clinical significiance.

For case 16, the traditional karyotype analysis showed 47,XN,+mar accounted for 32% of all cultured amniocytes and 68% of cultured cells was 46,XN, that is the karyotype was mosaicism 47,XN,+mar[32]/46,XN[68]. SNP array revealed that there was a duplication of 8.9 Mb at arr[hg19]9p21.1p13.1(30498773–39411673) in 40% of the fetal cells and the duplicated chromosomal section contained 68 OMIM genes including *ACO1*, *ALDH1B1* and *APTX*. And there was insufficient evidence for Triplosensitivity. The gain was evaluated as a variant of unknown significance. There were no abnormal ultrasonic manifestations throughout the whole pregnancy. The family continued their pregnancy, and the baby was about 3.5 years old with normal intellectual and physical development. Therefore, in our study we could state that this gain was likely benign.

For case 17, the standard karyotype analysis displayed the fetus with abnormal karyotype of 47,XN,+mar. The SNP array reported that there was a duplication of 1.8Mb at arr[hg19]22q11.1q11.21(16878002–18656495) in the fetus cells. The duplicated chromosomal section, that is the sSMC, contained the cat eye critical region (CECR) (Type I) on chromosome 22q11.21, which was definitely pathogenic.

The effect of sSMC commonly depends on its origin, size, content, structure and the proportion of mosaicism, as well as the amounts of euchromatin[10]. Others has reported that the risk of an abnormal phenotype could be associated with the size of the euchromatin region (even less than 1 Mb) or the number of genes (even less than 10). Nevertheless, when the sSMC contain some euchromatin fragments which are inherited from the healthy parent, we usually predict it as likely benign[11]. As the morphology of sSMC is highly heterogeneous, therefore the correlation of genotype-phenotype is exceedingly problematic in diagnostics.

Ten sSMC-specific syndromes were introduced in detailed by Hamideh etal in 2015, including Turner syndrome, Marker chromosome 15 syndrome, Emanuel syndrome (derivative 11;22 syndrome), Cat Eye syndrome (CES), Der(22)t(8;22)(q24.1;q11.1) syndrome, Isochromosome i (5p), 9p isochromosome syndrome, Isochromosome 18p (tetrasomy 18p)syndrome Tetrasomy 15qter syndrome (neocentric sSMA), and Pallister-Killian syndrome[4]. Given the above theory, Turner syndrome (case 13), tetrasomy 18p syndrome(case 14) and Cat eye syndrome(case 17) were involved in our study. Patients with Turner syndrome have variable phenotypes, like short stature, infertility, cardiovascular health issues, neurocognitive and behavioral aspects[12; 13]. Tetrasomy 18p is a rare disorder. The frequency of tetrasomy 18p is 1/140000 in live newborns[14], which is the most common type among all these isochromosomes[15]. Their clinical characteristics include moderate to severe mental retardation, development delay, microcephaly, typical dysmorphic features, and other anomalies like muscle tone abnormality[16; 17]. Cat eye syndrome (CES) is a rare developmental disorder with the incidence of 1/150000 to 1/50000 in liveborn infants[18]. CES is occured with a bisatellite-dicentric sSMC(22) resulting in a trisomy or partial tetrasomy of chromosome 22[19]. The clinical presentations of CES included high forehead, downslanting palpebral fissures, epicanthus,

microphthalmia, cataract, and strabismus. Intellectual deficits, congenital heart defects and renal malformations may be involved in some severe cases[20–22].

It has been assumed that de novo sSMC, particularly those with UPD, resulted from incomplete trisomy rescue[23]. However whether UPD is coincidence or consequence, further study should be performed to explore it. As chromosomes 6,7,14,15,16,20 contain imprinting genes, so UPD of these chromosomes are most reported in sSMC cases[24]. When the sSMC contained only heterochromatic regions, it is necessary to exclude whether an imprinted chromosome is involved for UPD[25]. This is necessary, even though, in our experience we have not found UPD cases for case 16 and 17 which were detected by SNP-array. We could not find UPD for cases 7–15 detected by CNV-seq, which is the big limitation in our research.

Mosaic condition was presented in 5 cases (29.4%), which was a little lower than that reported in 2005[7]. At the same time the sSMC cell line level varied from 4–74%, which was similar to what displayed in previous literature[7].

Earlier researchers found that mosaicism was the reason for the normal or mild clinical manifestations in carriers of sSMC with well-defined syndromes. In only 2% of cases, there were no clinical signs or much less severe outcomes than expected in low-mosaic samples[26]. Herein, five cases out of 17 were mosaic sSMC. The mosaic level of case 12 is 70%, and the sSMC contained a gain of about 10Mb with a variant of unknown significance which turned out to be normal phenotypes expressing since the child was about 2 years old with normal mental and physical development. Similarly, in case 16, the duplication of the DNA sequence is about 8.9Mb containing 68 OMIM genes. Most likely due to mosaicism, the child was about 3.5 years old without any adverse clinical signs. Our results seemingly confirmed the previous conclusion that mosaicism would result in normal or minor clinical signs in carriers of sSMC.

SNP array or CNV-seq enable us to detect the origin and actually describe the genetic content of sSMC, and so forth make it easier for genetic counselor to establish the genotype-phenotype correlations, therefore it is an outstanding technique and will be tendency in prenatal genetic diagnositics.

A total of 11 sSMC were successfully detected by SNP array or CNV-seq in our survey, 3 were likely pathogenic, 3 were a variant of unknow significance and 5 were likely benign. In clinical, the outcomes of many cases were difficult to predict, due to their unclear origins, or different proportions of mosaicism and the possibility of uniparental disomy (UPD).

What noteworthy is that detailed molecular characterization of sSMC could contribute to precise prenatal counseling and help precise decision makings of the fetuses with sSMC. Whiles, there are some limitations in the current study. Firstly, the consequent CNV-seq analyses of the fetal tissues after abortion were not conducted. Secondly, the CNV-seq analyses of case 8–11 could not exclude the probability of UPD in the initial chromosome. Thirdly, we could not get the children's karyotyping of peripheral blood or other body tissues in case 12 and 16.

In conclusion, our research emphasize the combination of traditional cytogenetic and further molecular cytogenetic methods in characterization of small maker chromosome, which could make an easier

understanding of the relationships between sSMC and the resulting phenotypes.

Declarations

Ethics approval and consent to participate:

This study was approved by the Medical Ethics Committee in the First Affiliated Hospital of Zhengzhou University. All of the analysed samples were obtained with signed informed consent.

Consent for publication:

All of the analysed samples were obtained with signed informed consent.

Availability of data and material:

All data generated or analyzed during this study are included in the article.

Competing interests:

The authors declare that they have no competing interests

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Authors' contributions:

XK and SH conceived the study, SH summarized all the data and involved in histological analysis, SH and XK provided expertise for data interpretation and suggestions for manuscript preparation. SH wrote the manuscript.

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Figures

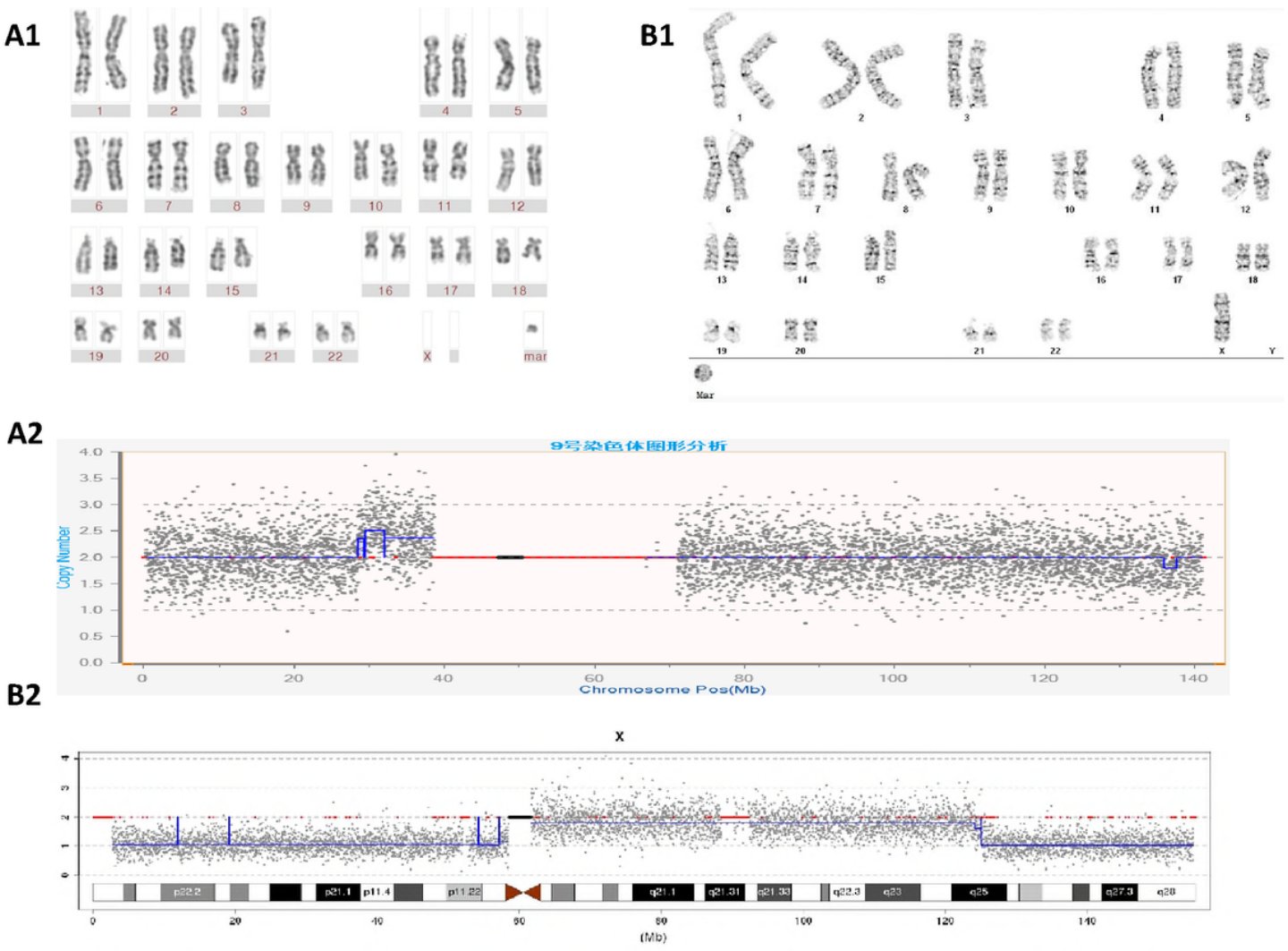

Figure 1

Cytogenetic and CNV-seq results of SMCs of case 12 and 13. （A1）G-banded karyotyping of case 12 showed a karyotype of 47（XN）+mar[28]/46（XN[12]. （A2）The SMCs of case 12 is partial triploid: seq[hg19] 9p12p21(28600000-38780000)×3（40%）.（B1） G-banded karyotyping of case 13 showed a karyotype of 46（X） +mar in all cells. （B2）The SMCs of case 13 was a partial deletion of X chromosome: seq[hg19] 46,XX; Xp22.33p11.1(2700000-58560000)x1（Xq25q28(125100000-154940000)x1.

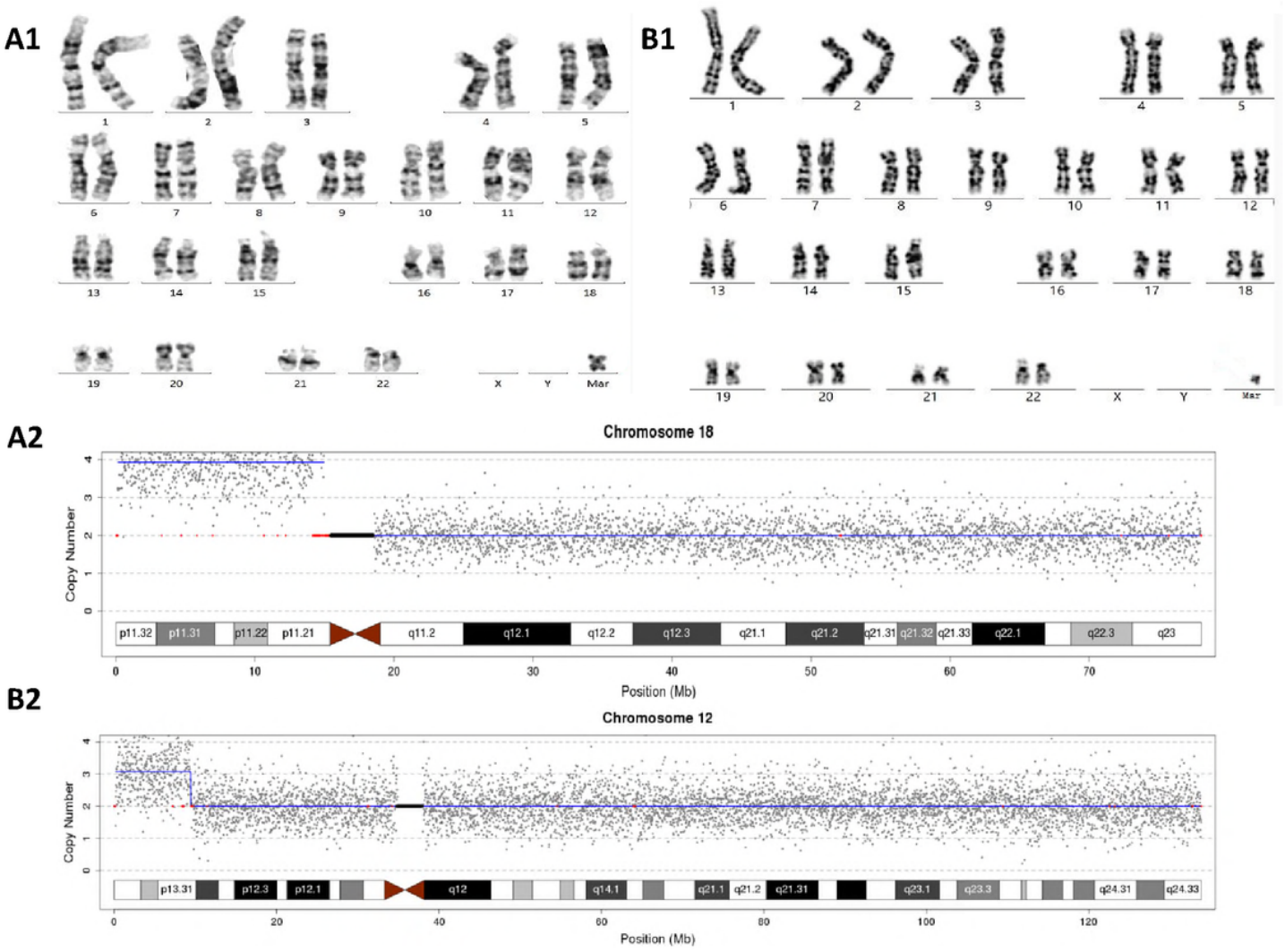

Figure 2

Cytogenetic and CNV-seq results of SMCs of case 14 and 15. （A1）G-banded karyotyping of case 14 showed a karyotype of 47（XN）+mar. （A2）The SMCs of case 12 is a partial tetraploid : seq[hg19] 18p11.32p11.21(120000-14980000) x4 （B1）G-banded karyotyping of case 15 showed a karyotype of 47（XN） +mar. （B2）The SMCs of case 15 is a partial triploid : seq[hg19] 12p13.33p13.31(160000-9420000)x3.

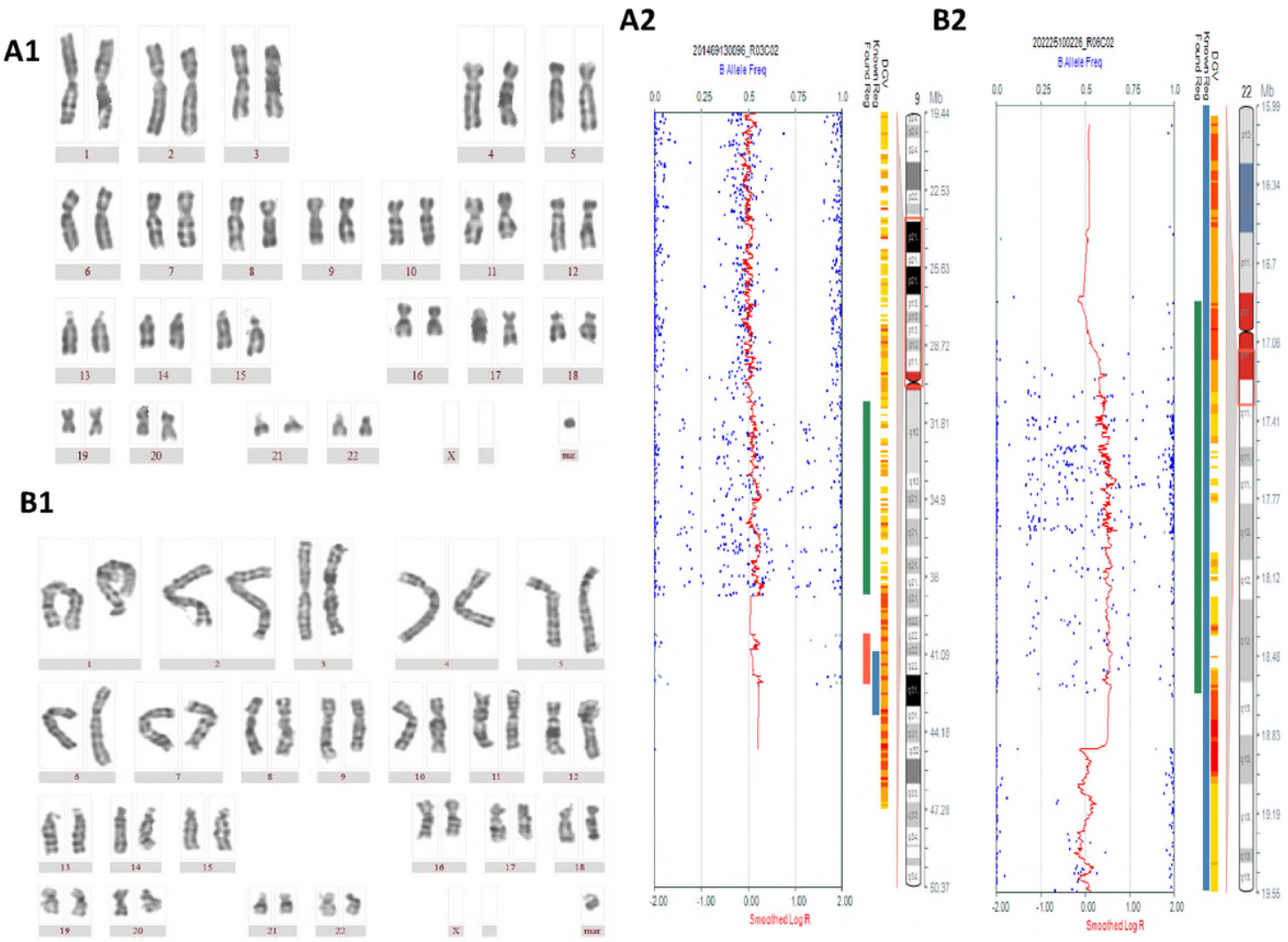

Figure 3

Cytogenetic and SNP array results of SMCs of case 16 and 17. （A1） A1 G-banded karyotyping of case 16 showed a karyotype of 47，XN，+mar[16]/46，XN[44]. （A2）The SMCs of case 16 is a partial triploid : arr[hg19]9p21.1p13.1(30498773-39411673)X(2-3). （B1）G-banded karyotyping of case 17 showed a karyotype of 47，XN，+mar（B2）The SMCs of case 15 is a partial tetraploid or pentaploid : arr[hg19]22q11.1q11.21(16878002-18656495)×(4~5).