Genotype-phenotype correlation in 75 patients with small supernumerary marker chromosomes

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Abstract

Background: Small supernumerary marker chromosomes (sSMCs) are rare structural abnormalities in the population; however, they are frequently found in children or fetuses with hypoevolutism and infertile adults. sSMCs are usually observed first by karyotyping, and further analysis of their molecular origin is important in clinical practice. Next-generation sequencing (NGS) combined with Sanger sequencing helps to identify the chromosomal origins of sSMCs and correlate certain sSMCs with a specific clinical picture.

Results: Karyotyping identified 75 sSMCs in 74,266 samples (0.1% incidence). The chromosomal origins of 27 of these sSMCs were detected by sequencing-related techniques (NGS, MLPA and STR). Eight of these sSMCs are being reported for the first time. sSMCs mainly derived from chromosomal X, Y, 15, and 18, and some sSMC chromosomal origins could be correlated with clinical phenotypes. However, the chromosomal origins of the remaining 48 sSMC cases are unknown. Thus, we will develop a set of economical and efficient methods for clinical sSMC diagnosis.

Conclusions: This study details the comprehensive characterization of 27 sSMCs. Eight of these sSMCs are being reported here for the first time, providing additional information to sSMC research. Identifying sSMCs may reveal genotype-phenotype correlations and integrate genomic data into clinical care.

Keywords: Small supernumerary marker chromosomes, Next-generation sequencing, Prenatal diagnosis, Genetic counseling

Background

Small supernumerary marker chromosomes (sSMCs) are structural abnormalities whose origins cannot be characterized by conventional cytogenetics alone but require molecular approaches. It is known that 70% of sSMCs are de novo, 20% are inherited from the mother, and 10% come from the father [1]. sSMCs are often derived from maternal meiosis I/II errors, trisomic/monosomic rescue, or fertilization errors [2, 3]. sSMCs are equal to or smaller than chromosome 20 in size and often have abnormal morphology (e.g., inverted duplication, minute, or ring). Many of them are derived from the short arms or pericentromeric regions of chromosomes. Nearly 70% of sSMC carriers are clinically normal; however, 30% are abnormal. Patients carrying sSMCs have developmental delays, intellectual disabilities, mixed gonadal dysgenesis (MGS), or infertility, depending on the origin of the sSMC. The treatment of these patients was based on different symptoms until the molecular characterization of sSMCs was developed.

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In this study, we identified 75 sSMC cases in 74,266 patients seen in our department from 2015 to 2018 by karyotyping. Fifty-seven of the cases were subjected to molecular analysis, and the remaining 18 were not characterized further. Next-generation sequencing (NGS) is a fast high-output sequencing technique used to determine copy number variations [4]. We combined NGS, multiplex ligation-dependent probe amplification (MLPA), and short tandem repeat (STR) analysis to identify the origins of the sSMCs in our study. The molecular components of 27 of the sSMCs were identified. Thirty of the sSMCs subjected to molecular analysis did not have any pathogenic information in original chromosomal.

sSMCs were first detected by conventional cytogenetic banding analysis, which is weak for identifying their molecular component. This study aimed to identify the origins of sSMCs diagnosed in our department over the last 4 years. This application may help recognize syndromes from which sSMC patients suffer, establish suitable and specific therapy, or even predict syndromes that will develop in the future. Such an application will be of great value in clinical genetic diagnosis and genetic counseling.

Results

Distribution of cases
A total of 74,266 samples were analyzed for genetic diagnosis from the infertility, pediatrics, and obstetrics departments of Shengjing hospital (Fig. 1). In particular, we studied 75 sSMC carriers (0.1% in total), including 23 adults with infertility or habitual abortion (23/75, 30.67%), 20 children with severe developed delay, MGS or gynandromorphism (20/75, 26.67%), 23 fetuses with intrauterine growth retardation or abnormal ultrasonic structures (23/75, 30.67%), and nine unsyndromic sSMC cases (9/75, 12%). We performed NGS, MLPA, and STR on 57 sSMCs and identified the chromosomal origins for 27 of these cases (Table 1). The chromosomal origins of the remaining 48 cases are still unknown (Table 2). These data suggested that most sSMC cases have clinical syndromes, which might be correlated with their clinical phenotypes.

sSMCs from chromosome Y
Twelve sSMCs were derived from chromosome Y. Patients 61166 and W02938 were sexually abnormal boys, showing similar characteristics to Turner syndrome with androgynous. Results showed the sSMCs were derived from a minute Y chromosome with SRY (Fig. 2A, B). Patient 69433 grew up as a girl. The MLPA analysis indicated that the sSMC was derived from min(Y) (Fig. 2C). Patients 61680, 62091, 77297, 80794, 98139 and W01824 were adult men with azoospermia and infertility. STR analysis showed that their sSMCs came from min(Y) (Fig. 2D-I, Table 3). Samples 150677, 162047, and 171276 were from amniotic fluid. The STR analysis results demonstrated that the sSMCs were from min(Y) (Fig. 2J-L).
| Patient NO. | Gender/age at diagnosis | Studied material | Cytogenetics | Final result of the sSMC | Test methods and results | Clinical symptoms | Age of gravida/karyotypes of parents | De novo/inherited |
|------------|-------------------------|------------------|--------------|--------------------------|--------------------------|-------------------|------------------------------------|------------------|
| 61166      | male/14 m               | PBL              | 45,X[2]/46,X,+mar[15] | del(1q)(pter→q11.222::q11.223→qter), first report | NGS:del(1)(p11.2)x0.5 (2.7 Mb), del(1)(p11.222→q11.223)x0 (2.2 Mb), AZF b, d and c regions: deleted. STRAMEL (Xp22.2:Yp11.2): 2:1. SRY (Yp11.31): positive. | Hypospadias, cryptorchidism. Term birth (BW 2.15 kg). | n.a. n.a. | n.a. |
| W02938     | male/13 m               | PBL              | 45,X[2]/46,X,+mar[19] | min(1) with SRY          | STRAMEL(Xp22.2:Yp11.2): 2:1. SRY (Yp11.31): positive. | Hypospadias, congenital testicular hypoplasia. His small penis was bent towards the abdomen side, and showed phimosis. | n.a. n.a. | n.a. |
| 69433      | female/6y               | PBL              | 45,X[1]/46,X,+mar[8] | min(1) with SRY          | MLPA: Y was normal. | Pygmyism, acita. H:106 cm, W:17.2 kg, BW:2.9 kg. | n.a. n.a. | n.a. |
| 61680      | male/29y                | PBL              | 45,X[8]/46,X,+mar[7] | min(1) with SRY          | AZF-b, d and c regions: deleted. STRAMEL (Xp22.2:Yp11.2): 1:1. SRY (Yp11.31): positive. | Azoospermatism | n.a. n.a. | n.a. |
| 77297      | male/25y                | PBL              | 46,X,+mar?          | min(1) with SRY          | AZF all regions: deleted. STRAMEL (Xp22.2:Yp11.2): 1:1. SRY (Yp11.31): positive. | Azoospermatism, infertility | n.a. n.a. | n.a. |
| 80794      | male/32y                | PBL              | 46,X[1]/46,X,+mar[7] | min(1) with SRY          | AZF-b, d and c regions: deleted. STRAMEL (Xp22.2:Yp11.2): 1:1. SRY (Yp11.31): positive. | Azoospermatism | n.a. n.a. | n.a. |
| 98139      | male/28y                | PBL              | 46,X,+mar?          | min(1) with SRY          | AZF all regions: deleted. STRAMEL (Xp22.2:Yp11.2): 1:1. SRY (Yp11.31): positive. | Infertile, azoospermatism. | n.a. n.a. | n.a. |
| W01824     | male/31y                | PBL              | 46,X[15]/46,X,+mar[10] | min(1) without SRY       | AZF all regions: deleted. STRAMEL (Xp22.2:Yp11.2): 1:1. SRY (Yp11.31): positive. | Infertile, azoospermatism. He had undergone remedial surgery for hypospadias and cryptorchidism when he was 5 years old. Magnetic resonance imaging (MRI) showed right spermatophore hypogenesys, and left spermatophore containing a mass. | n.a. n.a. | n.a. |
| 150677     | n.a./prenatal           | AF               | 45,X[1]/46,X,+mar[19] | min(1) with SRY          | STRAMEL (Xp22.2:Yp11.2): 1:1. SRY (Yp11.31): positive. | NIP indicated abnormal heterosomes. Gravida was G4P1 and had nature labour twice. Spousal AZF regions was normal. | 38/46XX; 46,XY de novo | n.a. |
| 162047     | n.a./prenatal           | AF               | 46,X,+mar[7?]       | min(1) with SRY          | STRAMEL (Xp22.2:Yp11.2): 1:2. SRY (Yp11.31): positive. | NIP indicated abnormal heterosome. Gravida was G2P1. | 33/n.a. n.a. | n.a. |
| 171276     | n.a./prenatal           | AF               | 45,X[2]/47,X,+mar[1],+mar[1]/46,X,+mar[47] | min(1) with SRY          | NGS: dup(15q11.2→q13.3;4.2 Mb), dup(15q13.3)x3 (1.6 Mb) | NIP: 4.7 mm (3.0 mm). Gravida underwent NGS in another hospital. | n.a. n.a. | n.a. |
| 69813      | male/6y                 | PBL              | 47,XY,+mar          | inv dup(15)(q11.2→q13.3), dual(15q13.3) | NGS: polymorphism du(15)(q11.21→p11.22):22740001–23520000x4 (0.78 Mb). AZF: normal. SRY: positive. | Hypoevolutism, hypophrenia, epilepsy. He could only say a few words. His EEG demonstrated epilepsy changes. | n.a. n.a. | n.a. |
| W03987     | male/31y                | PBL              | 47,XY,+mar          | inv dup(15)(q11.2) | NGS: polymorphism du(15)(q11.21→p11.22):22740001–23520000x4 (0.78 Mb). AZF: normal. SRY: positive. | Infertile, asthenospermia. | n.a. n.a. | n.a. |
| W04210     | female/25y              | PBL              | 47,X,+mar           | min(1) with SRY          | NGS: dup(15)(q11.2→q13.3) | Hyperspasms. She had hypterspasms for twenty years. Her hyperspasms occurred during sleep, with tongue biting, foaming at the mouth, and gatism, looking like epilepsy. | n.a. n.a. | n.a. |
| 70532      | male/2y                 | PBL              | 47,X,+mar           | inv dup(15)(q11.2) | MLPA: 3 points (two of SNRPN) | Autism | n.a. n.a. | n.a. |
| Patient NO. | Gender/age at diagnosis | Studied material | Cytogenetics | Final result of the sSMC | Test methods and results | Clinical symptoms | Age of gravida/| karyotypes of parents | De novo/ | inherited |
|------------|-------------------------|-----------------|-------------|--------------------------|--------------------------|-------------------|-----------------|-----------------|-----------|-----------|
| 83411      | female/5y PBL           | 47,XX,+mar      | inv dup(15)(q11.2) | MLPA: 3 points of 15q11.2 were heterozygous duplicated mutation. | Hypoechovolumism and mental retardation. She could not sit on her own at 1 year old and could not walk at 3 years old. MRI showed that her left lobe of the frontal lobe was partly demyelinated. Ultrasound revealed a ventricular septal defect, left to right ventricle shunt, wide coronary sinus, and persistent left superior vena cava. | n.a. | n.a. |
| 96862      | female/18m PBL          | 47,XX,+mar      | inv dup(15)(q11.2) | MLPA: 3 points of 15q11.2 were heterozygous duplicated mutation. | Hypoechovolumism. She could not walk steadily or pick up things with her hands, and had poor communication. MRI of the cerebrum showed that both sides of the hemisphere were not full. | n.a. | n.a. |
| 92568°     | female/12y PBL          | 45,X[7]/46,X, +mar[13] | r(X)(p11.23→q21.1); first report | NGS: 45,X[57%]/46,X, r(X)(p11.23→q21.1)[43%] | She was suspected Turner syndrome, and injected GH for 1 year. | n.a. | n.a. |
| W09834°    | female/14m PBL          | 45,X[4]/46,X, +mar[28] | min(X)(p11.2→q13.2); first report | NGS: partly 45,X: Xpter→p11.21, x1, X(q13.2→qter)x1. SRY: negative. | Turner syndrome. | n.a. | n.a. |
| 61259      | male/57d PBL            | 47,XY,+mar      | inv dup(18)(pter→p11.21→p11.21→pter) | NGS: dup(18)(p11.32→p11.21)x4 (15.3 Mb) | Neonatal feeding problem, pneumonia. He had microcephaly, low-set ears and often gazed look. | n.a. | n.a. |
| 172168     | female/4y AF prenatal    | 47,XX,+mar      | inv dup(18)(pter→p11.21→p11.21→pter) | NGS: dup(18)(p11.32→p11.21)x4. STR: normal. | NIP: the high risk of 18-trisomy syndrome (Edwards syndrome). | 38/46,X. | n.a. |
| 96932°     | female/4y PBL           | 45,X[21ps+] [14]/46,X, +mar, [21ps+] [6] | min(0), min(Y), first report | NGS: 45,X[65%]/46,XX[18%] | Hypoechovolumism. She grew slowly after birth, with W: 12.5 kg, H: 93 cm, (H/A ≤ 2SD). She had skin rash on the face, webbed neck, and short stature, looking like Turner syndrome. Her bone age was 3.5 years old, and 4 left carpals were sclerotized. Ultrasound showed vestigial uterus and no ovary. | n.a. | n.a. |
| 172990°    | female/4y AF prenatal    | 47,XX,+mar      | min(9)(pter→p13.1); first report | NGS: dup(9)(p24.3→p13.1)x3. STR: normal. | NIP indicated abnormal chromosome 9. | 37/46,XX. | n.a. |
| 70963°     | female/8y PBL           | 47,XX,[mar1qh+] [18]/46, XX[1qh+] [12] | min(20)(p12.3→q11.22); first report | NGS: mosaic duplication (20)(p12.3→q11.22)x3 (20.1 Mb) | Pygmyism, asitia. She had asitia and was sickly; W: 21.7 kg, H: 115.5 cm, H/A ≤ -2SD. Her 7 left carpals were sclerotized. Her mother’s height was 158 cm and father’s 178 cm. NGS was done at another hospital. | n.a. | n.a. |
| 160246°    | female/4y AF prenatal    | 160246: 47, XX,+mar | min(11)(q23.3→qter), first report | NGS: dup(11)(q23.3→q25)x3. STR: normal. | In 2016, her mother got pregnant (numbered 160246). Ultrasound showed that there was a fluid sonoluent area in the nuchal region of 160246. NGS performed at another hospital. In 2017, her mother got pregnant again (numbered 173026). The fetus carried the same balanced translocation, and his NGS results were normal. | 29/46,XX, t(11;22)(q23; q12)46,XY | de novo |
| 184290     | male/5y AF prenatal      | 47,XY,+mar      | inv dup(22)(q11.1 → 11.21) | NGS: dup(22)(q11.21)x3(2.46 Mb), dup(22)(q11.1→q11.21)x4. STR: normal. | NT: 3.1 mm. Gravida aborted a fetus with congenital heart disease in 2017. | 32/46,XX; 46,XY | de novo |

°The sSMC was reported for the first time

Abbreviations: PBL peripheral blood, AF amniotic fluid, y year, m month, d day, n.a not available, NIPT non-invasive prenatal testing, NT nuchal translucency
| Patient NO. | Gender/age at diagnosis | Studied material | Cytogenetics | Test methods and results | Clinical symptoms | Age of gravida/ karyotypes of parents | De novo/ inherited |
|------------|------------------------|------------------|--------------|--------------------------|-----------------|----------------------------------------|------------------|
| 150234     | male/prenatal          | AF               | 47,XX,+mar[23]/46,XX[21] | STR: normal            | Diabetes of type II | 36/n.a.                                | n.a.             |
| 150693     | female/prenatal        | AF               | 48,XX,+18,+mar | STR: 18-trisomy syndrome (Edwards syndrome) | Down's syndrome screening: high-risk, Advanced maternal age. | 43/n.a.                                | n.a.             |
| 151434     | male/prenatal          | AF               | 47,XY,+mar | STR: normal. SRY: positive | Ultrasound: ventricular septal defect, small kidney. | 31/46,XX;46,XY de novo |                               |
| 153225     | female/prenatal        | AF               | 47,XX,+mar[5]/46,XX[39] | STR: normal            | Ambryo develop delay | 28/n.a.                                | n.a.             |
| 161045     | n.a./prenatal          | AF               | 45,X[11]/46,X, +mar[21] | STR: 45,X              | NIPT: abnormal heterosome, NT:2.9 mm | 36/n.a.                                | n.a.             |
| 163110     | male/prenatal          | AF               | 47,XY,+mar | STR: normal. SRY: positive | Cerebromedullary tube anisotrophy. | 30/46,XX.                                | n.a.             |
| 170574     | n.a./prenatal          | AF               | 45,X[30]/46,X, +mar[3] | STR: 45,X              | Single umbilical artery (SUA), seroperitoneum of fetus | 30/n.a.                                | n.a.             |
| 172376     | n.a./prenatal          | AF               | 46,X,+mar[17]/45, X[12] | STR: 45,X              | NT > 3 mm | 33/46,XX.                                | n.a.             |
| 173060     | female/prenatal        | AF               | 47,XX,+mar | STR: normal            | Down's syndrome screening: high-risk | 26/n.a.                                | n.a.             |
| 180036     | female/prenatal        | AF               | 47,XX, +mar[1][SC]/46, XX[35] | STR: normal            | Oligohydramnios. | 30/n.a.                                | n.a.             |
| 180748     | female/prenatal        | AF               | 47,XX, +mar[3][MC]/46, XX[22] | STR: normal            | Twins | 28/n.a.                                | n.a.             |
| 181010     | female/prenatal        | AF               | 47,XX,+mar[1]/46, XX[29] | NIPT: low risk. STR: normal | Ventricular septal defect | 37/n.a.                                | n.a.             |
| 183584     | male/prenatal          | AF               | 47,XY,+mar[1]/46, X[29] | STR: normal            | Down's syndrome screening: high-risk(1/346). | 31/n.a.                                | n.a.             |
| 184082     | female/prenatal        | AF               | 47,XX,+mar[1]/46, XX[29] | STR: normal            | Down's syndrome screening: high-risk | 26/n.a.                                | n.a.             |
| 184172     | male/prenatal          | AF               | 47,XY, +mar[1][SC]/46, X[24] | NGS: dup(11)(p15.3→p15.3)x3, dup(6)(p12.32)(32400000–32780000)x3 | NT: 2.5 mm | 27/n.a.                                | n.a.             |
| A1045      | female/prenatal        | UCB              | 47,XX,+mar |                        | Develop delay for one month. | 30/n.a.                                | n.a.             |
| 61200      | male/32y               | PBL              | 47,XY,+mar[6]/36, X[13] | AZF: normal. SRY: positive | Infertile. | n.a.                                    | n.a.             |
| 61397      | male/24y               | PBL              | 47,XY,+mar | AZF: normal. SRY: positive | Azoospermatisim, hyperprolactinemia. | n.a.                                    | n.a.             |
| 62254      | female/3y              | PBL              | 47,XY,+mar | MLPA: normal            | Global developdelay | n.a.                                    | n.a.             |
| 63001      | male/29y               | PBL              | 47,XY,+mar | AZF: normal. SRY: positive | Asthenospermia | n.a.                                    | n.a.             |
| 63411      | female/22y             | PBL              | 47,XX,+mar |                        | The mother of a patient with develop delay . | n.a.                                    | n.a.             |
| 65676      | female/3y              | PBL              | 48,XX, + 21, +mar[13]/47,XX, +mar[7] |                        | Heart malformation | n.a.                                    | n.a.             |
| 67979      | female/9y              | PBL              | 46,XX,+mar[14]/45, X[11] |                        | Runtishness | n.a.                                    | n.a.             |
| 69235      | female/12m             | PBL              | 46,XX,+mar[8]/45, X[12] | MLPA: X was abnormal | Developdelay | n.a.                                    | n.a.             |
| 72699      | male/3y                | PBL              | 48,XY,+mar1, +mar2 | MLPA: normal            | Autism. | n.a.                                    | n.a.             |
sSMCs from chromosome 15

The sSMCs of six patients were derived from chromosome 15. NGS identified duplications on chromosome 15 for patients 69813 and W03987 (Fig. 3A, B). MLPA revealed that patients 70532, 83411, and 96862 had a heterozygous duplicated mutation at 15q11.2 (Fig. 3D-F). These five patients carried sSMCs derived from inv dup(15). The sSMC of patient W04210 was from min(15) (Fig. 3C). Five of these cases showed clinical features of Dup15q syndrome (e.g., hypoevolutism or autism). In

| Patient NO. | Gender/ age at diagnosis | Studied material | Cytogenetics | Test methods and results | Clinical symptoms | Age of gravida/ karyotypes of parents | De novo/ inherited |
|------------|--------------------------|-----------------|--------------|--------------------------|------------------|----------------------------------------|------------------|
| 73431      | male/10y                 | PBL             | 48,XY,+mar1, +mar2 | SRY: positive            | Astigia and hypometropia. | n.a. | n.a. |
| 73940      | female/59y               | PBL             | 47,XX,+mar     |                           | n.a.              | n.a. | n.a. |
| 7300       | male/33y                 | PBL             | 47,XY,+mar     | NGS: No obvious abnormal was detected. AZF: normal. SRY: positive. | Asthenospermia, teratospermia. | n.a. | n.a. |
| 80039      | male/33y                 | PBL             | 47,XY,+mar     | NGS: A 0.46 Mb section deleted in 6q12, no pathopoiesia information. | Infertile, asthenospermia. | n.a. | n.a. |
| 81882      | female/15y               | PBL             | 46, X, mar[11]/45, X[9] |                          | Primary amenorrhoe | n.a. | n.a. |
| 85773      | male/32y                 | PBL             | 47,XY,+mar     | NGS: No obvious abnormal was detected. | Infertile, azoospermatism. | n.a. | n.a. |
| 90074      | female/9 m               | PBL             | 45, X[8]/46, X, +mar[19] | NGS: 45,X              | Hypoevolutism     | n.a. | n.a. |
| 91473      | female/20y               | PBL             | 46, X,+mar[11]/45, X[10] | SRY: negtive            | Primary amenorrhoe, Vestigial uterus. | n.a. | n.a. |
| 92243      | female/34y               | PBL             | 47,XX,+mar[19]/46,XX[13] | NGS: No obvious abnormal was detected. | Infertile         | n.a. | n.a. |
| 92638      | female/25y               | PBL             | 45, X,+mar[1]/46, XX[16]/47,XX, +mar[3] |                          | Infertile         | n.a. | n.a. |
| 93162      | n.a./9 m                 | PBL             | 45, X[1]/46,X, +mar [9] |                          | Gynandromorphism  | n.a. | n.a. |
| 96704      | female/2y                | PBL             | 45, X[7]/46,X, +mar[13] |                          | Pygmyism          | n.a. | n.a. |
| 97858      | male/30y                 | PBL             | 47,XY,+mar     | AZF: normal. SRY: positive | Infertile, azoospermatism. | n.a. | n.a. |
| W00311     | female/30y               | PBL             | 47,XX,+mar     | NGS: A 0.14 Mb section deleted in 2q32.1, no pathopoiesia information. | G1P0 embryonic stop develop at 11 weeks. | n.a./47,XX, + mar | maternal |
| W00880     | female/53y               | PBL             | 47,XX,+mar     |                          | W00311’s mother    | n.a. | n.a. |
| W02523     | female/21y               | PBL             | 47,XX,+mar[2]/46, XX[23] |                          | G4P0. Habitual abortion, arrested embryo. | n.a. | n.a. |
| W03572     | female/7 m               | PBL             | 47,XX,+mar[1]/46, XX[29] |                          | Develop delay.    | n.a. | n.a. |
| W06115     | female/30y               | PBL             | 47,XX,+mar     |                          | G3P1. Arrested embryo twice. | n.a. | n.a. |
| W06490     | female/29y               | PBL             | 47,XX,+mar[26]/46,XX[12] |                          | G2P0. Arrested embryo twice. | n.a. | n.a. |
| W07384     | male/30y                 | PBL             | 47,XY,+mar[3]/46, XY[36] |                          | Spouse had one time hydatidiform mole. | n.a. | n.a. |
| W13749     | female/4y                | PBL             | 47,XX,+mar     |                          | Developmental retardation. | n.a. | n.a. |
| W13804     | male/18 m                | PBL             | 45,X[12]/46,X, +mar[18] |                          | Hypospadia        | n.a. | n.a. |
| W14357     | female/28y               | PBL             | 47,XX,+mar     |                          | Pregnant preparation | n.a. | n.a. |

Abbreviations: PBL peripheral blood, AF amniotic fluid, UCB umbilical cord blood, y year, m month, d day, n.a not available, NIPT non-invasive prenatal testing, NT nuchal translucency
Fig. 2 (See legend on next page.)
contrast, case W03987 with inv dup(15)(q11.2) was polymorphic without the features of Dup15q syndrome.

**sSMCs from chromosome X**

The sSMCs of two patients were derived from chromosome X. These patients showed characteristics of Turner syndrome. NGS indicated that the sSMC of patient 92568, which was mosaic (45,X/46,X,+mar), might be from r(X) (Fig. 4A). The sSMC of patient W09834 was partial 45,X and composed of min(X) (Fig. 4B).

**sSMCs from chromosome 18**

The sSMCs of patient 61259 and fetus 172168 were derived from inv dul(18) (Fig. 5A, B). NGS showed that they had the genotype dup(18)(p11.32→p11.21)×4. It has been reported that the clinical symptoms are likely isochromosome 18p [i(18p)] syndromes or tetrasomy 18p syndrome, which feature neonatal feeding problems, hypoevolutism, and high risk of infections [5, 6].

**sSMCs from other chromosomes**

NGS showed that patient 96932 had a complex sSMC that might be derived from min(X) and min(Y) (Fig. 6A). This patient displayed similar characteristics to Turner syndrome. The sSMC of fetus 172990 was derived from min(9) (Fig. 6B). The sSMC of patient 70963, who showed compound features of partial trisomy 20p and 20q11.22 duplication syndrome with pygmyism and asitia, was derived from min(20) (Fig. 6C). The sSMC of fetus 160246 was derived from min(11) (Fig. 7A-a, b). When her mother got pregnant again, the fetus carried the same balanced translocation (Fig. 7A-c). The sSMC of fetus 184290 was derived from inv. dup(22) (Fig. 7B).

**sSMCs of unknown chromosomal origin**

Although several techniques were used to identify the origin of the different sSMCs, 48 patients could not be diagnosed (Table 2). Amniotic fluid samples containing sSMCs were submitted for STR analysis, and only seven sSMCs were identified. From karyotyping, these unidentified sSMCs were classified into three groups (Fig. 8).

**Discussion**

In this study, we identified the origins of 27 sSMCs, of which, eight sSMCs are being reported for the first time (Table 1). Of the 27 defined sSMC origins, 12 were derived from the Y chromosome and two from the X chromosome. The infertile patients showed azoospermia, and their original Y sSMCs were detected. Azoospermia factor (AZF), which is located on the long arm of Y (Yq11.23), regulates spermatogenesis [7]. These patients had deletions of AZF-a region (the Sertoli cell-only syndrome), AZF-b region (sperm-maturation-arrest syndrome), or all AZF regions resulting in azoospermia.

| Regions | 61166 | 61680 | 62091 | 77297 | 80794 | 98139 | W01824 |
|---------|-------|-------|-------|-------|-------|-------|-------|
| AZFa    | sY84  | +     | +     | +     | –     | +     | –     |
| sY86    | +     | +     | +     | –     | –     | –     | –     |
| AZFb    | sY127 | –     | +     | –     | –     | –     | –     |
| sY134   | –     | +     | –     | –     | –     | –     | –     |
| AZFd    | sY145 | +     | +     | –     | –     | –     | –     |
| sY152   | –     | –     | –     | –     | –     | –     | –     |
| AZFc    | sY157 | –     | –     | –     | –     | –     | –     |
| sY254   | –     | –     | –     | –     | –     | –     | –     |
| sY255   | +     | –     | –     | –     | –     | –     | –     |
| SRY     | +     | +     | +     | +     | +     | +     | –     |

|Table 3 The results of AZF|
Thus, artificial insemination with donor sperm or adoption was suggested for clinical management. The pediatric patients carrying sSMCs from min(Y) or chromosome X or complex sSMCs from min(X) and min(Y) had similar characteristics to Turner syndrome; however, they had different phenotypes depending on their sSMC origins. The short arm of X harbors the short stature-homeobox gene (SHOX on Xp22.33) and lymphogenic gene (forkhead box P3, FOXP3 on Xp11.23), which are associated with stature and immunodeficiency or polyendocrinopathy [8]. Patient W09834 with min(X) had a loss of FOXP3 and an immunological problem. A similar sSMC derived from r(X)(::p11.21→q13.1::) was reported in craniofrontonasal syndrome (CFNS) [9]. The methyl-CpG binding protein-2 gene (MECP2 on Xq28) is located on the long arm of X. This gene correlates with RETT syndrome and the premature ovarian failure gene POF (POFI: Xq21→qter, POF2: Xq13.3→Xq21.1) [10]. As the min(X) from patient W09834 (p11.2→q13.2::) and r(X) from patient 92568 ([p11.23→q21.1::]) did not contain SHOX and MECP2, both patients had growth retardation and a high risk of RETT syndrome. As they had the part of POFS, so being attention to ovarian function. Patient 96932 had a complex sSMC from min(X) and min(Y), resulting in a high risk of type II germ cell tumors [11, 12]. All the pediatric patients were recommended for individualized treatment according to their genotype-related phenotypes.

Our sSMC patients with the 47,XN,+mar karyotype typically had special duplication syndrome, and six sSMCs were identified from inv dup(15). The region 15(q11.2→q13.3) is a known hot breakpoint. This region harbors the GABAAR genes, the paternal gene SNRPN, and the maternal gene UBE3A, which regulate central neural system development and function [13]. It was rare that two neocentric sSMCs derived from inv dup(18) had the same duplication fragment. There may be a hot breakpoint located at 18(p11.21). In region 18p, approximately 67 genes can contribute to the phenotypes, including AFG3L2, MC2R, and TGIF1, which are associated with developmental disorders [5, 6]. So, when taking care of patient 61259, pay attention to artificial feeding, avoiding infections, and evaluating affected organs and systems. The region of 20(p12.3→q11.22) comprises more than 2 hundred genes. Duplication of JAG1, BTBD3, and FLRT3, or ASXL1 induces Alagille syndrome, neurological dysfunction or chromatin re-modeling [14, 15]. Patient 70963 with the genotype min(20)(::p12.3→q11.22::) showed moderate symptoms due to 60% mosaic.

The identification of sSMCs is vital in prenatal diagnosis. Of the 75 sSMC cases from this study, 23 were from fetuses with intrauterine growth retardation or abnormal
ultrasonic structure, and seven fetal sSMC cases were found to have Y, 18, 9, 11, or 22 chromosomal origins. However, most sSMCs failed to define the original chromosome. Three fetal sSMCs from the Y chromosome needed careful evaluation. If the sSMCs correlated with androgyneity or AZF deletion, it was better to complete the pregnancy. However, if a fetus had an inv dup(18) genotype, termination of the pregnancy was suggested because of the i(18p) syndrome. Fetus 172990 had a duplicated region 9(p24.3→p13.1) that correlated with 9p duplication syndrome, which contains a potential autism spectrum disorder (ASD) and a normal IQ individual region [16, 17]. The sSMC of fetus 160246 was de novo and arose from a maternal balanced translocation t(11;22)(q23;q12), leading to three copies of 11(q23.3→q25). The sSMC derived from the inv dup(22) chromosome was also de novo. The fetus carrying this sSMC had similar regions to the 22q11.2 duplication syndrome (22DupS), which usually produces birth defects, such as congenital heart disease, hearing loss, hypophrenia, or high risk of psychosis (including autism) [18, 19]. A similar sSMC arising from inv dup(22)(q11.1~11.2) was reported with mild clinical signs [20].

Most sSMCs in fetuses are de novo, but a few are inherited from their parents. Thus, prenatal diagnosis and genetic counseling are critical. In our department, parents are asked to fill out a form to collect genetic information. Amniotic fluid is then submitted for both karyotyping and STR analysis. If an sSMC is diagnosed, further testing (e.g., NGS) is suggested, and the karyotypes of the parents are requested. If the parents are sSMC or translocation carriers, the fetus should take further testing. Preimplantation genetic screening (PGS)
and preimplantation genetic diagnosis (PGD) would help reduce the chances of miscarriage.

Although several sequencing-related techniques were used in our study, there were still 30 sSMCs for which pathogenic information could not be generated. It is possible that the sequencing primers did not cover the sSMC regions in the MLPA or STR (AZF) methods. Also, inverted duplicated chromosomes (acrocentric chromosomes), iso chromosomes, or minute chromosomes (centromere-nearby regions) might not have been detected by NGS due to the highly repeated sequences at the centromere regions, which will be improved in read depth, inducing read pair, split pair, or assembly-based analysis of NGS. Thus, a set of efficient techniques should be developed for further sSMC identification.

Conclusions
In summary, the sSMCs of the study patients were different in origin, size, replication times, affected genes, and mosaicism levels. Thus, their clinical manifestations varied. This study detailed the comprehensive characterization of 27 sSMCs. Eight of these sSMCs are being reported here for the first time, which provides additional information for sSMC research. The identification of sSMCs could reveal genotype-phenotype correlations and integrate genomic data into clinical care.

Methods
Patients’ collection
This research investigated 74,266 patients’ specimens in our department from 2015 to 2018, including 50,794 peripheral bloods from adults, 6,350 peripheral bloods from pediatrics, 14,759 amniotic fluids, and 2,363 cord bloods. 75 sSMC carriers were diagnosed by karyotyping (Tables 1 and 2), containing 52 live births, and 23 fetuses. Some of them took further detection (e.g., NGS, MLPA, or STR). Then we identified the molecular component of 27 sSMC cases. They were compared with the information in http://cs-tl.de/DB/CA/sSMC/0-Start.html. These retrospective studies were approved by the
Fig. 6 (A) Cytogenetic and molecular results for patient 96932. (a) The karyotype was revealed by G-banding. (b) NGS detected chromosome X and Y. (B) Cytogenetic and molecular results for patient 172990. (a) The karyotype was revealed by G-banding. (b) NGS identified duplication on chromosome 9. (c) The location of the sSMC on chromosome 9 is highlighted in red. (C) Cytogenetic result for patient 70963. (a) The karyotype was revealed by G-banding. (b) The location of the sSMC on chromosome 20 is highlighted in red.
ethical commission of the Shengjing Hospital of China Medical University (NO.2019PS423K).

**Chromosome karyotyping**
Patients’ peripheral blood and amniotic fluid samples were cultured, harvested, and stained with Giemsa (G-banded) (at the resolution of approximately 300–400 bands) following the standard protocols. Then scanned in Lieca Cyto Vision (German) and analyzed according to the ISCN 2013.

**STR and AZF detection**
In our department first-generation sequencing (FGS) (3730 DNA Analyzer, Singapore) was used to detect STR on five chromosomes (13, 18, 21, X, and Y in amniotic fluid), AZF(Yq11.2) and SRY(Yp11.31) of Y (in azoospermia adult). DNA was extracted by kit (BioBase, Chengdu, China) in Auto-Pure32A (ALL SHENG, Hangzhou, China), mixed with sequencing primers and Tag DNA Polymerase (Transgen, Beijing, China), and then did PCR (S1000 Thermal Cycler) and sequenced.

**MLPA**
MLPA was performed in FGS (3730 DNA Analyzer, Singapore) by the protocol of “SALSA® MLPA® P245 Microdeletion Syndromes-1” kit (MRC Holland, Amsterdam, the Netherlands). The preparation of DNA samples was same as STR. MLPA could suggest 23 kinds of deletion or duplication syndrome. Sequencing primers were illustrated in protocol, including one of Xp21.1, three of Xq28, three of 15q11.2 (one UBE3A probe and two SNRPN probes), and one (Y-fragment S0135-
L16766) for the Y chromosome. MLPA data were presented with ratio.

Next generation-sequencing
NGS was performed in accordance with the protocols of a commercial NGS sequencing kit (Berry Genomics, Hangzhou, China). DNA samples were prepared by the extract kit (Axygen, MA, USA), purified and enriched library, then sequenced in the Illumina NextSeq CN500 (Berry Genomics, Hangzhou, China). Sequencing data were analyzed with Software VI (Berry Genomics, Hangzhou, China) in h19 database, blasted and searched information of disease in DGV, DECIPHER, OMIM, UCSC and Pubmed. Data were presented with log2 ratio or copy numbers (SCN).

Abbreviations
sSMC: Small supernumerary marker chromosomes; NGS: Next-generation sequencing; MLPA: Multiplex ligation-dependent probe amplification; STR: Short tandem repeats; AZF: Azoospermia factor; FGS: First-generation sequencing; NT: Nuchal translucency; NIPT: Non-invasive prenatal testing; MGS: Mixed gonadal dysgenesis; ART: Assisted reproductive technology; PGS: Preimplantation genetic screening; PGD: Preimplantation genetic diagnosis; LCRs: Low copy repeats

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Authors’ contributions
YanZ and TL conceived and designed the study. HS participated in data analysis and performed statistics. TL, GC, YZ, MQ, XL and WC performed the genetic diagnosis. TL analyzed data and drafted the manuscript. YanZ and TL and revised it. All authors read and approved the final manuscript.

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

Ethics approval and consent to participate
This retrospective study was approved by the ethical commission of the Shengjing Hospital of China Medical University (NO.2019PS423K).

Consent for publication
Not applicable.

Competing interests
The authors have no conflicts of interest to declare.

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References
1. Jafari-Ghahfarokhi H, Moradi-Chaleshtori M, Liehr T, et al. Small supernumerary marker chromosomes and their correlation with specific syndromes. Adv Biomed Res. 2015;4:140.
2. Hochstenbach R, Nowakowska B, Volleth M, et al. Multiple small supernumerary marker chromosomes resulting from maternal meiosis I or II errors. Mol Syndromol. 2016;6(5):210–21.

3. Bartels I, Schlueter G, Liehr T, et al. Supernumerary small marker chromosome (SMC) and uniparental disomy 22 in a child with confined placental mosaicism of trisomy 22: trisomy rescue due to marker chromosome formation. Cytogenet Genome Res. 2003;101:103–5.

4. Kerkhof J, Schenkel LC, Reilly J, et al. Clinical validation of copy number variant detection from targeted next-generation sequencing panels. J Mol Diagn. 2017;19(6):905–20.

5. Sebold C, Roeder E, Zimmerman M, et al. Tetrasomy 18p: report of the molecular and clinical findings of 43 individuals. Am J Med Genet A. 2010;152A(9):2164–72.

6. Bawazeer S, Alshalan M, Alkhaldi A, et al. Tetrasomy 18p: case report and review of literature. Appl Clin Genet. 2018;11:9–14.

7. Yu XW, Wei ZT, Jiang Y, et al. Y chromosome azoospermia factor region microdeletions and transmission characteristics in azoospermic and severe oligozoospermic patients. Int J Androl. 2015;38(9):1463–14646.

8. Yu TY, Lin HS, Chen PL, et al. An isodicentric X chromosome with gonadal dysgenesis in a lady without prominent somatic features of Turner’s syndrome. A case report. J Formos Med Assoc. 2015;114(1):77–80.

9. Evers C, Jungwirth MS, Morgensteller J, et al. Craniofrontonasal syndrome in a male due to chromosomal mosaicism involving EFNB1: further insights into a genetic paradox. Clin Genet. 2014;85(4):347–53.

10. Chauhan P, Jaiswal SK, Lakhotia AR, et al. Molecular cytogenetic characterization of two Turner syndrome patients with mosaic ring X chromosome. J Assist Reprod Genet. 2016;33(9):1161–8.

11. Bertelloni S, Dati E, Valetto A, et al. Long-term growth hormone treatment in a boy with 45,X/46,XiY(idYP) mixed gonadal dysgenesis: comparison with growth pattern of an untreated patient. Hormones (Athens). 2015;14(1):142–7.

12. Fukui S, Watanabe M, Yoshino K, et al. 45,X mosaicism with Y chromosome presenting female phenotype. J Pediatr Surg. 2015;50(7):1220–3.

13. Bonuccelli A, Valetto A, Orsini A, et al. Maternally derived 15q11.2–q13.1 duplication in a child with Lennox-Gastaut-type epilepsy and dysmorphic features: clinical-genetic characterization of the family and review of the literature. J Pediatr. 2017;173(2):155–60.

14. Bartolini L, Santoni S, Lenzin E, et al. De novo trisomy 20p characterized by array comparative genomic hybridization: report of a novel case and review of the literature. Gene. 2013;524(2):368–72.

15. Avila M, Kirchhoff M, Malte N, et al. Delineation of a new chromosome 20q11.22 duplication syndrome including the ASXL1 gene. Am J Med Genet A. 2013;161A(7):1594–8.

16. Abu-Amero KK, Hemani AM, Salih MA, et al. A de novo marker chromosome derived from 9p in a patient with 9p partial duplication syndrome and autism features genotype-phenotype correlation. BMC Med Genet. 2010;11:135.

17. Huick PJ, Noonan KM, Kulkarni S, et al. Cytogenetic and array-CGH characterization of a complex de novo rearrangement involving duplication and deletion of 9p and clinical findings in a 4-month-old female. Cytogenet Genome Res. 2009;126(3):305–12.

18. Wenger TL, Miller JS, DePolo LM, et al. 22q11.2 duplication syndrome: elevated rate of autism spectrum disorder and need for medical screening. Mol Autism. 2016;7:27.

19. Nguyen LT, Fleshman R, Flynn E, et al. 22q11.2 microduplication syndrome with associated esophageal atresia/tracheo-esophageal fistula and vascular ring. Clin Case Rep. 2017;5(3):351–6.

20. Lohmann L, Chelloug N, Rossales B, et al. Dentric marker derived from chromosome 22 associated with mild clinical signs: a case report. Prenat Diagn. 2000;20(2):156–8.