Fetal Aberrant Right Subclavian Artery: Associated Anomalies, Genetic Etiology, and Postnatal Outcomes in a Retrospective Cohort Study

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Abstract

Aberrant right subclavian artery (ARSA) is becoming more common in fetuses. However, there are relatively few studies on the genetic etiology of ARSA. We performed genetic analysis on fetuses with ARSA and followed up the pregnancy outcome to evaluate the prognosis of the fetuses, providing information for prenatal and eugenics consultations. A retrospective study was conducted on 112 pregnant women with fetuses diagnosed with ARSA from December 2016 to February 2021. Karyotype analysis and single-nucleotide polymorphism array (SNP-array) were performed in 112 fetuses. The 112 fetuses were divided into two groups: ARSA group, 48 (42.9%) and ARSA with other ultrasound abnormalities group, 64 (57.1%) cases. The total rate of pathogenic copy number variation (CNV) was 7.1% (8/112) using karyotype analysis (3/8) and SNP-array (5/8). The rate of pathogenic CNV in isolated ARSA and ARSA combined with other ultrasound abnormalities were 4.2% (2/48) and 9.4% (6/64), respectively. There was no significant difference between the two groups ($P=0.463$). The results of genetic analysis influence parents' decision to terminate the pregnancy. During follow-up, fetuses with ARSA without pathogenic CNV were found to have normal growth and development after birth. Therefore, prenatal genetic counseling and SNP-array should be recommended to better assess fetal prognosis.

Introduction

Aberrant right subclavian artery (ARSA) is one of the common congenital anomalies of the aortic arch, accounting for 0.5–1.4% of healthy people [1–3]. ARSA can either be a normal vascular variation or part of a complex heart malformation or genetic syndrome. Normal ARSA arises from the aortic arch, one of the branches of the cephalic arm trunk, and is the first branch of the aortic arch. ARSA originates at the beginning of the descending aorta, and after diverging from the left subclavian artery, the fourth branch of the aortic arch passes close to the trachea and goes to the right shoulder [4].

In recent years, with the rapid development of prenatal ultrasound diagnosis in maternal and fetal medicine, more fetal structural malformations can be diagnosed in the prenatal period, which can help clinicians better formulate further scientific and effective diagnosis and treatment plans, reduce the occurrence of adverse pregnancy outcomes, and enhance the prevention and control of birth defects [5]. ARSA usually has no apparent clinical symptoms; however, in rare cases, it can compress the esophagus or trachea, causing difficulty swallowing or breathing [6]. Prenatal ultrasound can accurately diagnose fetal ARSA. It has been reported that fetuses with ARSA have an increased risk of trisomy 21 syndrome and copy number variation (CNV) [7–10]. It is believed that ARSA can be used as one of the soft ultrasound indicators for prenatal screening of fetal chromosomal abnormalities [11].

In this study, fetuses with ARSA diagnosed by prenatal ultrasound were included, and genetic etiology of fetuses with ARSA were detected by karyotype analysis and single-nucleotide polymorphism array (SNP-array). This research aimed to analyze the correlation between ARSA and genetic etiology, postnatal outcome and prognosis, and provide information for prenatal and eugenics consultations.

Materials And Methods

Participants

A retrospective study was conducted on 112 pregnant women with intrauterine fetuses diagnosed with ARSA during ultrasound examination in Fujian Maternal and Child Health Hospital from December 2016 to February 2021. The age of pregnant women ranged 18–46 years, with an average age of 28 ± 5 years. The gestational age of pregnant women ranged from 17 weeks to 35 weeks, with an average gestational age of 24.6 weeks. After the pregnant women and their family members signed the informed consent, amniotic fluid or cord blood was collected for karyotype analysis and SNP-array according to different gestational weeks. The 112 cases were divided into the ARSA group (48 cases) and ARSA combined with other ultrasonic abnormalities group (64 cases). This study was approved by the Ethics Committee of Fujian Maternal and Child Health Hospital, and the subjects signed the informed consent forms. Confirms that all experiments were performed in accordance with relevant named guidelines and regulations.

Karyotype analysis

The amniotic fluid or cord blood samples of 112 fetuses with ARSA were cultured, harvested, prepared, and g-banded according to the conventional methods established by our center [12]. Karyotype was collected and analyzed by GSL-120 automatic chromosome scanning platform. Twenty karyotypes were counted in each case, five were analyzed, and the count and analysis were increased in case of abnormality.

SNP-array

Genomic DNA was extracted from fetal cells using QIAamp DNA Blood Mini Kit. Digestion, amplification, purification, fragmentation, labeling, hybridization with microchips, washing, scanning, and data analysis of genomic DNA samples were performed according to the instructions provided by Affymetrix. Cyto Scan HD chip consisting of CNV and SNP-array probes, which can detect not only CNV but also chimera (chimera ratio > 10%) and loss of heterozygosity (LOH). Chromosome analysis suite (ChAS) V3.2 was used to analyze the results, and related databases were used to analyze the CNV properties. CNV can be divided into five categories [13, 14]: pathogenic CNV, likely pathogenic CNV, variants of uncertain clinical significance CNV (VUS), likely benign CNV, benign CNV. Regarding VUS, SNP-array is recommended for peripheral blood samples of parents, combined with pedigree analysis, to further clarify the nature of CNV.

Follow-up of postnatal outcome

All cases were followed up by telephone to understand the information of ARSA fetal development, pregnancy outcome, and postpartum growth and development.

Statistical analysis

SPSS 25.0 software was used for statistical analysis of the data. The detection rate of pathogenic CNV between groups was statistically analyzed by chi-square test (Fisher's test), and the difference was considered statistically significant when $P < 0.05$.

Results

ARSA associated with cardiac and other ultrasound abnormalities in fetuses

Among the 112 fetuses with ARSA, 48 (42.9%, 48/112) were in the ARSA group and 64 (57.1%, 64/112) in the ARSA with other ultrasound abnormalities group. In the group of ARSA combined with other ultrasound abnormalities, 17 cases (15.2%, 17/112) were associated with congenital heart defects, and 47 cases (42.0%, 47/112) were associated with extracardiac abnormalities. Ventricular septal defect (9.8%, 11/112) was the most common in fetuses with congenital heart defects, and ultrasonographic soft markers (34.8%, 39/112) were the most common in fetuses with extracardiac abnormalities (Table 1).

Table 1
Fetuses (64 cases) with ARSA combined with other ultrasound abnormalities

Classification	Number of fetuses
Combined with congenital heart defects	17
Ventricular septal defect	11
Left aortic arch	3
Pulmonary stenosis	2
Hydropericardium	1
Combined with extracardiac abnormalities	47
Ultrasonographic soft markers	39
Intrauterine growth restriction	1
Central nervous system	1
Acromphalus	1
Urogenital system	2
Polyhydramnios	1
Cleft lip	1
Strephenopodia	1

Karyotype analysis

Chromosome karyotype analysis was performed successfully in all 112 samples, and a total of three abnormalities (2.7%, 3/112) were detected, including one case of trisomy 21, one case of trisomy 18, and

one case of large fragment duplication (46, XY, add (22)(q12). All three fetuses with ARSA and chromosomal abnormalities were associated with other ultrasound abnormalities (Table 2).

Table 2
Karyotype analysis detected in fetuses with ARSA

case	karyotype analysis	SNP-array	Ultrasonic phenotype	P classification
1	47,XY,+21	arr[hg19] (21)x3	ARSA, Nasal bone dysplasia, the transparent layer of the neck thickened, mild tricuspid regurgitation	P
2	47,XY,+18	arr[hg19] (18)x3	ARSA, ventricular septal defect, pulmonary artery widening with little pulmonary valve regurgitation	P
3	46,XY,add(22)(q11)	arr[hg19]22q11.1q11.21(16,888,899-18,649,190)x4	ARSA, ventricular septal defect	P
ARSA: Aberrant right subclavian artery, P: pathogenic.				

Results of SNP-array

SNP-array was performed on 112 samples, and abnormal results were detected in 10 cases (8.9%, 10/112), including eight cases of pathogenic CNV and two cases of VUS (Tables 2 and 3). The eight cases of pathogenic CNV included two cases of aneuploidy, one case of large fragment duplication, two cases of microdeletion, two cases of microduplication, and one case of LOH.

The total detection rate was 7.1% (8/112) using karyotype analysis and SNP-array. Results of the two methods were identical in three cases, including two cases of aneuploidy and one case of large fragment duplication (Table 2). SNP-array also detected five additional cases of pathogenic CNV and two additional cases of VUS. Of the five pathogenic CNV fetuses, two were isolated ARSA, and the other three were fetuses with ARSA associated with other ultrasound abnormalities (Table 3).

Table 3
SNP-array detected in fetuses with ARSA

case	SNP-array	Size (Mb)	Ultrasonic phenotype	P classification	Inheritance
1	arr[hg19] 16p11.2(28,786,703-29,032,280)x3	0.2	ARSA	P	Maternal
2	arr[hg19]1p36.21p35.2(15,728,288-31,781,279)x2 hmz, 4p15.2p11(25,981,952-49,063,479)x2 hmz	16, 23	ARSA	P	-
3	arr[hg19] 17p12p11.2(15,759,453-20,547,625)x3	4.7	ARSA, Strephenopodia	P	-
4	arr[hg19]22q11.21(18,636,749-21,800,471)x1	3.16	ARSA, double renal pelvis separation	P	-
5	arr[hg19] 2q13(111,397,196-113,111,856)x1	1.7	ARSA, ventricular septal defect	P	Maternal
6	arr[hg19]7q34(139,340,641-139,769,640) ×3	0.4	ARSA	VUS	Maternal
7	arr[hg19]4q24(106,284,925-107,545,257)x3	1.2	ARSA, ventricular septal defect	VUS	Maternal
ARSA: Aberrant right subclavian artery, P: pathogenic, VUS: variants of uncertain clinical significance.					

Comparison of pathogenic CNV between ARSA and ARSA combined with other ultrasound abnormalities

The fetuses were divided into two groups: ARSA group and ARSA with other ultrasound abnormalities. Among the 48 cases of the ARSA group, two cases of pathogenic CNV were detected, with a positive rate of 4.2%. Regarding the 64 cases of ARSA combined with other ultrasound abnormalities, six cases of pathogenic CNV were detected, with a positive rate of 9.4%. Although the pathogenic CNV in the ARSA combined with other ultrasound abnormalities was higher than that in the ARSA group, and there was no statistical significance between the two groups ($P=0.463$, $P>0.05$) (Table 4).

Table 4
Phenotypic characteristics of 112 fetuses with ARSA

Classification	Number of fetuses	Number of pathogenic CNV
Fetuses with ARSA	48	2(4.2%)
Fetuses with ARSA and other ultrasound abnormalities	64	6(9.4%)
Total	112	8(7.1%)
ARSA: aberrant right subclavian artery, CNV: copy number variation. Fisher's test, $P=0.463$, $P > 0.05$.		

Follow-up of postnatal outcome

Of the 112 fetuses with ARSA, 110 fetuses with ARSA were successfully followed up except two fetuses lost to follow-up. The parents of eight fetuses with pathogenic CNVs chose to terminate the pregnancies. In addition, there were four cases of fetuses with ARSA, although the karyotype analysis and SNP-array were normal, the ultrasound showed severe malformations, and the parents of the fetuses also chose to terminate the pregnancies. The parents of the two fetuses with VUS chose to continue their pregnancies, and the fetuses had good growth during postnatal follow-up. The remaining 96 fetuses with ARSA with normal karyotype analysis and SNP-array all grew well after birth.

Discussion

With the development of ultrasound technology and improvement of people's understanding regarding fetal ARSA, the prenatal detection rate of ARSA is increasing daily. In this study, 112 fetuses were diagnosed with ARSA by prenatal ultrasound. Trisomy 21 syndrome has been reported in 14–20% of fetuses with ARSA [15, 16]. Thus, ARSA is closely related to chromosomal abnormalities. In this study, karyotype analysis and SNP-array were used to detect the genetic etiology of 112 fetuses with ARSA. Chromosomal abnormalities were detected in three cases (2.7%, 3/112) by karyotype analysis. The rate of chromosome abnormalities in this study was significantly lower than those reported in the literature [17]. However, SNP-array was used to detect five additional cases of pathogenic CNV, including two cases of microdeletion, two cases of microduplication, and one case of LOH. Conventional karyotype analysis can only detect chromosome fragment abnormalities of over 5-10MB, while SNP-array can detect low copy number abnormalities of over 10KB and normal copy number abnormalities such as LOH [18, 19]. Therefore, SNP-array has advantages in the etiological detection of fetuses with ARSA.

In this study, a total of eight cases of pathogenic CNV were detected in 112 fetuses with ARSA, including trisomy 21, trisomy 18, large fragment 22q12 duplication, 22q11.21 microdeletion, 17p12p11.2 microduplication, 16p11.2 microduplication, 2q13 microdeletion, and LOH of 1p36.21p35.2 and 4p15.2p11. The presence of ARSA may increase the risk of trisomy 21 syndrome. In this study, one case of trisomy 21 syndrome was detected in a fetus with ARSA, which is consistent with previous studies [8, 17, 20–22]. ARSA has also been reported in 22q11 deletion syndrome (22q11DS) [23]. The manifestations

of patients with 22q11DS were varied, mainly including congenital heart defects, thymus hypoplasia, parathyroid dysfunction with hypocalcemia, and developmental delay [24, 25]. Moreover, patients with 22q11DS may have vascular abnormalities, such as the right aortic arch and ARSA [26–29]. One case of 22q11DS was also detected in a fetus with ARSA in this study. The 17p12p11.2 microduplication contains the RAI1 gene [30], which can lead to the occurrence of Potocki-Lupski syndrome. The main clinical characteristics of Potocki-Lupski syndrome are abnormal heart development, low intelligence, triangular face, high zygomatic arch, and palatal dysplasia [31]. To date, no studies have reported the relationship between Potocki-Lupski syndrome and ARSA; however, in this study, one case of 17p12p11.2 microduplication was detected in a fetus with ARSA. Similarly, there is no relevant research on the relationship between 16p11.2 microduplication, 2q13 microdeletion, and LOH of 1p36.21p35.2 and 4p15.2p11 and ARSA, which needs to be confirmed by more researchers in the future.

Chaoui et al. [32], Rembouskos et al. [21], and Gul et al. [20] each reported a case of isolated ARSA with trisomy 21. Therefore, some scholars believe that ARSA can be used as one of the soft ultrasound markers for prenatal screening of fetal chromosome abnormalities, and prenatal chromosome examination should be recommended even if it is found alone. However, other studies do not recommend invasive prenatal testing for fetuses with isolated ARSA unless accompanied by other ultrasound abnormalities [17, 33]. Combined with the results of our study, two fetuses with isolated ARSA were detected with 16p11.2 microduplication, and LOH of 1p36.21p35.2 and 4p15.2p11, respectively. The rate of pathogenic CNV in isolated ARSA group was 4.2% (2/48), the rate of pathogenic CNV in ARSA combined with other ultrasound abnormalities was 9.3% (6/64). Although the rate of the pathogenic CNV in ARSA combined with other ultrasound abnormalities was higher than that of the isolated ARSA group, there was no statistical significance between the two groups. Therefore, SNP-array should be recommended for ARSA with other ultrasound abnormalities in fetuses, although the possibility of isolated ARSA with pathogenic CNV should not be ignored.

In addition to being associated with pathogenic CNV, ARSA is also closely associated with congenital heart defects [34]. Borenstein et al. [35] reported that the incidence of ARSA with congenital heart defects reached 16%, but due to the small number of cases, the types of congenital heart defects commonly associated were not counted. In this study, the incidence of ARSA combined with congenital heart defects was slightly lower, and 17 cases (15.2%, 17/112) were combined with congenital heart defects, among which ventricular septal defect was the most common type. The results of this study showed that 34.8% (39/112) of fetuses with ARSA were associated with extracardiac abnormalities, among which the most common complication was abnormal soft ultrasound markers, and the risk of pathogenic CNV increased with other ultrasound abnormalities. In this study, pathogenic CNV was detected in six fetuses with ARSA combined with other ultrasound abnormalities, including three fetuses with congenital heart defects, two fetuses with abnormal soft ultrasound markers, and one fetus with strephenopodia. Therefore, when prenatal ultrasound detects ARSA in the fetus, it is necessary to be highly alert to the possibility of other ultrasound abnormalities and carefully observe the fetus for other ultrasound abnormalities.

VUS has always been controversial, and the detection rate of VUS depends on the type of microarray chip and the population studied [36, 37]. Some studies have reported that VUS accounts for 1–12% of the population [38], and this study found that VUS accounts for 1.8% (2/112), which is consistent with the literature. The two fetuses with VUS in this study had good growth and development during postnatal follow-up. The C-type vascular ring formed by ARSA belongs to the incomplete vascular ring, which partially surrounds the trachea and esophagus and usually does not cause compression to the trachea and esophagus [35]. In this study, 96 fetuses with ARSA excluding pathogenic CNV had good growth and development after birth and during follow-up after genetic counseling. However, there were four cases of fetuses with ARSA combined with other ultrasound abnormalities. Although the genetic analysis was normal, there were severe ultrasound abnormalities, and the parents chose to terminate the pregnancies. In recent years, next-generation sequencing has been used to detect single-gene mutations and copy number variation [39–41], which may provide a more comprehensive prenatal genetic diagnosis for fetuses with ARSA and a better evaluation of fetal prognosis.

In conclusion, ARSA is a common soft ultrasound marker. Isolated ARSA in fetuses has a low probability of pathogenic CNV. However, when ARSA is complicated with other ultrasound abnormalities, the risk of pathogenic CNV is greatly increased. Prenatal genetic counseling and SNP-array should be recommended to better assess fetal prognosis.

Declarations

Acknowledgments

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Declaration of Conflicting Interests

The authors declare that they have no competing interest.

Consent for publication

All authors consented to publish.

Data Availability Statement

All data generated during and/or analyzed during the current study are available upon request by contact the corresponding author.

Author contributions

MC wrote the manuscript. HH searched literature. XF collected data. XF and SX managed study. LX designed study. XC interpreted data. N L revised the manuscript.

References

1. Zalel, Y., Achiron, R., Yagel, S. & Kivilevitch, Z. Fetal aberrant right subclavian artery in normal and Down syndrome fetuses. *Ultrasound Obstet Gynecol*, **31** (1), 25–29 (2008).
2. Arribas, C. B., Alderete, F. G., Pérez, R., Ortiz-Quintana, L. & León-Luis, J. [Diagnosis of isolated fetal aberrant right subclavian artery]. *Ginecol Obstet Mex*, **80** (6), 425–429 (2012).
3. Zapata, H., Edwards, J. E. & Titus, J. L. Aberrant right subclavian artery with left aortic arch: Associated cardiac anomalies. *Pediatr Res*, **14** (3), 159–161 (1993).
4. Chaoui, R., Rake, A. & Heling, K. S. Aortic arch with four vessels: aberrant right subclavian artery. *Ultrasound Obstet Gynecol*, **31** (1), 115–117 (2008).
5. Carin, Y. Whole-exome sequencing on deceased fetuses with ultrasound anomalies: expanding our knowledge of genetic disease during fetal development. *Genet Med*, **19** (10), 1171–1178 (2017).
6. González-Sánchez, M., Pardal-Refoyo, J. L. & Martín-Sánchez, A. The Aberrant Right Subclavian Artery and Dysphagia Lusoria. *Acta Otorrinolaringol Esp*, **64** (3), 244–5 (2013).
7. Wang, X., Zhang, H., Jin, Y. & Lu, Z. Successful endovascular treatment of popliteal artery entrapment syndrome: a case report with 3-years follow-up. *J Thromb Thrombolysis*, **44** (5), 1–6 (2017).
8. Cavoretto, P., Scala, C., Ferrero, S., Greco, T. & Maggiore Aberrant right subclavian artery in fetuses with Down syndrome: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*, **46** (3), 266–276 (2015).
9. Cohen-Vig, L., Maya, I., Tenne, T., Yeshaya, J. & Sharony Chromosomal microarray analysis in fetuses with aberrant right subclavian artery. *Ultrasound Obstet Gynecol*, **49** (3), 337–341 (2017).
10. Ranzini, A. C., Hyman, F., Jamaer, E. & Mieghem, T. V. Aberrant Right Subclavian Artery: Correlation Between Fetal and Neonatal Abnormalities and Abnormal Genetic Screening or Testing. *J Ultrasound Med*, **36** (4), 785–790 (2017).
11. Kosinski, P., Agathokleous, M., Poon, L., Chaveeva, P. & Nicolaides, K. H. Meta-analysis of second-trimester markers for trisomy 21. *Ultrasound Obstet Gynecol*, **41** (3), 247–61 (2013).
12. Cai, M., Lin, N., Lin, Y., Huang, H. & Xu, L. Evaluation of chromosomal abnormalities and copy number variations in late trimester pregnancy using cordocentesis. *Aging (Albany NY)*, **12** (15), 15556–15565 (2020).
13. South, S. T., Lee, C., Lamb, A. N., Higgins, A. W. & Kearney, H. M. ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. *Genet Med*, **15** (11), 901–909 (2013).
14. Kearney, H. M., Thorland, E. C., Brown, K. K., Quintero-Rivera, F. & South, S. T. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. *Genet Med*, **13** (7), 680–685 (2011).
15. Verbitsky, M. *et al.* The copy number variation landscape of congenital anomalies of the kidney and urinary tract. *Nat Genet*, **51** (1), 117–127 (2019).

16. Song, M. J., Han, B. H., Kim, Y. H., Yoon, S. Y. & Bo, K. P. Prenatal diagnosis of aberrant right subclavian artery in an unselected population. *Ultrasonography*, **36** (3), 278–283 (2017).
17. Yazcolu, H. F. *et al.* Aberrant right subclavian artery in Down syndrome fetuses. *Prenat Diagn*, **33** (3), 209–13 (2013).
18. Angie, J. Chromosomal Microarray Analysis Results From Pregnancies With Various Ultrasonographic Anomalies. *Obstet Gynecol*, **133** (4), 827–828 (2019).
19. Ganapathi, M., Nahum, O. & Levy, B. Prenatal Diagnosis Using Chromosomal SNP Microarrays. *Methods Mol Biol*, **1885**, 187–205 (2019).
20. Gul, A., Cor Ba Cioglu, A., Ba Kirci, I. T. & Ceylan, Y. Associated anomalies and outcome of fetal aberrant right subclavian artery. *Arch Gynecol Obstet*, **285** (1), 27–30 (2012).
21. Rembouskos, G. *et al.* Aberrant right subclavian artery (ARSA) in unselected population at first and second trimester ultrasonography. *Prenat Diagn*, **32** (10), 968–975 (2012).
22. Paladini, D. *et al.* Aberrant right subclavian artery: incidence and correlation with other markers of Down syndrome in second-trimester fetuses. *Ultrasound Obstet Gynecol*, **39** (2), 191–195 (2012).
23. Svirsky, R., Reches, A., Brabbing-Goldstein, D., Shira, A. & Yaron, Y. Association of aberrant right subclavian artery with abnormal karyotype and microarray results. *Prenat Diagn*, **37** (8), 808–811 (2017).
24. Lopez-Rivera, E. *et al.* Genetic Drivers of Kidney Defects in the DiGeorge Syndrome. *N Engl J Med*, **376** (8), 742–754 (2017).
25. Haskololu, Z. U. Chromosome 22q11.2 Deletion Syndrome (DiGeorge Syndrome/Velocardiofacial Syndrome). *Turkish Journal of Immunology*, **2** (3), 57–66 (2014).
26. Hachamdiolu, B., Hachamdioglu, D. O. & Delil, K. 22q11 deletion syndrome: Current perspective. *Appl Clin Genet*, **8**, 123 (2015).
27. Habel, A., Herriot, R., Kumararatne, D., Allgrove, J. & Tsai-Goodman, B. Towards a safety net for management of 22q11.2 deletion syndrome: guidelines for our times. *Eur J Pediatr*, **173** (6), 757–765 (2014).
28. Cancrini, C., Puliafito, P. & Digilio, M. C. Clinical Features and Follow-Up in Patients with 22q11.2 Deletion Syndrome. *J Pediatr*, **164** (6), 1475–1480 (2014).
29. Botto, L. D. *et al.* A population-based study of the 22q11.2 deletion: phenotype, incidence, and contribution to major birth defects in the population., **112** (1), 101 (2003).
30. Joseph, T. *et al.* Copy number loss upstream of RAI1 uncovers gene expression regulatory region that may impact Potocki-Lupski syndrome diagnosis. *Molecular cytogenetics. Mol Cytogenet*, **8**, 75 (2015).
31. Twentyman, L. R. Neurodevelopmental Disorders Associated with Abnormal Gene Dosage: Smith–Magenis and Potocki–Lupski Syndromes. *J Pediatr Genet*, **4** (03), 159–167 (2015).
32. Chaoui, R. *et al.* Aberrant right subclavian artery as a new cardiac sign in second- and third-trimester fetuses with Down syndrome. *Digest of the World Latest Medical Information*, **192** (1), 257–263 (2017).

(2005).

33. León-Luis, J. D. *et al.* Second-trimester fetal aberrant right subclavian artery: original study, systematic review and meta-analysis of performance in detection of Down syndrome. *Ultrasound Obstet Gynecol*, **44** (2), 147–153 (2014).
34. Pico, H. *et al.* Prenatal Associated Features in Fetuses Diagnosed with an Aberrant Right Subclavian Artery. *Fetal Diagn Ther*, **40** (3), 187–194 (2016).
35. Borenstein, M., Minekawa, R., Zidere, V., Nicolaides, K. H. & Allan, L. D. Aberrant right subclavian artery at 16 to 23 + 6 weeks of gestation: a marker for chromosomal abnormality. *Ultrasound Obstet Gynecol*, **36** (5), 548–552 (2010).
36. Ronald, J. *et al.* Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis. *Obstet Gynecol Surv*, **68** (4), 2175 (2013).
37. Ganapathi, M., Nahum, O. & Levy, B. Prenatal Diagnosis Using Chromosomal SNP Microarrays. *Methods Mol Biol*, **1885**, 187–205 (2019).
38. Wapner, R. I., Martin, C. L., Levy, B. & Ballif, B. Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis. *N Engl J Med*, **367** (23), 2175–2184 (2012).
39. Spa, F. *et al.* Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study - ScienceDirect.Lancet. 2019
40. Lord, J., McMullan, D. J. & Eberhardt, R. Y. Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study., **393** (10173), 747–757 (2019).
41. Wapner, R. *et al.* Whole exome sequencing in the evaluation of fetal structural anomalies: A prospective study of sequential patients. *Am J Obstet Gynecol MFM*, **216** (1), S5–S6 (2017).