Chromosomal Microarray Analysis of Fetuses with Nasal Bone Anomaly

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Research

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Abstract

Objective: To explore the significance and value of fetal nasal bone anomaly (absence or hypoplasia) as indications of prenatal diagnosis.

Methods: A total of 102 fetuses diagnosed with nasal bone absence or hypoplasia by ultrasonography underwent chorionic, amniotic, or umbilical cord blood puncture. Single nucleotide polymorphism microarray (SNP-array) was used to analyze fetal chromosomes.

Results: Of the 102 fetuses with nasal bone absence or hypoplasia, 25 (24.5%) had chromosomal abnormalities, including 15 cases of trisomy 21, one trisomy 18 case, and 9 cases of other copy number variations. Among the 52 cases with isolated nasal bone absence or hypoplasia, 7(13.5%) had chromosomal abnormalities. In 50 cases, abnormal nasal bone with additional soft markers or structural abnormalities was observed, while 18 cases (36.0%) had chromosomal abnormalities, which were significantly higher than that among the fetuses with isolated nasal bone abnormality.

Conclusion: Fetal nasal bone absence or hypoplasia can be used as an indication for prenatal diagnosis. The detection rate of chromosomal abnormalities increases with additional soft markers or structural abnormalities. This study demonstrates that fetal nasal bone absence or hypoplasia is associated with micro-deletions or micro-duplications of chromosomes. Application of single nucleotide polymorphism microarray (SNP-array) technology can reduce the rate of missed prenatal diagnoses.

1 Introduction

At present, routine prenatal ultrasound scanning has become an integral component of antenatal care. In addition to fetal ultrasond structural abnormalities, many ultrasond soft markers are closely related to fetal chromosomal abnormalities. Fetal nasal bone anomalies which include both nasal bone absence and hypoplasia has been proved to be a more significant indicator of fetal aneuploidy, especially Down's syndrome [1–4]. The likelihood ratio of nasal bone absence or hypoplasia in patients with Down syndrome has previously been reported to be 11.6–50.5 [5, 6].

Single nucleotide polymorphism microarray (SNP-array) is a high-resolution technology for whole genome.It allows the detection of micro-deletions micro-duplications and uniparental disomy(UPD) that are not routinely seen on karyotyping[7]. It is possible to investigate the whole genome for copy number variations, as small as 50–100 kb, with a 100-fold magnification in resolution compared with standard G-band karyotyping[8, 9].

In this retrospective study, single nucleotide polymorphism microarray was used to detect the chromosome of 102 fetuses with abnormal nasal bone development. The relationship between nasal bone development abnormalities and chromosomal aberrations was investigated based on the results of the SNP-array. At the same time, our study was performed to explore the necessity of prenatal diagnosis with fetal nasal bone abnormality.

2 Methods

2.1 Study population

This study included a group of 102 pregnant women admitted to Fujian Provincial Maternity and Children's Hospital from May 2017 to December 2019. All fetuses in this group were diagnosed with fetal nasal bone absence or hypoplasia by ultrasonographic screening. Among the 102 pregnant women, the average age was 30.8 years, ranging from 22 to 42 years. Gestational age ranged from 13 to 34 weeks.

2.2 Interventional surgery

Under the guidance of ultrasonography, 2 study participants underwent choriocentesis at 9–13 weeks of gestation, 84 participants underwent amniocentesis at 18 to 24 weeks of gestation, and umbilical vein puncture was used on 16 participants after 24 weeks of gestation.

2.3 SNP-array and interpretation of SNP-array results

Fetal DNA was extracted using the QIAGEN DNA mini kit (250; Qiagen, Valencia, CA, USA). DNA samples were then scanned and analyzed using the CytoScan 750K (Affymetrix Inc., CA, USA) gene chip detection platform. Copy number variation (CNV) thresholds were set to report deletions greater than 200 Kb or duplications greater than 500 Kb. Data was analyzed using Chromosome Analysis Suite (ChAS) software by Affymetrix. SNP-array results were assessed in reference to the following databases: Database of Genomic Variants (DGV), Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER), International Standards for Cytogenomic Arrays concortium (ISCA), and Online Mendelian Inheritance in Man (OMIM). Peripheral blood from the parents of the fetus will be extracted for SNP-array detection when appropriate. Systematic evaluation of CNV clinical significance were refered to database, literatures and ultrasonography finding.

2.4 Statistical analyses

Data were expressed by frequency and rate, and the chi-square test was used to compare the differences between groups. $P < 0.05$ was considered statistically significant.

3 Results

3.1 SNP-array detection results

Of the 102 fetuses with nasal bone absence or hypoplasia, 25 (24.5%) were detected to have chromosomal abnormalities. Of these cases, there were 15 trisomy 21 (14.7%), one case of trisomy 18 (1.0%), and 9 cases (8.8%) of other copy number variations. Of the 9 copy number variation cases, 4 were pathogenic and we observed a 2.0 Mb deletion within chromosome 15q13.2q13.3 (Fig. 1), a 14.2 Mb deletion in the p15.31p14.3 region combined with a 3.1 Mb deletion in the q14.3 region of chromosome 15 (Fig. 2**(a)**, **2(b)**), a 37 Mb deletion in chromosome 4p16.3p14 (Fig. 3), a 2.6 Mb deletion in chromosome 15q24.1q24.2 (Fig. 4). In these 4 cases, both parents showed negative SNP-array results,

indicating that the CNV were *de novo*. One case carried a 994 Kb deletion in chromosome 16p12.2 (Fig. 5) which involved susceptibility sites for neurocognitive impairment, likely pathogenic, but parental verification was refused. Another 2 cases carried an 869 Kb deletion in chromosome 15q26.1 (Fig. 6) and a 422 Kb duplication in chromosome 15q13.3 (Fig. 7), respectively. The same variation was confirmed in both parents by family verification and the mutations were considered as likely benign. The SNP-array results of the remaining 2 cases revealed a 908 Kb duplication in chromosome 3p25.3p25.2 (Fig. 8) and a 1.4 Mb duplication in chromosome 16p13.13p13.12 (Fig. 9). The clinical significance of these two CNV was uncertain and the parents rejected family validation.

3.2 Relationship between ultrasonography and chromosomal anomaly

Among 102 cases with fetal nasal bone absence or hypoplasia, 52 cases had solitary nasal bone anomaly, while 7 of the 52 cases had chromosomal abnormalities (13.5%). Additional soft ultrasound markers or other structural abnormalities were detected in 50 cases, and 18 of those had chromosomal abnormalities (36.0%). A significantly higher rate of chromosomal abnormalities ($\chi^2 = 7.0$, $P < 0.05$) was observed in fetuses with nonsolitary nasal bone anomaly when compared with those with solitary nasal bone anomaly (Table 1). Ultrasonography results of the 25 fetuses with chromosomal abnormalities are summarized in Table 2.

Table 1
The detection rates of chromosomal abnormalities in fetuses with solitary nasal bone anomaly and nonsolitary nasal bone anomaly

Ultrasonography findings	Normal	Abnormal	Total	Detection rate (%)
Solitary nasal bone anomaly	45	7	52	13.5%[a]
Nonsolitary nasal bone anomaly	32	18	50	36.0%[b]

* There was a significant difference between the detection rate of solitary nasal bone anomaly ([a]) compared with nonsolitary nasal bone anomaly ([b]) ($\chi^2 = 7.0$, $P < 0.05$).

Table 2
Ultrasonography findings and SNP-array detection results of 25 fetuses with abnormal chromosomes

Ultrasonography findings	SNP-array results	Numbers	Pregnancy outcomes
Solitary nasal bone anomaly	arr[hg19](21)x3	4	TOP
	arr[hg19]3p25.3p25.2(11,583,017 – 12,491,402)x3	1	Live birth
	arr[hg19]15q13.2q13.3(30,386,398 – 32,444,261)x1 dn	1	TOP
	arr[hg19]15q13.3(32,021,609 – 32,444,043) x3 mat	1	Live birth
Nonsolitary nasal bone anomaly			
+FGR, ASD, SUA	arr[hg19](18)x3	1	TOP
+Bilateral ventricular choroid plexus cyst	arr[hg19](21)x3	1	TOP
+Intestinal hyperechogenicity; Left ventricular echogenic foci	arr[hg19](21)x3	1	TOP
+Bilateral renal parenchymal hyperechogenicity; Liver parenchyma echo thickened	arr[hg19](21)x3	1	TOP
+ Increased NF thickness; aberrant right subclavian artery	arr[hg19](21)x3	1	TOP
+ Abnormal blood flow signals in the right chamber; right coronary right atrial fistula cannot be excluded; Left ventricular echogenic foci	arr[hg19](21)x3	1	TOP
+FL/BPD64% (71–87%), FL/HC18% (19–21%); cardiac malformation: VSD, aortic straddle, pulmonic stenosis; duodenal stenosis or atresia; hydramnios	arr[hg19](21)x3	1	TOP
+ Increased NT thickness; ECD; systemic hydroderma; deeper notchα-wave of the venous catheter	arr[hg19](21)x3	1	TOP
+ Increased NT thickness	arr[hg19](21)x3	4	TOP

TOP, termination of pregnancy; FGR, fetal growth restriction; ASD, atrial septal defect; SUA, single umbilical artery; NF, nuchal fold; FL, femur length; BPD, biparietal diameter; VSD, ventricular septal defect; NT, nuchal translucency; ECD, endocardial cushion defect; AC, abdomen circumference; SD, standard deviations.

Ultrasonography findings	SNP-array results	Numbers	Pregnancy outcomes
+ Increased NT thickness	arr[hg19]5p15.31p14.3(8,701,919 – 22,948,182)x1, 5q14.3(85,819,360 – 88,999,900)x1 dn	1	TOP
+Oligoamnios	arr[hg19]16p12.2(21,816,542 – 22,710,614)x1	1	Continued gestation
+Lagger cholecyst	arr[hg19]16p13.13p13.12(11,528,493 – 12,934,811)x3	1	Live birth
+VSD	arr[hg19]15q26.1(90,211,822 – 91,080,606)x1 pat	1	Live birth
+ Cardiac malformation: coarctation of aorta, smaller left ventricle; increased NF thickness; SUA	arr[hg19]4p16.3p14(68,345 – 37,141,015)x1 dn	1	TOP
+ AC was less than 2 SD below the normal predicted value; increased cardiothoracic ratio, VSD, pulmonic stenosis with incomplete closure, narrow inner diameter of the aortic arch, small amount of pericardial effusion; intestinal hyperechogenicity; oligoamnios	arr[hg19]15q24.1q24.2(72,965,465 – 75,567,135)x1 dn	1	TOP

TOP, termination of pregnancy; FGR, fetal growth restriction; ASD, atrial septal defect; SUA, single umbilical artery; NF, nuchal fold; FL, femur length; BPD, biparietal diameter; VSD, ventricular septal defect; NT, nuchal translucency; ECD, endocardial cushion defect; AC, abdomen circumference; SD, standard deviations.

4 Discussion

Previous studies have shown that fetal nasal bone anomaly can be used as an ultrasound soft marker for screening of fetal chromosomal aberrations, particularly trisomy 21 syndrome. Nasal bone abnormality is closely associated with Down syndrome in fetuses with special facial features such as low and flat nasal root, widened eye distance, and glassy eyes [2, 10–13]. In this study, chromosome abnormalities were found in 25 (24.5%) of 102 fetuses with abnormal nasal bone development, among which the majority (n = 15) had trisomy 21. The ratio was similar to some reports at home and abroad [5, 10, 14].

Fetal nasal bone anomaly has been reported to be associated with other rare diseases or syndromes with facial deformities such as Cri du chat (5p-) syndrome, Wolf-Hirschhorn (4p-) syndrome, and Fryns syndrome [1, 15, 16]. In our study, copy number variation was detected in 9 cases (8.8%), 4 of which were clearly pathogenic, resulting in diseases that also overlapped with the above reports. The first case was a 2.0 Mb deletion in chromosome 15q13.2q13.3. This missing region contains 7 OMIM genes and can cause 15q13.3 micro-deletion syndrome which is manifested as developmental retardation, mild to

moderate learning disabilities, epilepsy, finger and toe deformities, and minor facial abnormalities. Another case had a 14.2 Mb deletion in the p15.31p14.3 region combined with a 3.1 Mb deletion in the q14.3 region of chromosome 15. The former partially overlaps with Cri du chat (5p-) syndrome and contains 20 OMIM genes including CTNND2 which is associated with mental retardation in Cri du chat syndrome. The latter contains 5 OMIM genes, including RASA1 and MEF2C, which are related to mental retardation, epilepsy, hypotonia, vascular malformation and more. One fetus with a 37 Mb deletion in chromosome 4p16.3p14 includes 115 OMIM genes and covers a key pathogenic region for Wolf-Hirschhorn syndrome. The last case had a 2.6 Mb deletion in the 15q24.1q24.2 region and includes 30 OMIM genes associated with 15q24 micro-deletion syndrome.

In addition to the four clearly pathogenic cases mentioned above, we found an additional case with a likely pathogenic mutation. This case carried a 994 Kb deletion in 16p12.2 which is a susceptibility site of neurocognitive disorder. The region contained 3 OMIM genes, including UQCRC2, EEF2K, and CDR2, which are found in less than 1% of the general population. Though the penetrance of the mutation was reportedly 12%, incomplete penetrance and variable expressivity make it difficult to predict the postnatal outcome [17], and family validation was refused. But the pathogenicity will remain unclear even if family verification is performed. This CNV was also found to be inherited in many cases from a phenotypically normal or only a mildly affected parent complicating phenotypic association and causality.

Four cases with uncertain clinical significance were detected in total. Of these cases, 2 (908 Kb duplication in 3p25.3p25.2 and 1.4 Mb duplication in 16p13.13p13.12), did not include parental verification and pathogenicity could not be determined. The other 2 cases carrying an 869 Kb deletion in 15q26.1 and a 422 Kb duplication in 15q13.3, respectively, were verified to be from parents with normal phenotypes. The q26.1 region of chromosome 15 contains 23 OMIM genes, and the fetuses carrying this CNV only showed signs of ventricular septal defects during ultrasonography not including nasal bone hypoplasia. The fetus with the duplication in 15q13.3, which harbors 2 OMIM genes (OTUD7A and CHRNA7), only displayed nasal bone abnormality during ultrasonography. This CNV partially overlapped with the recurrent region 15q13.3 when referring to the ClinGen database and the relapsed region had a triple dose effect score of 1 with an estimated penetrance of 5–10%. Fetuses with this variation develop phenotypes such as speech disorders, cognitive impairment, epilepsy, schizophrenia, and autism [18–22] although the pathogenicity of both of the above mutations remains unclear because they were found in the general population as well as in reportedly healthy parents [23]. We can only infer that it is potentially benign based on the results of family verification and ultrasonography. Family verification is known to be important for variants of unknown significance (VUS). Previously, It was reported that VUS accounted for about 1.6–4.2% of total CNV [7, 24, 25] and was reduced to less than 1% by parental diagnoses although the clinical significance of 0.5-1% remains unclear [26].

In this retrospective study, 52 cases of solitary nasal bone anomaly were detected, of which 7 (13.5%) were chromosome aberrations. There were 50 cases of nonsolitary nasal bone anomaly, 18 (36.0%) of which had chromosomal abnormalities. The detection rate of nonsolitary nasal bone anomaly was significantly different from solitary nasal bone anomaly ($\chi^2 = 7.0, P < 0.05$), consistent with the results of

Ting et al [27]. We considered that the risk of chromosomal abnormalities was not limited to solitary nasal bone anomaly but was significantly increased when combined with other structural malformations. The detection of chromosomal abnormalities may also be related to ultrasonographic anomalies or a combination of prenatal diagnosis indications. Therefore, we believe that prenatal diagnosis is feasible for fetuses with nonsolitary nasal bone anomaly, and solitary nasal bone absence or hypoplasia cannot be ignored. The high prevalence of chromosomal abnormalities (13.5%) demonstrates that doctors experienced in genetic counseling should inform patients of the relationship between nasal bone anomaly and chromosomal aberrations. We recommend that doctors pay close attention to other indications for prenatal diagnosis, such as high risk for serology screening or age, then continue to follow-up and fetal chromosome testing when necessary.

In recent years, the application of SNP-array technology has greatly improved the detection rate of prenatal diagnosis of chromosomal abnormalities. Unlike traditional karyotype analysis, SNP-array can detect both micro-deletions and micro-duplicates less than 5 Mb and uniparental disomy (UPD). In our study, 9 cases had CNVs in the genome and only 1 case was missing a large segment. We observed 1 case missing a large fragment combined with a micro-deletion. The remaining 7 cases had submicroscopic chromosomal aberrations which were not detected by karyotype analysis. Among them, only 2 were likely to be benign and inherited from parents, 2 were VUS, and the remaining 3 CNVs were reported as clearly or likely pathogenic. SNP-array proved to be a necessary and effective supplement to prenatal diagnosis. Chromosome micro-array analysis technology should be the preferred diagnostic method for genetic testing, even when fetal abnormal soft markers or other structural abnormalities can be detected by prenatal ultrasound [28–30].

Abbreviations

SNP: single nucleotide polymorphism; CNV: Copy number variation; DGV: Database of Genomic Variants; DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources; ISCA: International Standards for Cytogenomic Arrays concortium; OMIM: Online Mendelian Inheritance in Man; UPD: uniparental disomy; TOP: termination of pregnancy; FGR: fetal growth restriction; ASD: atrial septal defect; SUA: single umbilical artery; NF: nuchal fold; FL: femur length; BPD: biparietal diameter; VSD: ventricular septal defect; NT: nuchal translucency; ECD: endocardial cushion defect; AC: abdomen circumference; SD: standard deviations

Declarations

Acknowledgements

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Authors’ contributions

Xiaorui Xie and Liangpu Xu designed the research; Linjuan Su and Meiying Cai performed experiments; Xiaoqing Wu and Hailong Huang analyzed the data; Xiaorui Xie wrote the manuscript; Ying Li provided the genetic counseling; all authors reviewed and approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present study was approved by the Protection of Human Ethics Committee of Fujian Provincial Maternity and Children's Hospital, affiliated Hospital of Fujian Medical University. Written informed consent for participation was received for all patients.

Competing interests

The authors declare no conflict of interest.

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Figures

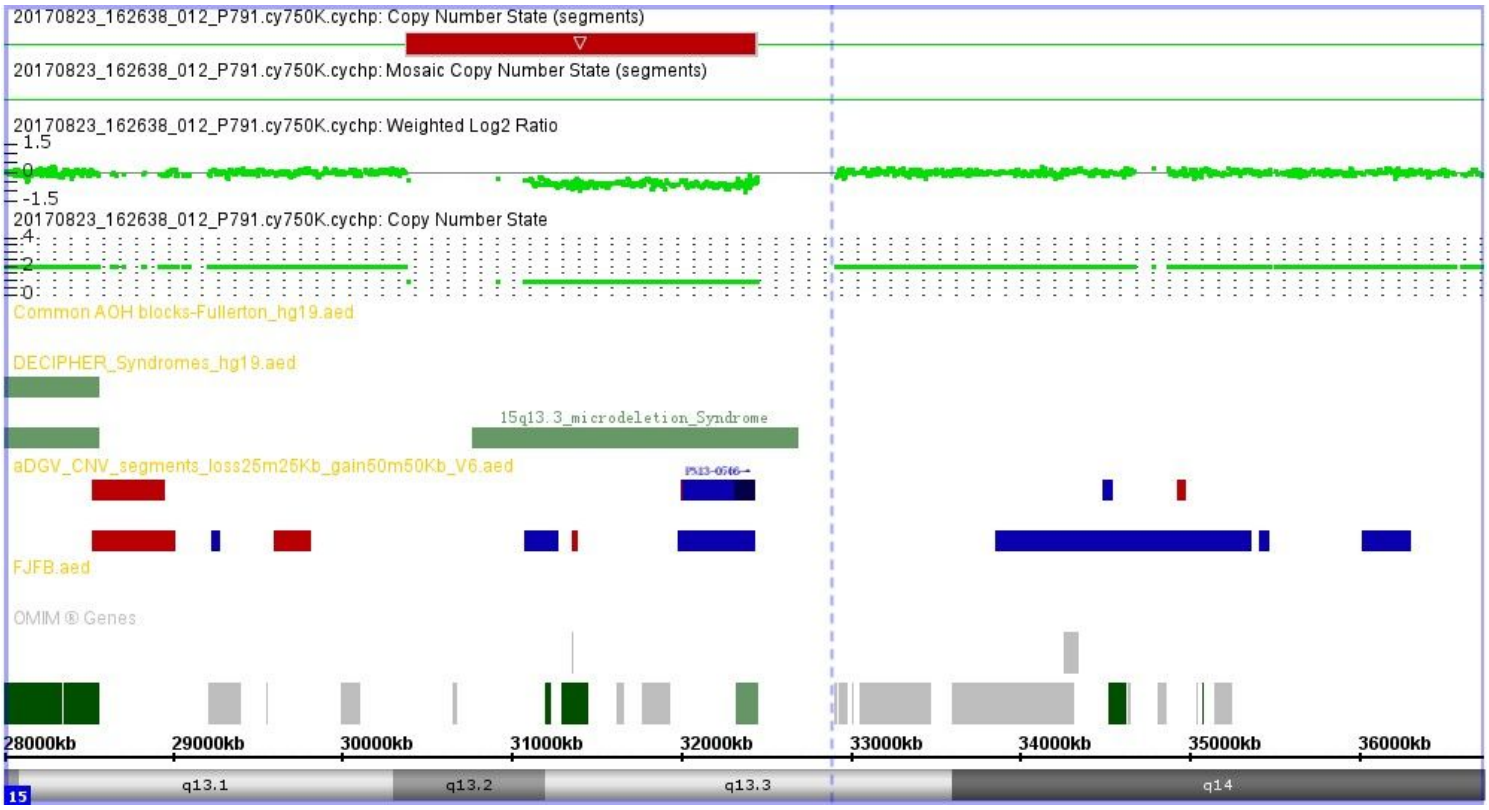

Figure 1

Deletion in the 15q13.2q13.3 region

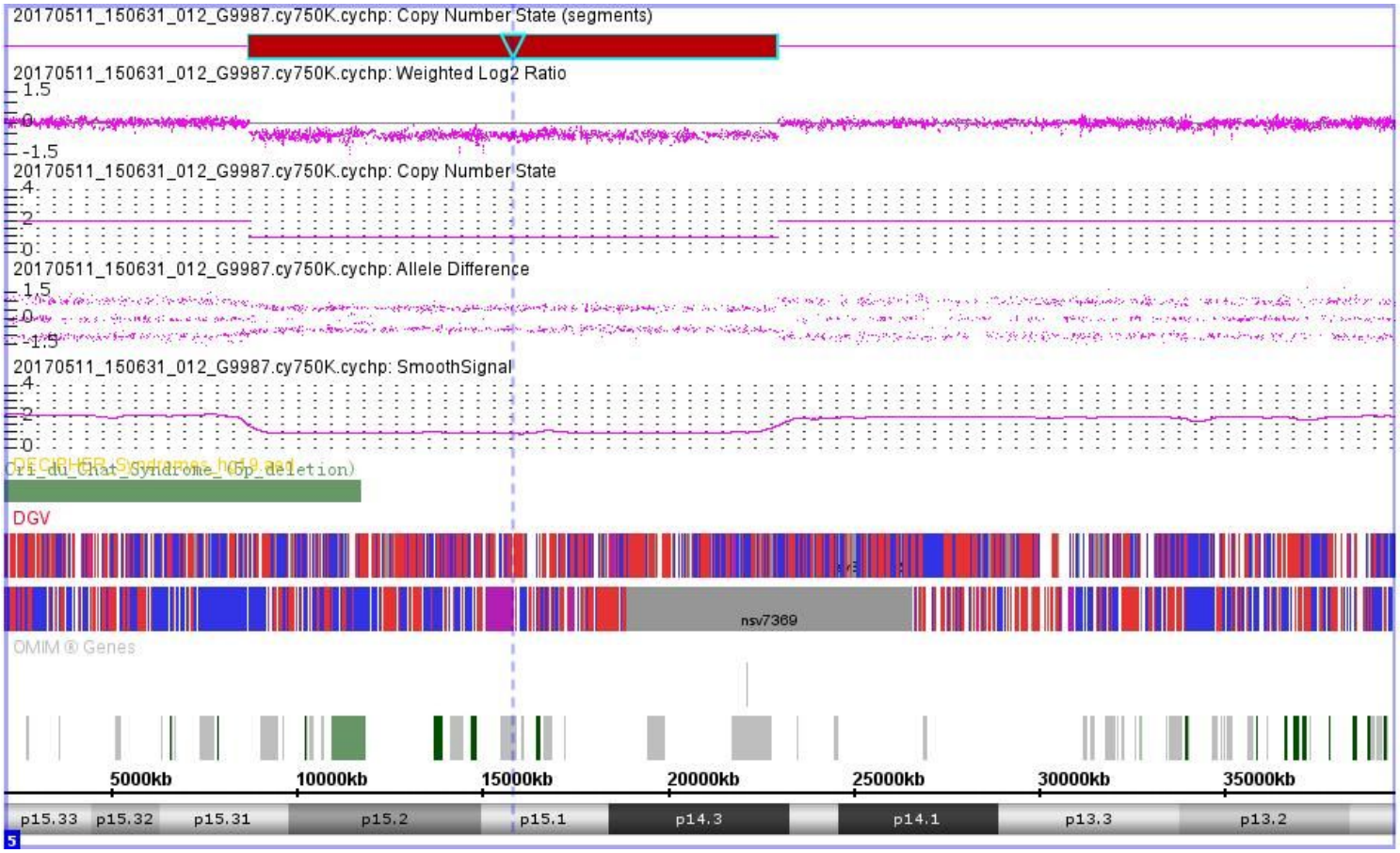

Figure 2

Deletion in the 5p15.31p14.3 region

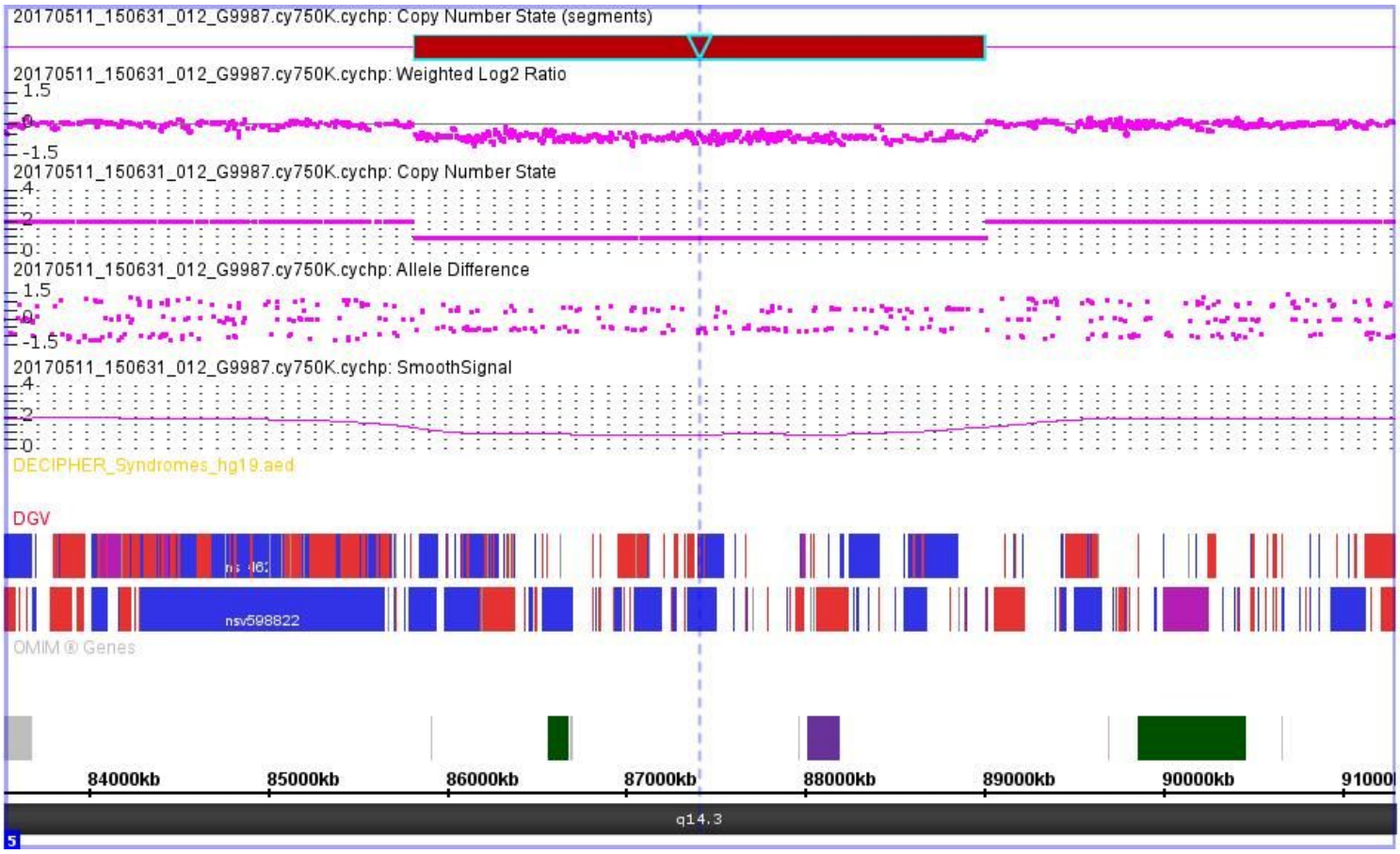

Figure 3

Deletion in the 5q14.3 region

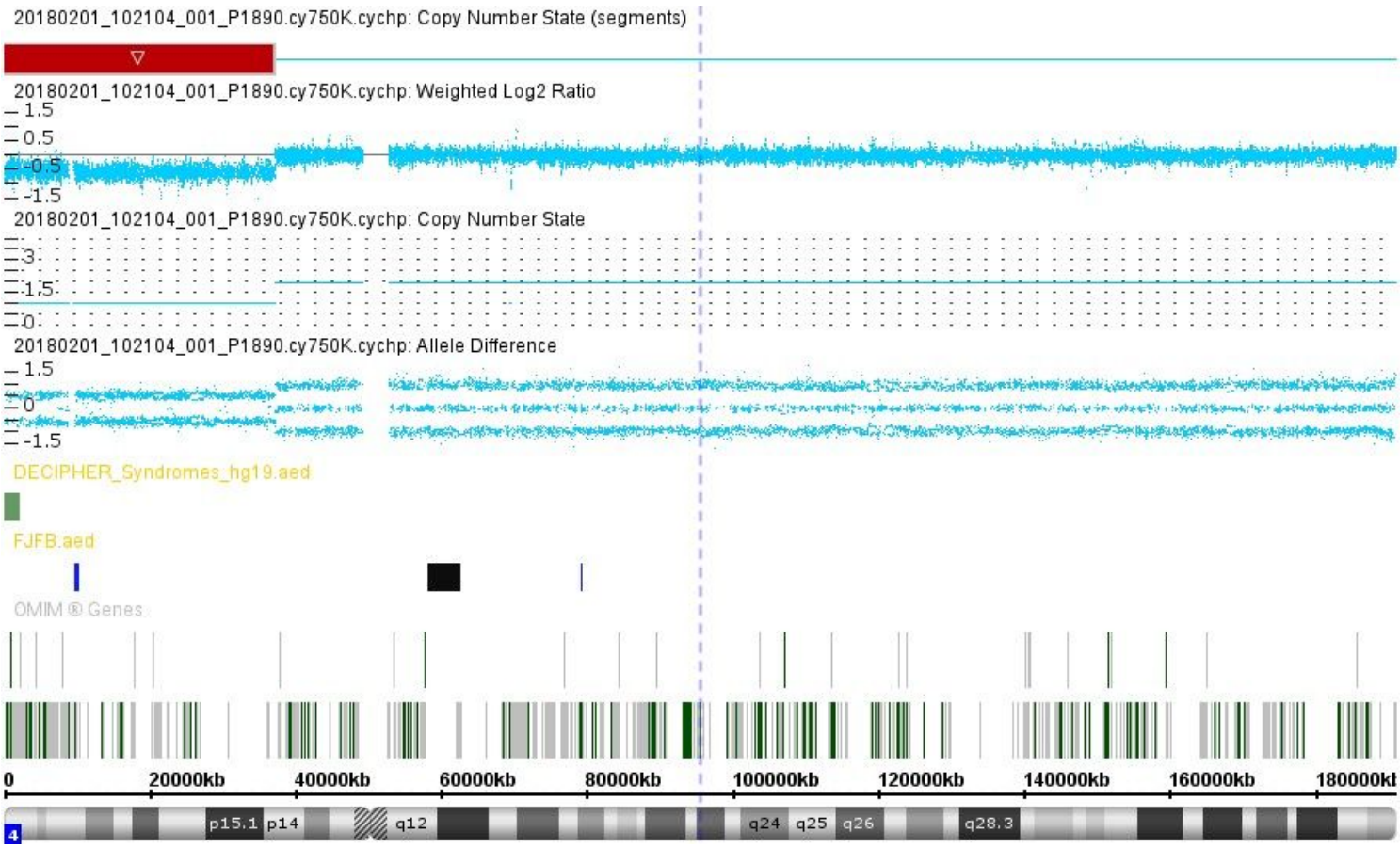

Figure 4

Deletion in the 4p16.3p14 region

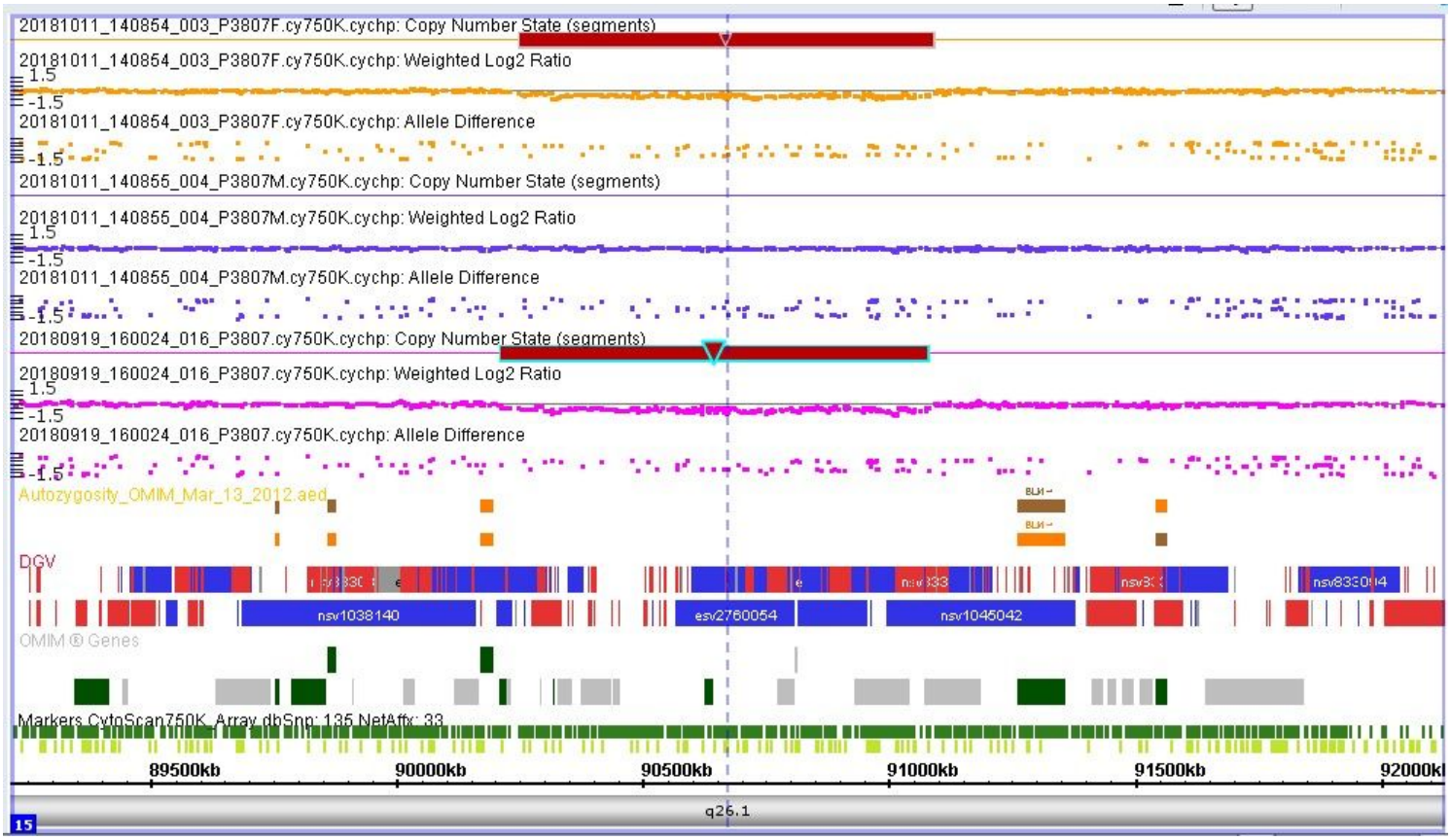

Figure 5

Deletion in the 15q24.1q24.2 region

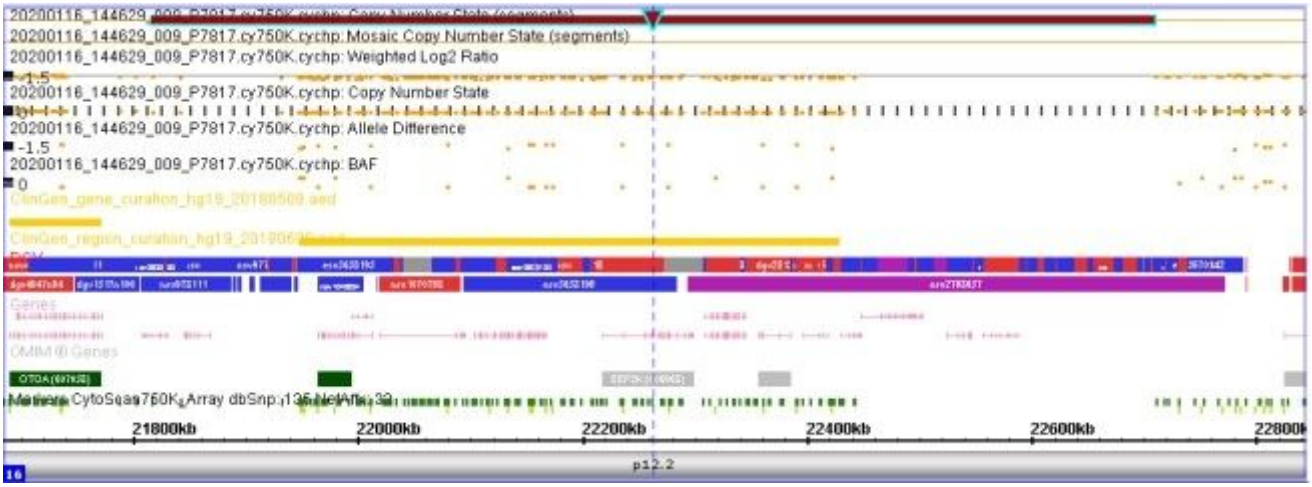

Figure 6

Deletion in the 16p12.2 region

Figure 7

Deletion in the 15q26.1 region

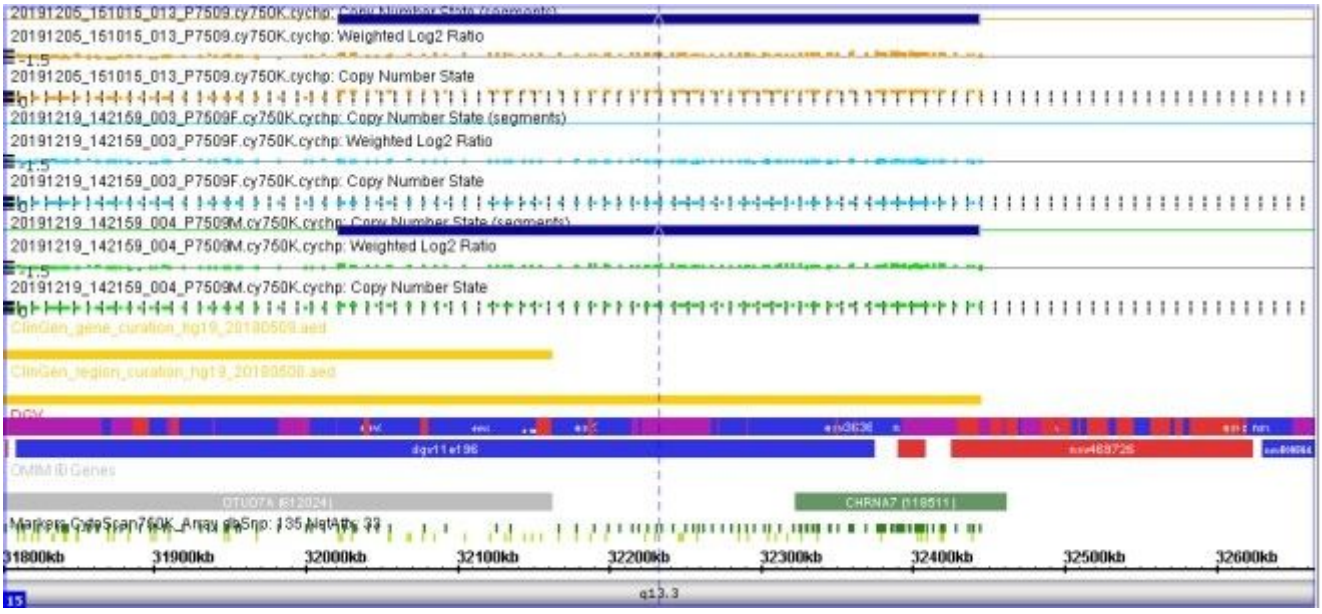

Figure 8

Duplication in 15q13.3 region

Figure 9

Duplication in the 3p25.3p25.2 region

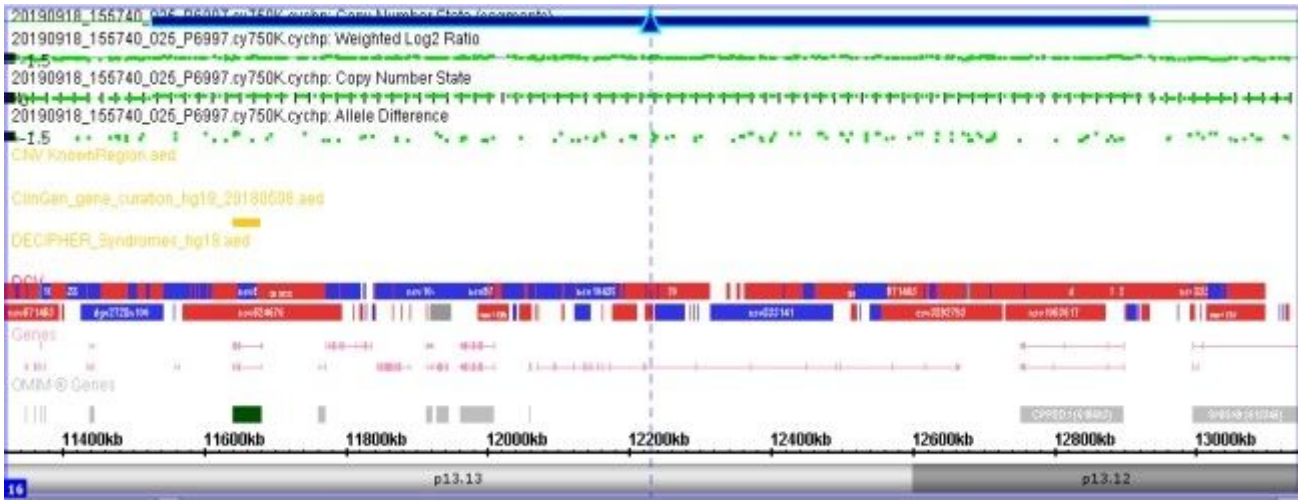

Figure 10

Duplication in the 16p13.13p13.12 region