Copy number variations of chromosome 17p11.2 region in children with development delay and in fetuses with abnormal imaging findings

Yuanyuan Zhang

Shengjing Hospital of China Medical University

Xiaoliang Liu

Shengjing Hospital of China Medical University

Haiming Gao

Shengjing Hospital of China Medical University

Rong He

Shengjing Hospital of China Medical University

Guoming Chu

Shengjing Hospital of China Medical University

Yanyan Zhao (✉ yyzhao@sj-hospital.org)

Shengjing Hospital of China Medical University

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Abstract

Background: Deletion and duplication of the -3.7 Mb region in 17p11.2 result in two reciprocal syndromes, Smith-Magenis syndrome and Potocki-Lupski syndrome, which are well-known development disorders. The purpose of this study is to identify the prevalence, genetic characteristics and clinical phenotype of 17p11.2 deletion/duplication in Chinese children with development delay and in fetuses with potential congenital defects.

Methods: 7077 children with development delay and/or intellectual disability were screened by multiplex ligation-dependent probe amplification (MLPA) P245 assay. 7319 fetuses with potential congenital defects were detected using next generation sequencing (NGS) technique.

Results: 417 of 7077 children patients were identified to carry chromosome imbalance. Among them, 28 (28/7077, 0.4 %) cases had imbalance at chromosome 17p11.2, of which 12 cases (42.9 %) had heterozygous deletions and 16 (57.1 %) had heterogeneous duplications. The phenotypes of these 28 children were variable, including neurobehavioral disorders, craniofacial/skeletal anomalies, immunologic defects, ocular problems, and organ malformations. 263 of 7319 fetuses were recognized to have genomic copy number variations (CNV). Only 2 of them were found to harbor 17p11.2 imbalance. The fetus with deletion presented with ventricular septal defect and the fetus with reciprocal duplication had cerebral ventricle dilation.

Conclusion: Our study highlights the phenotypic variability associated with 17p11.2 variations in China. The results further expand the phenotypic spectrum of SMS/PTLS and increase awareness of these disruptive mutations among clinicians.

Background

Chromosome 17 band p11.2 is an unstable region that is prone to nonallelic homologous recombination (NAHR). This can produce recurrent deletion or duplication within the region and contribute to copy number variation (CNV) of corresponding gene clusters [1]. Genomic disorders map to this region are two reciprocal syndromes, Smith-Magenis syndrome (SMS, OMIM #182290) and Potocki-Lupski syndrome (PTLS, OMIM #610883) [2-3], which are caused in most cases by an approximately 3.7 Mb deletion or duplication [4]. SMS is a well known and complex neurobehavioral disorder characterized by developmental delay, intellectual disability, sleep disturbances and self-injurious behaviors [5-6]. Patients generally have craniofacial features [6]. While, the symptoms of PTLS have recently been recognized and include infantile hypotonia, sleep apnea, congenital cardiovascular anomalies, learning disabilities, short stature, and failure to thrive [7].

The incidences of SMS and PTLS are predicted to be 1 in 25,000 [8]. However, they are likely to be underestimated, given the poor resolution of conventional G-band karyotyping analysis in detecting subtle abnormalities. G-band karyotyping has been the method of choice for cytogenetic test, accurately detecting chromosomal aberrations larger than 5 Mb [9]. Before the utilization of array comparative genomic hybridization (aCGH) and next generation sequencing (NGS) in the clinic laboratory, multiplex ligation-dependent probe amplification (MLPA) assay is widely used to detect chromosome sub-microscopic deletions and duplications of targeted regions [10-11]. Specifically, the MLPA P245 kit is designed to screen patients presenting with developmental delay and/or intellectual disability for 31 kinds of common microdeletion or microduplication syndromes. P245 probemix includes 3 probes in 17p11.2 region, which are RAI1, LRRC48 (DRC3) and LLGL1. Ratio anomalies of these probes indicate copy number variations at chromosome 17p11.2.

In this study, we presented an overview of the genetic and clinical characteristics of Chinese patients that were identified to carry 17p11.2 imbalance by MLPA and NGS. The aim of this study was to estimate the prevalence and detailed clinical characteristics of 17p11.2 deletions/duplications in children with development delay as well as in fetuses with potential congenital defects, which could contribute to provide basic information for clinical evaluation.

Methods

Patients and samples

From July 2013 to December 2020, 7077 children patients were enrolled through the outpatients of pediatrics and clinic genetics of Shengjing Hospital of China Medical University. Most of these cases presented with mental retardation and/or development delay. 7319 pregnant women, who required amniocentesis for invasive prenatal diagnosis based on guideline published by Association for Clinical Cytogenetics (Prenatal Diagnosis Best Practice Guidelines 2009 v1.00), were recruited from outpatient of obstetrics from April 2018 to December 2020. The whole peripheral blood and amniotic fluid were retrieved from clinic genetics of Shengjing Hospital. This study was approved by Ethics Committee of Shengjing Hospital of China Medical University. Written informed consent to participate was obtained from all of the participants in this study (written informed consent to participate of individuals younger than the age of 16 was obtained from their parents or legal guardians).

DNA extraction

Genomic DNA was extracted from the whole peripheral blood and amniotic fluid using Automatic nucleic acid extractor (Allsheng Auto-Pure 32A) with UPure Blood DNA Extraction Kit (M2002-A32) and UPure Tissue DNA Extraction Kit (M2012-02) (BioBase Technologies Co., LTD) following the

manufacturer's instructions. The concentration of DNA was detected using a spectrophotometry method (NanoDrop 1000, Thermo Scientific, USA). A concentration of 10–50 ng/μL DNA was prepared for MLPA and NGS assay.

Multiplex Ligation-dependent Probe Amplification (MLPA) assay

MLPA assay and the subsequent results analyses were performed as previously described [12]. Briefly, The SALSA MLPA KIT P245 (MRC Holland, Amsterdam, Netherlands) were used for MLPA analysis according to the manufacturer's instructions. PCR amplification products were separated by capillary electrophoresis using ABI 3730 Genetic Analyser (Applied Biosystems, USA). Coffalyser. Net software (MLPA Holland, Amsterdam, Netherlands) was used to analyze and give an interpretation of the raw MLPA data. The detailed information of the probes included in MLPA kits was described in the manufacture's instructions.

Chromosome karyotype analysis

Conventional chromosome G-band karyotyping analysis of the cultures of peripheral blood and amniotic fluid blood cells was performed as previously described [12]. Leica CytoVision (Leica, USA) was used to capture images of mitotic metaphase chromosomes. The karyotyping results were identified and described upon agreement of the two examiners, with reference to the International System for Human Cytogenetic Nomenclature (ISCN 2016) [13].

Next generation sequencing (NGS) assay

Copy number variation sequencing was performed with next generation sequencing (NGS) as previously described [14]. Briefly, the DNA libraries were constructed using library prep kit (Berry Genomics). Then the DNA libraries were purified using the Purification DNA Libraries for NGS kit (Berry Genomics) and quantitated using the Kapa SYBR fast qPCR kit (Kapa Biosystems), with the standard of greater than 25nmol/L. The quantitated DNA libraries were pooled and loaded into Illumina Nextseq CN500 flow cells, and then were sequenced using the single-ended 36-bp sequencing protocol. No less than 2.5 million unique reads were analyzed by Illumina Sequencing Analysis Viewer software provided by Berry Genomics.

Results

Molecular analysis

MLPA P245 assay was used to screen on 7077 children patients with mental retardation and/or development delay. Totally, 417 (417/7077, 5.9 %) patients were found to carry chromosome imbalance and 41 (41/417, 9.8 %) patients had imbalance at chromosome 17. Among the 41 patients, 28 (28/41, 68.3%) had 17p11.2 deletions or duplications (Figure 1A). Their ages ranged from 30 days to 10 years. 12 of the 28 (42.9 %) patients had heterozygous deletions and the left 16 (57.1 %) patients had heterozygous duplications. RAI1, LRRC48 and LLGL1 probes were abnormal in all the patients with 17p11.2 imbalance. In particular, one child with 17p11.2 duplication was found to harbor three additional probes (CLDN5, GP1BB and SNAP29) abnormalities, suggesting that he also had 22q11.2 duplication. Using P245 assay, we confirmed that his 22q11.2 duplication was inherited from his mother, while the 17q11.2 duplication was de novo. In the same way, MLPA analyses of the other parental blood revealed no abnormality at 17p11.2. Figure 2 showed representative data from three patients and one normal control analyzed by MLPA. Moreover, 9 of the 28 patients were confirmed by NGS for more detailed information about sizes of imbalance. The results showed that the affected regions were all within chr17p11.2: 16560001-20520000 in the reference genome (GRCh7/hg19). Sizes of imbalance were mostly 3.4 Mb and 3.7 Mb, with the smallest 2.82 and the largest 3.96 Mb.

On the other side, 7319 fetuses with potential congenital defects were detected by NGS using DNA from amniotic fluid cells. 263 (263/7319, 3.6 %) samples were found to have chromosomal aberrations, and 24 (24/263, 9.1 %) of which were identified to harbor chromosome 17 imbalance. Only 2 (2/24, 8.3%) fetuses had 17p11.2 variations (Figure 1C). One had a 3.40 Mb deletion spanning an interval of 16820001-20220000, and the other one had a 3.42 Mb duplication ranging from 16800001 to 20220000 (Figure 3). NGS analyses of their parental blood indicated no abnormality at 17p11.2. In addition to 17p11.2, other loci at chromosome 17 were found to be affected in this fetal cohort including 17q12, 17p12, 17q11.2 and 17p13.3. Particularly, the frequencies of 17q12 and 17p12 were much higher than that of 17p11.2. And the number of cases with 17p11.2 was similar to that with 17q11.2 and 17p13.3. However, among children with development delay, 17p11.2 variation was more common than 17p13.3 and 17q11.2. The numbers of cases with various kinds of chromosome 17 chromosomal aberrations were detailed in Figure 1B and 1D.

Karyotype analysis

Conventional chromosome G-band karyotyping analysis of these 30 samples with 17p11.2 deletions/duplications showed normal karyotypes as 46, XX or 46, XY. Although in some cases, genomic imbalance were suspected because the short arm of one of the two chromosome 17 was smaller than the other one, but this could also occur when the staining was not done properly or the chromosome arm was not fully extended. The results proved again that G-band karyotyping analysis was not a first-line test in the diagnosis of 17p11.2 abnormalities.

Phenotype analysis

Among those 12 children with 17q11.2 deletions, the most common phenotype was neurobehavioral disorder, including speech and language development delay (8/12, 66.7 %), gross motor development delay (8/12, 66.7 %), intellectual disability (4/12, 33.3 %), hyperactivity (3/12, 25 %), attention deficit (3/12, 25 %). Besides, individual patients also had features of autism spectrum disorder (2), cognitive impairment (1), global developmental delay (1), temper tantrums (1) and sleep disturbance (1). Craniofacial and skeletal features were frequently observed that 5 (5/12, 41.7%) patients showed different appearance including wide eye distance, depressed nasal bridge, lateral canthus upwarping, square-shaped face, short stature, cleft lip/palate, tongue protrusion, antijaw, heavy eyebrows, cubitus valgus and rickets. Occasionally, some patients had immunologic defects and other manifestations containing cardiac defects, leukodystrophy, gallstone, tracheoesophageal fistula, arachnoid cyst of greater occipital pool. Detailed clinical information was listed in Table 1.

Table 1. Phenotypic characteristics of 13 cases with 17p11.2 deletion.

		17p11.2 deletion cases (13)														Number of cases
		1 F 7y	2 M 5y	3 M 10y	4 M 5y	5 M 5y	6 F 9y	7 F 30d	8 F 1y	9 M 19m	10 M 4y	11 M 3y	12 M 9y	13 Fetus		
Neurobehavioral	Speech and language development delay			x	x	x	x		x		x	x	x		8	
	Gross motor development delay			x	x	x	x		x	x		x	x		8	
	Intellectual disability			x	x	x	x								4	
	Hyperactivity			x	x		x								3	
	Attention deficit			x	x		x								3	
	Features of autism spectrum disorder										x	x			2	
	Cognitive impairment											x			1	
	Global developmental delay					x									1	
	Temper tantrums				x										1	
Craniofacial/ Skeletal	Sleep disturbance								x						1	
	Wide eye distance	x		x			x								3	
	Depressed nasal bridge	x													1	
	Lateral canthus upwarping						x								1	
	Square-shaped face												x		1	
	Cleft lip/palate							x							1	
	Tongue protrusion			x											1	
	Antijaw			x											1	
	Heavy eyebrows												x		1	
Immunologic	Short stature			x											1	
	Cubitus valgus			x											1	
	Rickets	x													1	
	Maxillary sinusitis		x									x			2	
	Sphenoid sinusitis		x												1	
	Others	Cardiac defects							x	x					x	3
		Leukodystrophy		x												1
		Gallstone		x												1
		Tracheoesophageal fistula							x							1
Arachnoid cyst of greater occipital pool												x			1	

The 16 patients with 17p11.2 duplications presented with similar spectrum of neurobehavioral disorders to reciprocal deletions. Most of them showed gross motor development delay (8/16, 50.0 %) and intellectual disability (8/16, 50.0 %). The symptoms of speech and language development delay (7/16, 43.8 %), global developmental delay (5/16, 31.2 %) and attention deficit (4/16, 25 %) were less common. Muscular hypertonia, stiff expression, hyperactivity, features of autism spectrum disorder and sleep disturbance could also be seen. Brain abnormalities and ocular defects were found in a smaller portion of patients. 4 patients had craniofacial and skeletal anomalies, including 3 cases of short stature and one case of ptosis. 2 of 10 male patients were diagnosed with cryptorchidism (See Table 2).

Table 2. Phenotypic characteristics of 17 cases with 17p11.2 duplication.

Phynotype		17p11.2 duplication cases (17)																		
		1 F 7y	2 M 5m	3 M 7y	4 M 16m	5 M 2y	6 M 2y	7 F 9y	8 F 9m	9 F 2y	10 F 1y	11 M 7y	12 M 8m	13 F 9y	14 M 40d	15 M 18m	16* M 9m	17 Fetus	Number of cases	
Neurobehavioral	Gross motor development delay	x			x		x		x		x	x				x	x		8	
	Intellectual disability	x		x	x	x		x			x		x				x		8	
	Speech and language development delay	x			x		x	x		x		x		x					7	
	Global developmental delay		x			x				x			x	x					5	
	Attention deficit	x		x							x		x						4	
	Muscular hypotonia								x			x					x		3	
	Stiff expression	x			x							x							3	
	Hyperactivity	x										x							2	
	Features of autism spectrum disorder						x												1	
Sleep disturbance		x																1		
Ocular	Strabismus							x				x							2	
	Amblyopia							x											1	
	Glaucoma											x							1	
Craniofacial/ Skeletal	Ptosis																x		1	
	Short stature									x			x	x					3	
Others	Cryptorchidism												x	x		x			2	
	Descending cerebellar tonsil	x																	1	
	Hydrocephalus	x																	1	
	Arachnoid cyst of greater occipital pool																x		1	
	Cerebral ventricle dilation																	x	1	

*This child had both 17p11.2 duplication and 22q11.2 duplication.

Fetal anomalies were usually difficult to determine and mostly suggested by imaging examination. Both of the two fetuses have abnormal imaging findings when they went through antenatal care. So they were speculated to have congenital defects. Specifically, the fetus with 17p11.2 deletion showed ventricular septal defect by three dimensional color Doppler ultrasound, while the fetus with reciprocal duplication had mild left ventricular dilatation detected by ultrasound as well as head CT scan.

Discussion

Deletion/duplication of chromosome 17p11.2 can lead to SMS/PTLS, which are multiple congenital anomalies usually recognized in infancy or early childhood because of craniofacial deformity and developmental delay. In our study, MLPA P245 assay was applied to screen in 7077 cases with intellectual disability and/or developmental delay. This cohort also included other phenotypes including congenital malformation, hypotonia and feeding difficulties, speech and motor deficits, cardiovascular defects, epilepsy, hearing impairment, craniofacial and skeletal features, and behavioral issues. The results revealed that 17p11.2 was the fourth frequent pathogenic copy number variations (CNVs) with a frequency of 0.40 % (28/7077), following 15q11.2 (1.21 %), 7q11.23 (1.19 %) and 22q11.2 (0.85 %). The prevalence of 17p11.2 deletions and duplications were 0.17 % (12/7077) and 0.23 % (16/7077), respectively, which were higher than the frequencies reported in literature (0.10 % and 0.06 %) [15]. Some analyses had suggested that NAHR-generated deletions occurred approximately twice as frequently as duplications [16]. While in the present study, the number of patients with duplications was more than that of patients with deletions.

Most 17p11.2 deletions and reciprocal duplications have common breakpoints, although deletions and duplications of different sizes have been identified. Both appear to involve a 2.1-4.9 Mb chromosome interval in 17p11.2 region that includes *RAI1* gene [4]. Our MLPA results showed that all the patients with 17p11.2 imbalance had RAI1, LRRC48 and LLGL1 probes abnormal. These probes located in the region of chr17: 17681458 to 18244875. NGS analyses of 9 children and 2 fetuses displayed that the affected regions were all in the range of chr17: 16560001-20520000, with the sizes of imbalance mostly 3.4 Mb and 3.7 Mb. *RAI1* gene is a main dosage-sensitive gene in this genomic interval. The protein of RAI1 is highly expressed in neurons during the early stage of neurodevelopment, which matches with its role in cognitive and motor skills neurodevelopment [17]. Haploinsufficiency or triplosensitivity of *RAI1* gene is believed to be responsible for most features of SMS and PTLS, including behavioural, craniofacial, and neurologic signs and symptoms [18-19]. In fact, about 10 percents of all the SMS patients carry an *RAI1* gene mutation responsible for the phenotype [20]. Phenotypic differences have been described between patients with 17p11.2 deletion and those with *RAI1* gene

mutation [6, 21-22]. Patients with *RAI1* mutations may have less severe motor delay and higher functioning. They are also more likely to exhibit overeating and overgrowth phenotypes, polyembolokoilamania, self hugging, muscle cramping, and dry skin compared with patients with deletions [6]. Although *RAI1* was shown to be responsible for most SMS/PTLS features, other genes in the 17p11.2 region also contribute to the variability and severity of the phenotype in 17p11.2 deletion/duplication cases [23-26].

It is considered that genomic duplications are generally better tolerated than deletions. Nevertheless, in our study, patients with duplications were clinically recognized earlier than those with deletions, with the average age were 3.4 vs. 5.4 except neonates. It was reported that PTLS patients can manifest with severe hypotonia and failure to thrive during infancy, and with significant behavioral abnormalities [27]. We found in both groups of patients with deletions and duplications, the most common neurobehavioral syndromes were gross motor development delay, intellectual disability and language development delay. Besides, patients with deletions were more prone to hyperactive and attention deficit, while global developmental delay, muscular hypotonia and stiff expression were usually seen in patients with duplications. Moreover, craniofacial features and cardiac defects were more common in patients with deletions compared to that with duplications. Although cardiovascular anomalies were reported in about 40 % of PTLS patients [28], no cardiovascular abnormalities were found in the 17 patients with duplications. In particular, there were 2 of 10 male patients with duplications had cryptorchidism. In decipher database, four male patients were reported to have the phenotype of micropenis and three female patients were reported to have the abnormality of labia (<https://www.deciphergenomics.org/search/patients/results?q=17p11.2>). They all were 17p11.2 deletion patients. Zhang et al [22] identified a newborn male patient with chr17p11.2 (15759453-20547625) deletion had micropenis and anus close to the perineal body. Based on these findings, it can be inferred that 17p11.2 variations may contribute to the development of external genital. However, the relationship of genes located in 17p11.2 with external genital has not been invested. Some genes, such as *SREBF1* and *MYO15A*, were hypothesized to be related to external genitalia defects [22], but the mechanism involved in the morphogenesis of external genitalia needs further investigation.

As far as we know, the direct and first-hand descriptions on neonatal phenotypes of SMS/ PTLS were very rare. The abnormal ultrasound findings of the fetuses with a deletion/duplication of 17p11.2 were even rarely reported. Most of the reported cases were retrospective reports of patients aged > 2 who were later diagnosed. In the present study, 2 neonates and 2 fetuses were identified. Among of them, 2 cases were referred to the doctors for cardiac defects and were detected to harbor 17p11.2 deletion. The other 2 cases had reciprocal duplications with milder appearance, that one had bilateral cryptorchidism and the other had cerebral ventricle dilation. The identification of neonatal and fetal cases may increase awareness of these rare disorders among clinicians so that patients are diagnosed early. An earlier diagnosis will lead to more timely developmental intervention and medical management, which will improve clinical outcomes.

In the cohort of 7319 fetuses with potential congenital defects, 263 cases (263/7319, 3.6 %) were identified to have genomic disorders, and this number is less than that of chromosome aneuploidy (384/7319, 5.2 %). 24 of 263 (9.1 %) cases with chromosomal aberrations had imbalance at chromosome 17, proving again that chromosome 17 was prone to variation. Interestingly, of the distinct genomic disorders map to chromosome 17, 17q12 was the most frequently affected region in this fetal cohort, followed by 17p12. Few fetuses carried 17p13.3, 17p11.2 and 17q11.2 variations. However, 17p11.2 imbalance was far more common than 17p13.3 and 17q11.2 among children patients with development delay. We could not get the data of 17p12 and 17q12 loci, as probes of these two region were not included in MLPA P245 kit. The differences of the ratio of 17p11.2 variations might be attributed to three possible reasons. (1) The inclusion criteria of these two cohorts were different. (2) Individuals with 17p11.2 variations had more obvious symptoms of neurobehavioral defects, so they could be suspected as patients with development disorders by clinicians in most cases. (3) Fetal symptoms of 17p11.2 imbalance may not be sufficiently severe to warrant a detailed examination, and hence, the prevalence may be underestimated.

Conclusion

Chromosome 17p11.2 deletion/duplication is a relatively common genomic disorder in patients with development delay in China. We conducted a study to provide prevalence, genotype and phenotype characteristics of 17p11.2 variations in children with development delay and/or intellectual disability and in fetuses with potential congenital defects. We hope our findings could further expand the phenotypic spectrum of SMS/PTLS and increase awareness of these disruptive mutations among clinicians. Once the diagnosis is confirmed, the patient should be referred to clinical geneticists and other allied health-care providers to get multidisciplinary management. In addition, the recurrence risks for the parents and the patients should be addressed, and the importance of family support should be highlighted.

Abbreviations

aCGH: array comparative genomic hybridization; CLDN5: claudin 5; CLTCL1: clathrin heavy chain like 1; CNV: copy number variation; DRC3: dynein regulatory complex subunit 3; LLGL1: LLGL scribble cell polarity complex component 1; MLPA: multiplex ligation-dependent probe amplification; MYO15A: myosin XVA; NAHR: nonallelic homologous recombination; NGS: next generation sequencing; PTLS: Potocki-Lupski syndrome; *RAI1*: retinoic acid-induced 1; SMS: Smith-Magenis syndrome; SNAP29: synaptosome associated protein 29; *SREBF1*: Sterol regulatory element-binding protein-1.

Declarations

Ethics approval and consent to participate

This study was approved by Ethics Committee of Shengjing Hospital of China Medical University. Written informed consent to participate was obtained from all of the participants in this study (written informed consent to participate of individuals younger than the age of 16 was obtained from their parents or legal guardians).

Consent for publish

Written informed consents for publication of identifying images or other personal or clinical details were obtained from all patients (written informed consents for publication of identifying images or other personal or clinical details of individuals younger than the age of 16 were obtained from their parents or legal guardians).

Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the figshare repository (https://figshare.com/articles/figure/Data_of_17q11_2_variation/14585979).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YYZ1 analyzed and interpreted the MLPA data and write the manuscript. XLL analyzed and interpreted the clinical data of patients. HMG analyzed and interpreted the karyotyping data. GMC and RH analyzed and interpreted the NGS data. YYZ2 modified the manuscript. We ensure that all authors have read and approved the manuscript.

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Figures

Figure 1

(A) The frequency of 17p11.2 imbalance in children patients with development delay. (B) Numbers of children patients harboring various kinds of chromosome 17 imbalances. (C) The frequency of 17p11.2 imbalance in fetuses with potential congenital defects. (D) Numbers of fetuses harboring various kinds of chromosome 17 imbalances.

Figure 2

Graphs represent results of analyzed by Multiplex ligation-dependent probe amplification (MLPA). X-axis represents MLPA probes (genes) and y-axis represents probe dosage quotient. The blue line indicates probe dosage quotient of 1.35 and any probes above this line represent duplication. The red line indicates probe dosage quotient of 0.65 and any probes below this line represent deletion. The probes between 0.85 and 1.15 are considered as normal controls. (A) A control with normal copy probes. (B) A patient carries 17p11.2 deletion. (C) A patient carries 17p11.2 duplication. (D) The patient carries both 17p11.2 duplication and 22q11.2 duplication.

Figure 3

Next generation sequencing (NGS) plot showing the deletion/duplication of 17p11.2 region. (A) The fetus carries a 3.40 Mb deletion ranging from 16820001 to 20220000. (B) The fetus carries a 3.42 Mb duplication ranging from 16800001 to 20220000.