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Cytogenetic analysis and family research for two cases of chromosome 6 microduplication and chromosome 9 microdeletion: Different clinical manifestations

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Dear editor:

Chromosomal abnormalities are the result of alteration in the number or structure of chromosomes causing diverse functional problems to various organs.[1] Recently, though strategies for prenatal diagnosis for chromosomal abnormalities have progressed rapidly, management of patients of chromosomal abnormalities still remains an important medical problem.[2] In medical genetics, cytogenetic analysis is becoming an essential source of diagnostic information and provides a genome-wide snapshot of an individual ' s chromosomes.[3]

At present, around 20 chromosomal microdeletion/microduplication syndromes have been reported, most of which are rare disease.[4,5] The number of new children with microdeletion/microduplication syndrome in China every year is astonishing. Since most of these children have severe chromosomal disorders, which carry an underappreciated psychological and financial burden for family and society. During the last decade, cytogenetic analysis has become a very important tool for genetic counseling. Compared with cytogenetic diagnosis analysis, single nucleotide polymorphism array (SNP-array) has higher resolution and accuracy in chromosome abnormalities analysis.[6,7] In addition, SNP-array has advantages in analyzing the genes involved in the chromosomal deletion/duplication and the clinical phenotypes of patients. Here we present two cases of children whose parents had previous abnormal deliveries.

The first parents had a 2-year-old boy, who was born at 37 weeks of gestation after an uneventful pregnancy. During his fetal period, the chromosomal analysis results indicated a low risk for trisomy 13,18 and 21. Ultrasound scan at 22 weeks of gestation showed fetal ventricular septal defect, single umbilical artery, and echocardiographic examination was not found other abnormalities except for ventricular septal defect. Ultrasonic at 35 weeks of gestation revealed suspicious fetal cerebellar dysplasia, and MRI did not reveal obvious pathological findings. Ultrasonic scan at 37 weeks of gestation showed intrauterine developmental retardation, and echocardiography investigation revealed fetal distress. The echocardiographic results of the boy's reexamination after birth showed that he had cardiac abnormalities including ventricular septal defect, atrial septal defect, patent ductus arteriosus, coarctation of the descending aortic arch, and pulmonary hypertension. The boy's cardiac were, surgically corrected on the 18th day after birth, but he presented weak physique, low immunity, feeding difficulties, and developmental retardation. Then he was diagnosed with Hirschsprung's disease. A biopsy of the colon indicated that the colonic nerves were absent and could not defecate independently. He is now more than 2 years old,showing a severe mental retardation, ignorance of parents, no language skills, sluggish expression, thick eyebrows, low nose, low ears, wide set eyes,hypotonia, unable to sit up or unable to walk and unable to defecate independently.

The second healthy nonconsanguineous couple had a 7-year-old girl who is the first child of them. Family history was noninformative. The girl's gestational condition is unknown. She was born at term after uneventful pregnancy and delivery. At birth, her weight was 3200 g. She presented a mild mental retardation, convergent squint, dysplasia in the middle of the face, able to answer questions but lags behind children of the same age, unstable working, no abnormalities in external genital.

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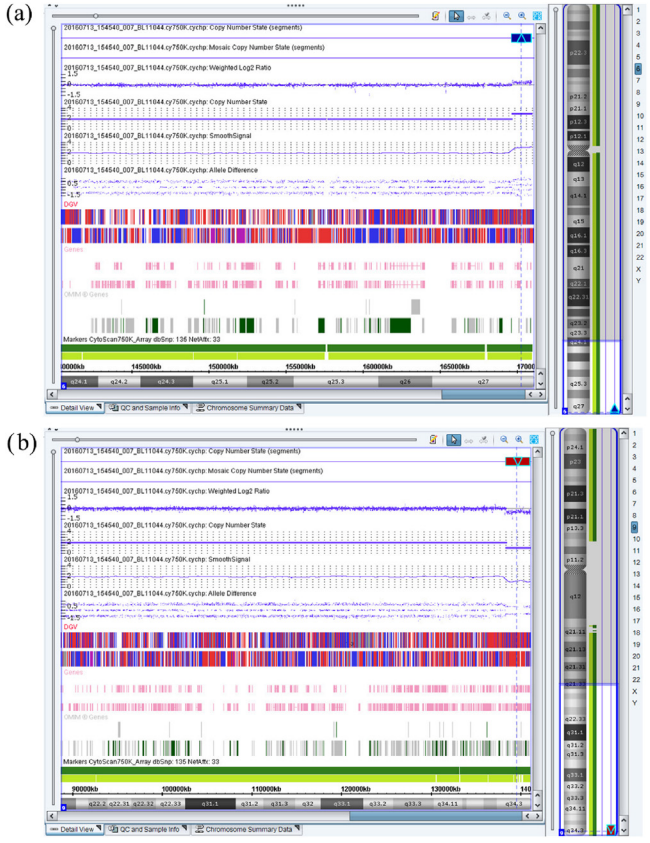

Fig. 1. SNP array detected Case 1. (a) SNP result showed the 6q27 duplication of chromosome 6, 6q27(169,640,336-170,914,297) × 3. (b) SNP result revealed the 9q34.3 deletion of chromosome 9, 9q34.3(138,376,144-141,018,648) × 1.

There are abnormal discharge waveforms on the electroencephalogram (EEG), but episode of no seizures not found yet.

Chromosome analysis was carried out for two kids and their patients. Chromosome analysis was performed following a standard method using G-banding technique from cultured peripheral lymphocytes.[8] To confirm the chromosomal abnormalities, FISH analysis was performed on peripheral blood metaphase chromosomes from the proband and both his parents using the commercial probe D15Z1/SNRPN/PML (Vysis, Abbott Molecular, USA).

In case 1, the SNP results displayed that the 2-year-old patient presented with 1.27Mb microduplication of 6q27 region from chromosome 6 and 2.64Mb microdeletion of 9q34.3 region from chromosome 9 (Fig. 1). The karyotype in patient 1 was 46, X Y, der (9)t(6; 9) (q27; q34.3). Chromosome analysis detected normal karyotype of the patient's parents with 46, XY and 46, XX, respectively (Fig. 2a–b). Further, FISH studies using probes 6pter/6qter and 9pter/9qter were performed on the chromosomes of the patient ‘s parents (Fig. 2c–d). The results revealed that no translations between chromosomes 6 and 9 or between other chromosomes were found in the mother. However, the father was a carrier of translocation at the end of the long arm of chromosomes 6 and 9.

The father of patient 1 had a reciprocal translocation of chromosomes 6 and 9, as a reciprocal translocation carrier, and form quadrivalents at meiosis. These complexes segregate by alternate, adjacent-1, adjacent-2, 3 : 1 or 4 : 0 modes to give sperms with different balanced or unbalanced chromosome complements, which will lead to abnormal offspring. The patient inherited a derivative chromosome 9 from the father through an unbalanced adjacent-1 segregation. Microduplication of chromosome 6/microdeletion of chromosome 9 was associated with clinical manifestations including a severe mental retardation, special facial features, multiple deformities, congenital heart disease, hypotonia, feeding difficulties, developmental retardation and multiple deformities. When patients of patient 1 reproduce again, the offspring still have a high

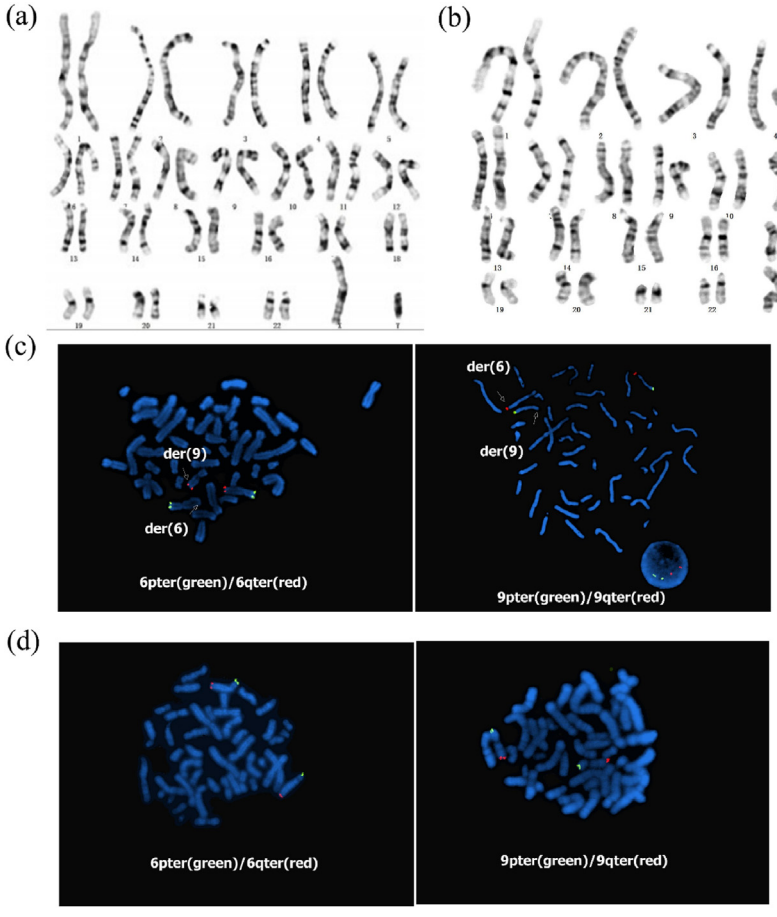

Fig. 2. Chromosomal analysis and FISH on the parents of Case 1. (a–b) Chromosome analysis detected normal karyotype of child'parents with 46, XY and 46, XX, respectively. (c) The results of FISH using probes 6pter/6qter and 9pter/9qter showed that the patient 1 ′ s father was a carrier of a translocation at the end of the long arm of chromosome 6 and 9, 6p25q27(6pter × 2, 6qter × 2), 9p24q34(9pter × 2, 9qter × 2). (d) The results of FISH using probes 6pter/6qter and 9pter/9qter showed no translocation between chromosomes 6 and 9 or with other chromosomes were found in the mother of the patient, 6p25q27(6pter × 2, 6qter × 2),9p24q34(9pter × 2, 9qter × 2).

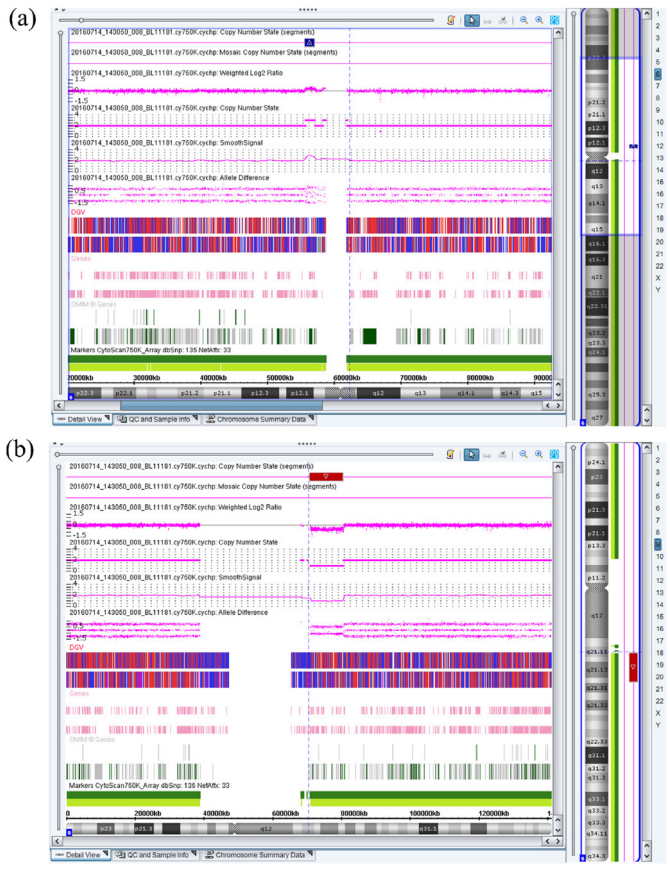

Fig. 3. SNP array detected Case 2. (a) SNP result showed the 6p12.1p11.2 duplication chromosome 6, 6p12.1p11.2(55,703,593-57,133,357) × 3. (b) SNP result revealed the 9q21.11q21.2 deletion of chromosome 9, 9q21.11q21.2(70,966,261-80,640,043) × 1.

probability of abnormal phenomena such as trisomy, monosomy and partial trisomy, and partial monosomy. It is recommended to adopt third-generation in vitro fertilization (IVF) assisted reproduction or prenatal diagnosis to avoid the birth of defective children.

In case 2, the results of SNP revealed that the duplication 1.42Mb in 6p12.1p11.2 region from chromosome 6 and the deletion 9.67Mb in 9q21.11q21.2 region from chromosome 9 in patient 2 (Fig. 3). The karyotype in patient 2 was 46, XX, dul (6) (p12.1; p11.2) del (9) (q21.11; q21.23). The patient's parents have normal karyotype with 46, XY and 46, XX, respectively (Fig. 4a–b). Further, FISH studies using bacterial artificial chromosome (BAC) clones RP11-978I13 and RP11-664L23 were performed on the chromosomes of patient's parents (Fig. 4c–d). FISH analysis showed no evidence for a deletion/duplication 6p12.1 and 9q21.13 or other chromosomes translocation in patient's father. However, her mother was carried of 6p12.1 duplication, and the deletion/translocation on 9q21.13 were not found.

The case 2 inherited the duplication of chromosome 6 from the mother. Meanwhile, chromosome 9 occurred deletion mutation. Microdeletion of chromosome 9 was associated with clinical manifestations including a mild mental retardation, convergent squint, unstable working and abnormal electrical discharge. However, the clinical significance of the microduplication of chromosome 6 is unknown. Because the patient's chromosome 9 microdeletion was a new mutation, the probability of re-pregnancy of the parents is low. It is recommended to standardize the prenatal care during re-pregnancy and prenatal diagnosis.

In the study, we presented 2 cases of chromosome 6 deletion and chromosome 9 microduplication. Case 1 was caused by one of the parents being a carrier of balanced translocations t (6; 9). Since the father carries a small balanced translocation region, it is difficult to detect by high-resolution chromosome analysis. Therefore, further FISH analysis using telomere probes 6 pter/6qter and 9pter/9qter were used to confirm the patient's father as a balanced translocation t (6; 9) carrier. The patient 1 inherited a normal chromosome 6 and a derived chromosome 9 from the father, resulting in part 6q trisomy and part 9q monosomy. The patient has a 1.27Mb duplication from 6q27 region, which contains 11 OMIM genes such as ERMARD and TBP(Table 1). Studies reported that patients with duplication of the long arm of chromosome 6 presenting distinctive features including microcephaly, acrocephaly, prominent forehead, flat facial profile, and mental retardation.[9] Patients with deletions of 6q27 region were mainly associated with structural brain abnormalities including periventricular nodular heterotopia (PNH), agenesis of the corpus callosum (ACC) and cerebellar malformations. Lee et al.[10] shown the patients with terminal deletion 1.2 Mb in 6q region presented with mental retardation, microcephaly, seizures, an enlarged cisterna magna, dimpling at elbows, and found TBP as a candidate gene for mental retardation. The patient also had a 2.64Mb deletion located in 9q34.3 region of chromosome 9, which contains 72 OMIM genes such as EHMT1, NOTCH1 and INPP5E. The clinical symptoms in our patient are similar to the cases described by Guterman et al.,[11] they found haploinsufficiency for EHMT1 is causative for 9q subtelomeric deletion syndrome, which is characterized by severe mental retardation, hypotonia, brachycephaly, flat face with hypertelorism, and so on. Villa A et al.[12] reported that individuals with duplication 9q34 presented slight low birth weight, dolichocephaly, psychomotor retardation, facial asymmetry and narrow horizontal palpebral fissures.

The case 1 shared clinical phenotype including microcephalus, developmental delay, mental retardation, congenital heart disease. The mother has an extremely high probability of unbalanced gamete binding in the fetus during the re-pregnancy because the father is a carrier of t (6; 9) balanced translocation. It is recommended that the mother undergo prenatal diagnosis during pregnancy to exclude chromosomal abnormalities in the fetus, or use third-generation IVF assisted reproduction to avoid the birth of children with birth defects caused by the balance of gametes.

The case 2 inherited the duplication 6p12.1 of chromosome 6 from the mother. Meanwhile, he presented a deletion mutation on chromosome 9. High-resolution chromosome analysis failed to detect translocation because the carrier has a small balanced translocations region. Therefore, after chromosome karyotype was tested, FISH analysis results confirmed that the duplication of chromosome 6 was inherited from his mother, and the deletion of chromosome 9 was a new mutation. The patient has a 1.42Mb the duplication of 6p12.1p11.2 region from chromosome 6, which contains 9 OMIM genes such as DST and RAB23. One case who with duplication of 6p12p21.3 presenting growth retardation, psychomotor delay and craniofacial, brain, limb, and genital anomalies.[12] Meanwhile, the patient was found to have 9.67Mb deletion located in 9q21.11q21.2 region of chromosome 9, which contains 34 OMIM genes such as FXN, TJP2 and TMC1. Here, we reported a patient with a 9.67 Mb microdeletion at 9q21.11q21.2 chromosomal region presenting low birth weight, mild intellectual disability, speech delay and characteristic facial features but no epilepsy behavior. The symptoms in our patient were similar to those seen in the other patients with a deletion at 9q21.11-q21.2.[13] However, Boudry-Labis et al.[14] reported that patients with 9q21 microdeletions presenting several common major characteristics including significant developmental delay, epilepsy, neuro-behavioural disorders and recognizable facial features. At present, no phenotypic abnormalities of partial duplication of chromosome 6 from the patient ' s mother were found. Therefore, we speculated that the deletion of chromosome 9 might contribute to clinical phenotypic abnormalities in our patient. In addition, since the deletion of chromosome

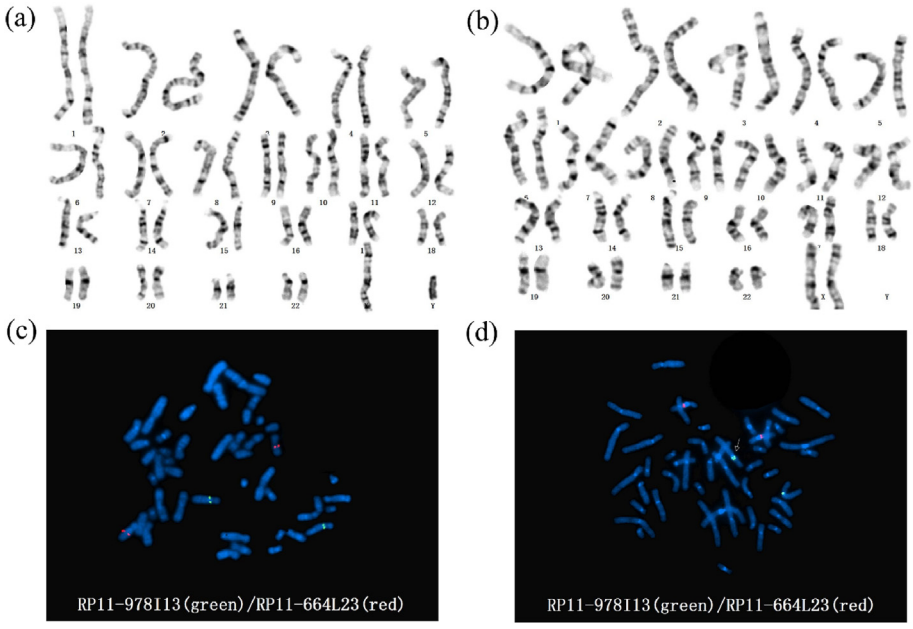

Fig. 4. Chromosomal analysis and FISH on the parents of Case 2. (a–b) Chromosome analysis detected normal karyotype of the parents with 46, XY and 46, XX, respectively. (c) The results of FISH using probes RP11-978I13 and RP11-664L23 showed that no deletions/duplications of 6p12.1 and 9q21.13 or translocations with other chromosomes were found in the father of the patient 2. (d) The results of FISH using probes RP11-978I13 and RP11-664L23 revealed that the patient's mother was a carrier of the 6p12.1 duplication, and no deletion or translocation of 9q21.13 were found.

Table 1
Details of gene duplications and deletions.

	Sex	Age(Y)	Duplication 6/ deletion 9	Gene
Case 1	Male	2	chr6:169,640,336-170,914,297 chr9:138,376,144-141,018,648	*THBS2,WDR27,C6orf120,PHF10,DYNLT2,ERMA,RD,DLL1,FAM120B,PSMB1,TBP,PDCD2,PPP1R26,C9orf116,MRPS2, LOC101928525,LCN1,OBP2A,PAEP,LINC01502,LOC102723971,GLT6D1,LCN9,SOHLH1,KCNT1,CAMSAP1,UBAC1, NACC2,TMEM250,LHX3,QSOX2,GPSM1,DNLZ,CARD9,SNAPC4,ENTR1,PMPCA,INPP5E,SEC16A,C9orf163,NOTCH1, MIR4673,MIR4674,EGFL7,MIR126,AGPAT2,DIP,K1B,SNHG7,LCN10,LCN6,LCN8,LCN15,TMEM141,CCDC183, CCDC183AS1,RABL6,MIR4292,AJM1,PHPT1,MAMDC4,EDF1,TRAF2,MIR4479,FBXW5,C8G,LCN12,PTGDS,LCNL1, PAXX,CLIC3,ABCA2,LINC02908,FUT7,NPDC1,ENTPD2,SAPCD2,UAP1L1,MAN1B1,DPP7,GRIN1,LRRC26,MIR3621,TMEM210, MAN1B1-DT*
Case 2	Female	7	chr6:55,703,593-57,133,357 chr9:70,966,261-80,640,043	*BMP5,NPM1P36,COL21A1,DST,BEND6,KIAA1586, ZNF451, BAG2, RAB23, PGM5,PIP5K1B,FXN,TJP2,BANCR,FAM189A2, APBA1,PTAR1,C9orf135,SMC5,KLF9,TRPM3,CEM,IP2,ABHD17B,C9orf85,C9orf57,GDA,ZFAND5,TMC1, ALDH1A1,ANXA1,RORB,TRPM6,CARNMT1,NMRK1,OSTF1,PCSKS,RFK,GCNT1,PRUNE2,PCA3,VPS13A,GN14,GNAQ,*

9 in the patient is a new mutation, the fetus whose parents are pregnant again should have no obvious abnormal phenotype even if they inherit the mother's 6 duplication, and the probability of reoccurring chromosome 9 deletion is extremely low.

Two children in this study both have chromosome 6 microduplication and chromosome 9 microdeletion, but their clinical manifestations were quite different. The different clinical phenotypes might be caused differences in the size and location of chromosome microdeletion/microduplication and related pathogenic genes. At the same time, the two patients had different genetic patterns. Furthermore, it is interesting that the risks of their parents being pregnant again were also different. Therefore, it plays an important role to detect and analyze the source of chromosomal abnormalities, which is associated with the analysis of genetic risk, genetic counseling and prenatal diagnosis methods.

In conclusion, the analysis of detailed clinical phenotype and cytogenetic analysis not only enables better analyze virulence gene involved in chromosomal abnormalities of the patients, but also locate the correlation between the genotype and phenotype of the virulence genes. In addition,the cytogenetic analysis of the source of the patient's chromosomal abnormalities are conducive to assessing the risk of recurrence and future eugenics.

Ethics statement and consent

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Peking University People's Hospital Ethics Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Declaration of competing interest

The authors declare no conflict of interest. All authors consent for publication.

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