Simultaneous 9p Deletion and 8p Duplication in a Seven-Year-Old Girl, Detected Using Multiplex Ligation-Dependent Probe Amplification: A Case Report

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Abstract
Deletion 9p syndrome is a rare chromosomal abnormality with a wide spectrum of manifestations such as craniofacial dysmorphism, congenital anomalies, and psychomotor delay. We report a case of a seven-year-old girl with simultaneous 9p24.3 deletion and 8p23.3 duplication detected using multiplex ligation-dependent probe amplification (MLPA). Chromosomal and cytogenetic analyses using MLPA are effective in assessing genetic abnormalities in patients with developmental delay and mental retardation. We found breakpoints at 9p24.3 and duplication in the 8p23.3 region, leading to a wide variety of manifestations including speech delay, upslanting palpebral fissures, hypertelorism, epicanthal fold, high arched eyebrows, flat nasal bridge, thin upper lip, and cleft palate. Simultaneous detection of 9p24.3 deletion and 8p23.3 duplication has been rarely reported. Clinical phenotypes of our patient resembled the features of Nicolaides-Baraitser syndrome, which might have been primarily caused by the haploinsufficiency of SMARCA2 (SWI/SNF-related, matrix associated, actin-dependent regulator of chromatin, subfamily A, member 2) gene located at 9p24.3.

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Keywords
- Chromosome disorders
- Chromosome 9p deletion syndrome
- Cytogenetics
- Chromosome aberrations
- Multiplex polymerase chain reaction

What’s Known
- Deletion 9p syndrome is a rare chromosomal abnormality manifested as craniofacial dysmorphism, congenital anomalies, and psychomotor retardation. Distal and various proximal breakpoints have been identified in patients with 8p inverted duplication syndrome.
- Mental retardation, facial anomalies, hypotonia, and corpus callosum are associated with 8p inverted duplication.

What’s New
- For the first time, we report a rare case of a seven-year-old girl with simultaneous 9p24.3 deletion and 8p23.3 duplication. It was detected using the multiplex ligation-dependent probe amplification technique.
Nicolaides-Baraitser syndrome in a child with simultaneous 9p deletion and 8p duplication

Breakpoints have been identified in patients with inverted 8p duplication using cytogenetic analysis. There is an association between 8p inverted duplication and different phenotypes such as mental retardation, facial anomalies, hypotonia, and corpus callosum. However, there is no significant association between this duplication and congenital heart defects, coloboma, scoliosis, and seizures.2

Nicolaides-Baraitser syndrome (NCBRS) is a condition with various clinical features. People with NCBRS can have a wide variety of signs and symptoms, but the most common are sparse scalp hair, microcephaly, distinct facial features, short stature, prominent finger joints, unusually short fingers and toes (brachydactyly), epilepsy, and moderate to severe intellectual disability with impaired language development. Facial features include a triangular face, deep-set eyes, a thin nasal bridge, wide nostrils, pointed nasal tip, and thick lower lip. Typical phenotypes include premature wrinkles, large finger joints, broad and oval shape fingertips, a wide gap between the first and second toes, and pale skin with veins visible on the skin surface due to the lack of subcutaneous fat.3, 4 Early developmental events such as crawling and walking are often achieved normally. However, further progress is limited, and language development is severely impaired or never develop in one-third of patients, and the remaining individuals may lose oral communication over time. People with NCBRS are generally happy and very friendly.5

For the first time, we herein present an individual with chromosomal aberrations derived from simultaneous 9p24.3 deletion and 8p23.3 duplication, which could be categorized in the spectrum of NCBRS.

Case Presentation

In 2017, a seven-year-old Iranian girl with multiple congenital anomalies and developmental delay was referred to the Medical Genetic Counseling Center and Social Welfare Organization (Bandar Abbas, Iran). She was the first child of non-consanguineous parents born at full term by cesarean section due to meconium aspiration. At birth, she weighed 2,600 g, had a severe cleft palate, and her one-minute Apgar score was nine. Since birth, she had been hospitalized several times due to cyclic vomiting, hypoglycemia, and upper respiratory infection. She suffered from a bilateral inguinal hernia, psychomotor retardation, and speech delay. Esophagography revealed distal esophageal stenosis, and the cardiac test showed a very small atrial septal defect (ASD). Magnetic resonance and ultrasound imaging of the abdomen and brain were unremarkable. At the age of three, she weighed 9,100 grams with a head circumference of 48 cm and a height of 83 cm. Upon admission to our center, she was diagnosed with microcephaly, scoliosis, and dysmorphic facial features (e.g., long philtrum, micrognathia, low-set ears, upslanting palpebral fissures, hypertelorism, epicanthal folds, high arched eyebrows, flat nasal bridge, thin upper lip, cleft palate, and irregular teeth). She had undergone cleft palate surgery twice and was unable to stand or walk without support. Muscular atrophy in both upper and lower extremities was observed (figure 1). Her hearing was normal, but she suffered from a speech disorder. Written informed consent was obtained from her parents for all laboratory and physical assessments.

Chromosomal analysis was performed on the phytohemagglutinin (PHA)-stimulated peripheral lymphocyte blood culture using standard cytogenetic methods. About 20 to 30 metaphases at 450-500 band resolution were analyzed.6 Genomic DNA from the peripheral blood was extracted at a concentration of 20 ng/μL using a kit from Kawsar Biotech Company (Tehran, Iran), according to the manufacturer’s instructions. The patient was screened for subtelomeric re-arrangements (SALSA MLPA P036 and P070 test kits) and microdeletion syndromes (SALSA MLPA P245 test kit).

Figure 1: The seven-year-old Iranian girl with multiple congenital anomalies and developmental delay. (a, b): Dysmorphic facial features such as long philtrum, micrognathia, low set ears, upslanting palpebral fissures, hypertelorism, epicanthal fold, high arched eyebrows, flat nasal bridge, thin upper lip, cleft palate, irregular teeth, and scoliosis. (c): The patient was unable to stand or walk without support. (d): Long fingers.
Amplified products were separated using a genetic analyzer (ABI 3500 XL, MRC Holland), and the data were analyzed using GeneMarker® software, version 1.85 (SoftGenetics LLC, State College, PA, USA). Healthy individuals were used as controls. The results showed that while the karyotype was normal, based on the result of the multiplex ligation-dependent probe amplification (MLPA) test, our patient had simultaneous 9p24.3 deletion and 8p23.3 duplication (figures 2 and 3).

**Figure 2:** The result of multiplex ligation-dependent probe amplification (MLPA) test using the SALSA MLPA P036 test kit. The Y chromosome probe was removed, since our patient was female.

**Figure 3:** The result of multiplex ligation-dependent probe amplification (MLPA) test using the SALSA MLPA P070 test kit. The Y chromosome probe was removed, since our patient was female.

disability are relatively common pediatric conditions, affecting 3% of the general population. Genetic factors are responsible for up to 40% of developmental disability cases.7 Multiple phenotypes in patients with 8p23.3 duplication or 9p24.3 deletion have been reported. In the present study, we compared the clinical features of our patient with those previously reported cases. Such cases are extremely rare, and studies reporting symptoms of simultaneous deletion 9p and duplication 8p are scarce, especially those reporting on upper respiratory tract infection as in our case.

**Discussion**

Global developmental delay and intellectual
Nicolaides-Baraitser syndrome in a child with simultaneous 9p deletion and 8p duplication

In our study, we used MLPA to detect 9p 24.3 deletion and 8p 23.3 duplications. The main characteristics of deletion 9p syndrome, first reported in 1973, are trigonocephaly, midface hypoplasia, long philtrum, and developmental delay. Clinical manifestations of deletion 9p syndrome in our patient corresponded with the reported phenotypic characterization in previous studies. Tables 1 and 2 present a list of known genes in chromosome 9. Deletion 9p syndrome is caused by partial monosomy of 9p with breakpoint sites mainly in the regions 9p22 to 9p24. These regions include many critical genes such as DMRT (doublesex and mab-3 related transcription factor), DOCK8 (dedicator of cytokinesis 8), KANK1 (KN motif and ankyrin repeat domains 1), SMARCA2 (SWI/SNF-related, matrix associated, actin-dependent regulator of chromatin, subfamily A, member 2), VLDLR (very low-density lipoprotein receptor), KCNV2 (potassium voltage-gated channel modifier subfamily V member 2), GLIS3 (GLIS family zinc finger 3), SLC1A1 (solute carrier family 1, member 1), JAK2 (Janus kinase 2), GLDC1 (glycine decarboxylase 1), TYRP1 (tyrosinase-related protein 1), Cerberus 1, CER1 (DAN family BMP antagonist), and MPDZ (multiple PDZ domain crumbs cell polarity complex component). DOCK 8, KANK1, and EHMT1 are associated with developmental delay. Based on the array comparative genomic hybridization technique, Mitsui and colleagues found that monosomy 9p syndrome of a suspect newborn girl was associated with 9p deletion with a breakpoint at 9p23 region. It is known that the 9p24.3-ppter is associated with aberrant sexual development. The DMRT domain of the DOCK8 gene function remains unclear. However, our patient had normal external genitalia. Mutations in the DOCK8 gene which are located on 9p24.3 and play an important role in the immune system, may lead to immune-related disorders. DOCK8 deficiency is associated with

| Article      | Region               | Sex, age | Head, neck, and extremities | Neurological Other anomalies | Other anomalies | Related gene |
|--------------|----------------------|----------|-----------------------------|-----------------------------|----------------|--------------|
| Aktas et al. | 9p24.3, duplication   | Female, 10 years | -                           | Mental retardation, psychomotor development delay, speech delay, squint, developmental delay, poor muscle tone, muscle weakness, short stature, microcephaly, short palpebral fissures, high arched eyebrows, long philtrum | Adult spinal deformity, scoliosis | -             | -            |
| Choi et al.  | 9p24.3, duplication   | Male, six years | -                           | Mental retardation, psychomotor development delay, speech delay, squint, developmental delay, poor muscle tone, muscle weakness, short stature, microcephaly, short palpebral fissures, high arched eyebrows, long philtrum | Adult spinal deformity, scoliosis | -             | -            |
| Hou et al.   | 9p24.3, duplication   | Male, six years | -                           | Mental retardation, psychomotor development delay, speech delay, squint, developmental delay, poor muscle tone, muscle weakness, short stature, microcephaly, short palpebral fissures, high arched eyebrows, long philtrum | Adult spinal deformity, scoliosis | -             | -            |
| Sirisena et al. | 9p24.3, duplication | Male, four months | -                           | Mental retardation, psychomotor development delay, speech delay, squint, developmental delay, poor muscle tone, muscle weakness, short stature, microcephaly, short palpebral fissures, high arched eyebrows, long philtrum | Adult spinal deformity, scoliosis | -             | -            |
high mortality at a young age due to frequent infections. This was also the case with our patient, since she had been hospitalized several times for recurrent vomiting, hypoglycemia, and upper respiratory infection. The identification of clustered mutations in a large number of patients with NCBRS supports the idea that this syndrome is a distinct clinical entity.11-13

| Clinical features | Involved genes | Phenotype | OMIM | Common clinical features in our proband |
|-------------------|----------------|-----------|------|-----------------------------------------|
| Deletion 9p24.3, long philtrum, micrognathia, retrognathia, low-set ears, upslanting palpebral fissures, hypertelorism, epicanthal folds, small palpebral fissures, high arched eyebrows, flat nasal bridge, anteverted nares, thin upper lip, microstomia, high and narrow palate, irregular teeth, atial septal defect, inguinal hernia, scoliosis and kyphosis, tapering fingers, pterygium, mental retardation, psychomotor development retardation, speech delay, hypotonia | 9 p24.3 deletion | Autosomal-recessive hyper-IgE syndrome (AR-HIES) | 611432 | She had been hospitalized several times for recurrent vomiting, hypoglycemia, and upper respiratory infection. |
| DMRT1 | Swyer syndrome UniProt form: 46, XY sex reversal 4 (SRXY4) is a condition characterized by male-to-female sex reversal in the presence of a normal 46, XY karyotype. Patients display complete or partial gonadal dysgenesis and a chromosome 9p deletion. [MIM:154230] | 602424 | - |
| DMRT2 | | | 604935 | - |
| DMRT3 | | | 614754 | - |
| KANK1 | Cerebral palsy, spastic quadriplegic, 2 (CPSQ2): Congenital hypotonia evolving over the first year to spastic quadriplegia with accompanying transient nystagmus and varying degrees of mental retardation. Neuroimaging shows brain atrophy and ventriculomegaly. [MIM:612900] | | 607704 | Hypotonia, varying degrees of mental retardation. |
| SMARCA2 | Nicolaides-Baraitser syndrome: Primarily sparse scalp hair, small head size (microcephaly), distinctive facial features, short stature, abnormal fingers, recurrent seizures (epilepsy), and moderate to the severe intellectual disability with impaired language development | 600014 | Primarily sparse scalp hair, small head size (microcephaly), distinctive facial features, short stature, abnormal fingers, recurrent seizures (epilepsy), and moderate to the severe intellectual disability with impaired language development |

Conclusion

The deletion breakpoint at the 9p24.3 band is associated with mutations in SMARCA2 causing Nicolaides-Baraitser syndrome. Due to the high proportion of phenotypic variation in patients with 9p deletions, no clear genotype/phenotype correlations have been established. Further studies are required to determine the role of genes involved in deletion 9p syndrome.

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Authors’ Contribution

M.S and F.M: Contributed to study concept and data acquisition; M.S: Contributed to drafting the manuscript; F.M: Contributed to critically revision of the manuscript. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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