IJMS
Vol 47, No 5, September 2022

Case Report

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

Mozhgan Saberi, MD; Frouzandeh Mahjoubi, PhD

Department of Medical Genetics, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotecnology, Tehran, Iran

Correspondence:
Frouzandeh Mahjoubi, PhD;
National Institute of Genetic Engineering and Biotechnology, Research Blvd., Science and Technology Research Town, 15th Km, Tehran-Karaj Highway,
Postal code: 14977-16316, Tehran, Iran
Tel: +98 21 44787301-3
Fax: +98 21 44787399
Email: frouz@nigeb.ac.ir
Received: 09 January 2021
Revised: 19 May 2021
Accepted: 20 August 2021

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.

Please cite this article as: Saberi M, Mahjoubi F. Simultaneous 9p Deletion and 8p Duplication in a Seven-Year-Old Girl, Detected Using Multiplex Ligation-Dependent Probe Amplification: A Case Report. Iran J Med Sci. 2022;47(5):494-499. doi: 10.30476/IJMS.2021.89353.2039.

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.

Introduction

Deletion 9p syndrome is a rare chromosomal abnormality with a wide spectrum of manifestations such as craniofacial dysmorphism, congenital anomalies, and psychomotor retardation. Patients with this syndrome exhibit trigonocephaly, small palpebral fissures, flat nasal bridge, low-set, and dysplastic ears, anteverted nostrils and long philtrum, cardiac defects, inguinal hernia, and sex development disorders. Patients often demonstrate 9p terminal deletions or 9p chromosomal re-arrangements with the breakpoints at 9p22-9p24 regions. The majority of cases demonstrate *de novo* deletion, which may arise from paternal or maternal meiosis, while in one-third of all cases deletion is associated with unbalanced chromosomal re-arrangements from a parent with a balanced translocation.[1]

Distal, often detected in 8p23 regions, and various proximal

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 the 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 such as wrinkling, 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 even 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)

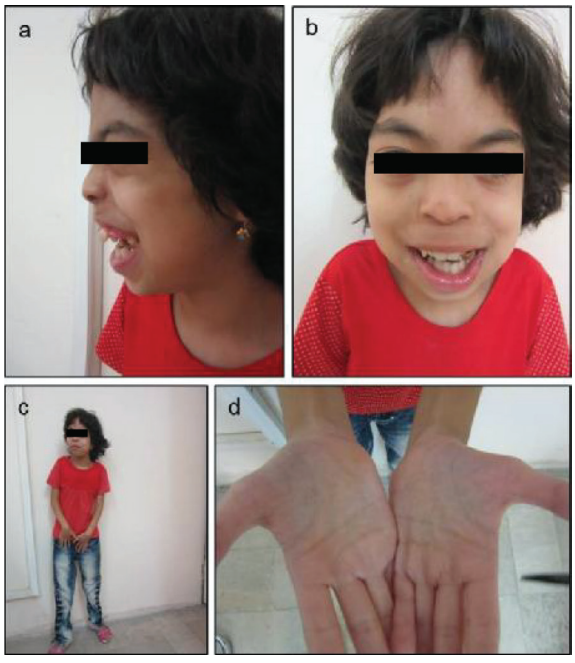

Figure 1: The seven-year-old Iranian girl with multiple congenital anomalies and developmental delay. (a, b): Dysmorphic facial features such as long philtrum, micrognathia, low set ears, upslanting palpebral fissures, hypertelorism, epicanthal fold, high arched eyebrows, flat nasal bridge, thin upper lip, cleft palate, irregular teeth, and scoliosis. (c): The patient was unable to stand or walk without support. (d): Long fingers.

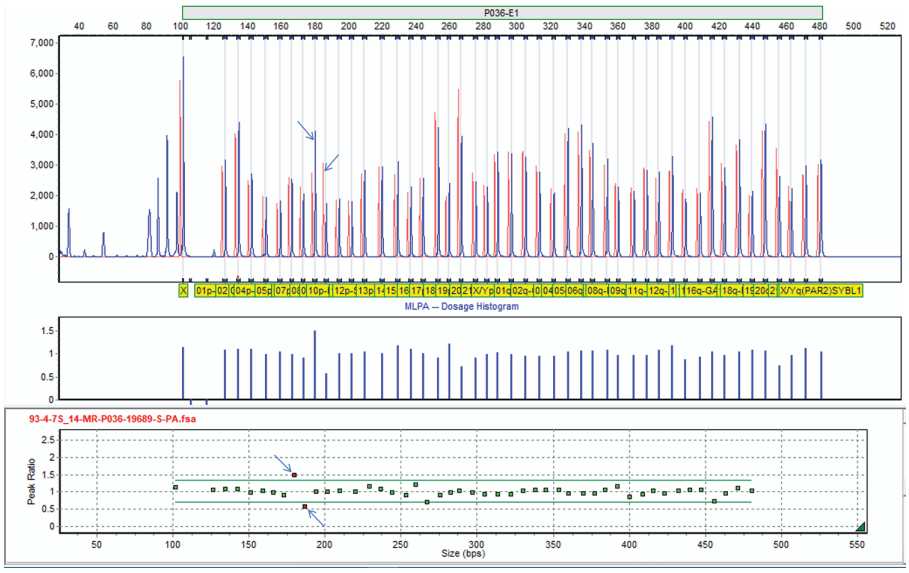

Figure 2: The result of multiplex ligation-dependent probe amplification (MLPA) test using the SALSA MLPA P036 test kit. The Y chromosome probe was removed, since our patient was female.

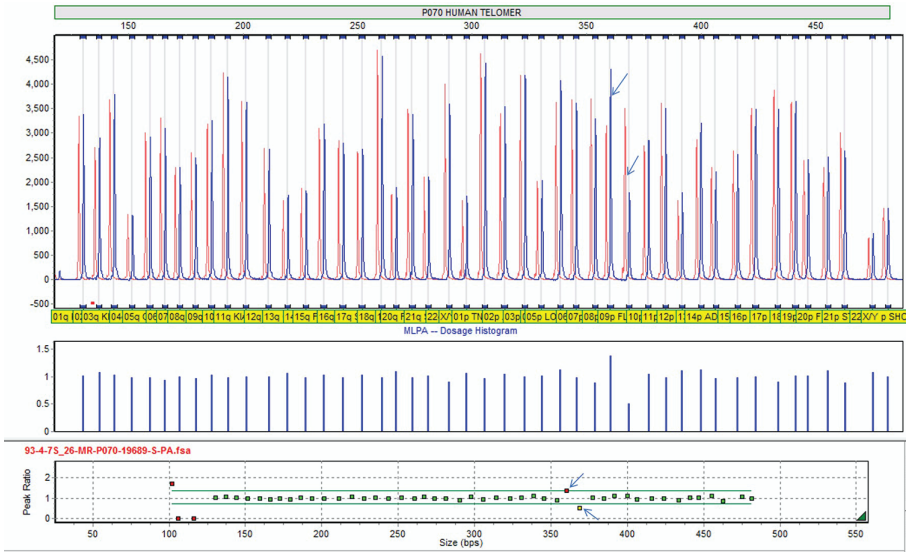

Figure 3: The result of multiplex ligation-dependent probe amplification (MLPA) test using the SALSA MLPA P070 test kit. The Y chromosome probe was removed, since our patient was female.

according to the manufacturer's instructions (MRC Holland, Amsterdam, The Netherlands). 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).

Discussion

Global developmental delay and intellectual 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.

MLPA assay is the gold standard for molecular diagnosis of a wide range of chromosomal abnormalities, especially those related to deletions or duplications of a specific

Table 1: A list of articles identifying chromosome 9p deletion and the corresponding features

Article	Region	Sex, age	Mouth	Nose	Ears	Lips	Eyes	Head, neck, and extremities	Neurological	Other anomalies	Related gene
Aktas et al.[2] (2009)	Deletion 9p24.3, duplication 8p23.3	Female, 10 years	Cleft palate	Flat nose	Low-set ears	Thin upper lip	Hypertelorism	Microcephaly, arched eyebrows, micrognathia, long philtrum	Mental retardation, psychomotor development retardation, speech delay, hypotonia	Adult spinal deformity (ASD), scoliosis	
Choi et al.[14] (2017)	Deletion 9p24.3, duplications 7q36.1-q36.3	Male, six years	-	Small nose	Small ears	-	-	Microcephaly, dysmorphic facial features (long face and high forehead)	Developmental delay, language delay, normal brain MRI	-	
Hou et al.[15] (2016)	Deletion 9p24.2p22.2, deletion 9p24.3p22.1, deletions 9p24.3-9p23	Case 1: 17 weeks of gestational age Case 2: Male, 4.5 years	-	Flat nose	Low-set ears	Thin upper lip	-	Trigonocephaly, flat midface, short palpebral fissures, high arched eyebrows, short neck, long fingers	Hypotonia, intellectual disability, speech delay, hyperactivity, mild hydrocephalus, hypotonia, gross motor delay	Omphalocele	*DOCK 8, KANK1, DMRT1, DMRT2, TYRP1, CER1, DMRT3, KDM4C*
Sirisena et al.[16] (2013)	46XX karyotype, deletion 9p22-pter	Male, four months	Downturned corners of the mouth	Flat nasal bridge, Anteverted nostrils	Malformed low-set ears	Thin upper lip	Hypertelorism	Trigonocephaly, short palpebral fissures, high arched eyebrows, long philtrum, long fingers, micrognathia, short neck, flat occiput, midfacial hypoplasia, bilateral epicanthal folds, down-slanting palpebral fissures, squared nail shape	Gross motor delay, social milestones delay	Omphalocele, asymmetrically flattened chest on the left side, wide-set nipples, ASD, complete collapse of the left lung	

gene. 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.[4] 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, *KANK*1, and *EHMT*1 are associated with developmental delay.[8] 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.[9] It is known that the 9p24.3-pter is associated with aberrant sexual development. This region contains the *DMRT* domain. Although *DMRT* genes are most prominent for the gonadal dysgenesis phenotype, the underlying molecular mechanism remains unclear.[10] 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

Table 2: Details of the genes involved in 9p24.3 deletion

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, atrial septal defect, inguinal hernia, scoliosis and kyphosis, tapering fingers, pale skin, mental retardation, psychomotor development retardation, speech delay, hypotonia	*DOCK8*	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

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]

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.

Acknowledgment

The present manuscript was extracted from a PhD thesis by M. Saberi. The study was supported by the Institute of Genetic Engineering and Biotechnology (Tehran, Iran) and the Iranian Ministry of Social Affairs. The authors would like to thank the parents of our patient for their collaboration.

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.

References

1 McKusick VA [Internet]. Online Mendelian inheritance in man, OMIM. [cited 2021 May

26]. Available from: http://www.ncbi.nlm.nih.gov/omim
2 Aktas D, Weise A, Utine E, Alehan D, Mrasek K, von Eggeling F, et al. Clinically abnormal case with paternally derived partial trisomy 8p23.3 to 8p12 including maternal isodisomy of 8p23.3: a case report. Mol Cytogenet. 2009;2:14. doi: 10.1186/1755-8166-2-14. PubMed PMID: 19566937; PubMed Central PMCID: PMCPMC2715415.
3 Ejaz R, Babul-Hirji R, Chitayat D. The evolving features of Nicolaides-Baraitser syndrome - a clinical report of a 20-year follow-up. Clin Case Rep. 2016;4:351-5. doi: 10.1002/ccr3.425. PubMed PMID: 27099726; PubMed Central PMCID: PMCPMC4831382.
4 Sanchez AI, Rojas JA. A SMARCA2 Mutation in the First Case Report of Nicolaides-Baraitser Syndrome in Latin America: Genotype-Phenotype Correlation. Case Rep Genet. 2017;2017:8639617. doi: 10.1155/2017/8639617. PubMed PMID: 28948053; PubMed Central PMCID: PMCPMC5602489.
5 National Library of Medicine (US) [Internet]. Genetics Home Reference. Bethesda (MD): The Library. [cited 2020 May 12]. Available from: https://ghr.nlm.nih.gov/
6 Schmid M, McGowan-Jordan J, Simons A. An International system for human cytogenomic nomenclature (ISCN 2016). Cytogenetic and Genome Research. 2016;149:1-2. doi: 10.1159/isbn.978-3-318-05979-3.
7 Miclea D, Peca L, Cuzmici Z, Pop IV. Genetic testing in patients with global developmental delay / intellectual disabilities. A review. Clujul Med. 2015;88:288-92. doi: 10.15386/cjmed-461. PubMed PMID: 26609258; PubMed Central PMCID: PMCPMC4632884.
8 Wang JC, Mahon LW, Ross LP, Anguiano A, Owen R, Boyar FZ. Enrichment of small pathogenic deletions at chromosome 9p24.3 and 9q34.3 involving DOCK8, KANK1, EHMT1 genes identified by using high-resolution oligonucleotide-single nucleotide polymorphism array analysis. Mol Cytogenet. 2016;9:82. doi: 10.1186/s13039-016-0291-3. PubMed PMID: 27891178; PubMed Central PMCID: PMCPMC5111223.
9 Mitsui N, Shimizu K, Nishimoto H, Mochizuki H, Iida M, Ohashi H. Patient with terminal 9 Mb deletion of chromosome 9p: refining the critical region for 9p monosomy syndrome with trigonocephaly. Congenit Anom (Kyoto). 2013;53:49-53. doi: 10.1111/j.1741-4520.2012.00362.x. PubMed PMID: 23480358.
10 Onesimo R, Orteschi D, Scalzone M, Rossodivita A, Nanni L, Zannoni GF, et al. Chromosome 9p deletion syndrome and sex reversal: novel findings and redefinition of the critically deleted regions. Am J Med Genet A. 2012;158A:2266-71. doi: 10.1002/ajmg.a.35489. PubMed PMID: 22821627.
11 Tassano E, Accogli A, Pavanello M, Bruno C, Capra V, Gimelli G, et al. Interstitial 9p24.3 deletion involving only DOCK8 and KANK1 genes in two patients with non-overlapping phenotypic traits. Eur J Med Genet. 2016;59:20-5. doi: 10.1016/j.ejmg.2015.11.011. PubMed PMID: 26656975.
12 Engelhardt KR, Gertz ME, Keles S, Schaffer AA, Sigmund EC, Glocker C, et al. The extended clinical phenotype of 64 patients with dedicator of cytokinesis 8 deficiency. J Allergy Clin Immunol. 2015;136:402-12. doi: 10.1016/j.jaci.2014.12.1945. PubMed PMID: 25724123; PubMed Central PMCID: PMCPMC4530066.
13 Van Houdt JK, Nowakowska BA, Sousa SB, van Schaik BD, Seuntjens E, Avonce N, et al. Heterozygous missense mutations in SMARCA2 cause Nicolaides-Baraitser syndrome. Nat Genet. 2012;44:445-9. doi: 10.1038/ng.1105. PubMed PMID: 22366787.
14 Choi A, Oh JY, Kim M, Jang W, Jang DH. Patient With Delayed Development Resulting From De Novo Duplication of 7q36.1-q36.3 and Deletion of 9p24.3. Ann Rehabil Med. 2017;41:881-6. doi: 10.5535/arm.2017.41.5.881. PubMed PMID: 29201829; PubMed Central PMCID: PMCPMC5698677.
15 Hou QF, Wu D, Chu Y, Liao SX. Clinical findings and molecular cytogenetic study of de novo pure chromosome 9p deletion: Pre- and postnatal diagnosis. Taiwan J Obstet Gynecol. 2016;55:867-70. doi: 10.1016/j.tjog.2016.11.001. PubMed PMID: 28040136.
16 Sirisena ND, Wijetunge UK, de Silva R, Dissanayake VH. Child with deletion 9p syndrome presenting with craniofacial dysmorphism, developmental delay, and multiple congenital malformations. Case Rep Genet. 2013;2013:785830. doi: 10.1155/2013/785830. PubMed PMID: 23984121; PubMed Central PMCID: PMCPMC3741698.