Case Report

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Overgrowth Syndrome with 9q22.3 Microdeletion Detected by Microarray Comparative Genomic Hybridization

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ABSTRACT

Microdeletion of 9q22.3 is a rare chromosomal disorder characterized by body overgrowth, facial dysmorphic features and psychomotor delay. The presence of genomic microdeletion or microdu-plication can not be identified by the conventional chromosomal analysis. Microarray comparative genomic hybridization (CGH) is a newly developed molecular cytogenetic technique that enables the identification of minute copy number variation (CNV) in the human genome. Here, we report a case of microdeletion in the 9q22.31-q22.33 region, which included a *patched homolog 1* (*PTCH1*) gene, as detected by CGH and confirmed by fluorescence in situ hybridization (FISH) analyses in a neonate with prenatal onset of macrosomia, dysmorphism, and muscle hypotonia. To the best of our knowledge, this is the first case report of 9q22.3 microdeletion detected by CGH in Korea.

Key Words: 9q22.3 microdeletion, Overgrowth, Psychomotor delay, Microarray CGH

INTRODUCTION

9q22.3 microdeletion has been recently reported with overgrowth syndrome, characterized by overgrowth, dysmorphic features, macrocephaly, and psychomotor delay[1,2]. There have been reports on congenital chromosomal abnormalities that cause overgrowth such as Sotos syndrome with an abnormality of *nuclear receptor binding SET domain protein 1 (NSD1)* gene on 5q35[2,3], and Beckwith-Wiedemann syndrome due to genomic alteration of chromosome 11q15[2,4]. The chromosomal location of the 9q22.3 deletion includes the *patched homolog 1 (PTCH1)* gene, which is reported to be responsible for basal cell nevus syndrome (BCNS)[1,2,5,6]. Recently developed high-throughout chromosomal analyses, such as comparative genomic hybridization (CGH), enable early detection of new phenotypic features in individuals with the 9q22.3 microdeletion, which are not

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Figure 1. (A) and (B) Facial image of the patient at the neonatal period showing frontal bossing, a small mouth with a thin upper lip, a protruding chin, low set ears with uplifted ear lobule, and a short neck. (C) Facial image at the age of 3years shows frontal bossing, small mouth with a thin upper lip, a protruding chin and pit of the nasal dorsum (black arrow).

consistent with basal cell nevus syndrome at an early age[5].

A neonate presented with prenatal onset of macrosomia, facial dysmorphism, vertebral abnormality, mild ventriculomegaly, and muscle hypotonia. Via microarray CGH, we identified a 4.70-Mb microdeletion in the 9q22.31-q22.33 region that included *PTCH1* and confirmed the deletion of this gene by fluorescence in situ hybridization (FISH).

CASE REPORT

A female neonate with Apgar scores 8 and 9 at 1 and 5 minutes, respectively, was delivered at 35 weeks of gestation via vaginal delivery 6 hours after preterm premature rupture of the membrane. The patient was the first baby of non-consanguineous and healthy 33-year-old parents with normal statures (paternal height of 183 cm and maternal height of 167 cm). The prenatal period was uneventful. The patient was transferred to our unit 9 hours after delivery and from that point on showed progressively aggravated breathing. Growth parameters at birth were as follows: weight of 2920 g (>90th percentile), length of 51 cm (>90th percentile), and head circumference of 36 cm (>90th percentile). Facial dysmorphic features were noted including frontal bossing, a small mouth with thin upper lip, a protruding chin, low set ears with uplifted ear lobule, and a short neck (Figure 1A, 1B). Spine roentgenogram revealed the spina bifida of the cervical vertebrae (Figure 2). Brain magnetic resonance imaging performed at the age of 1 month showed mild left lateral ventriculomegaly without other pathological lesions and follow-up imaging performed at the age of 22 months showed no interval change (Figure 3A, 3B). Cardiac echocardiography of the neonate performed on the third day following delivery showed the presence of patent ductus arteriosus (PDA). However, following fluid restriction and diuretic treatment, a follow-up study revealed the closing of the PDA. No abnormalities were found in the ophthalmological and auditory evaluations. The neonatal period was complicated by respiratory distress syn-

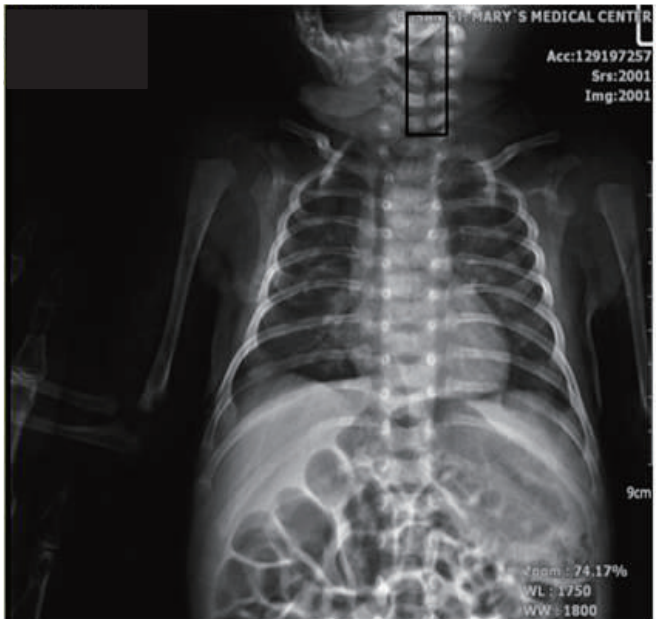

Figure 2. Spinal roentgenogram shows the spina bifida on the cervical vertebrae (in black rectangle).

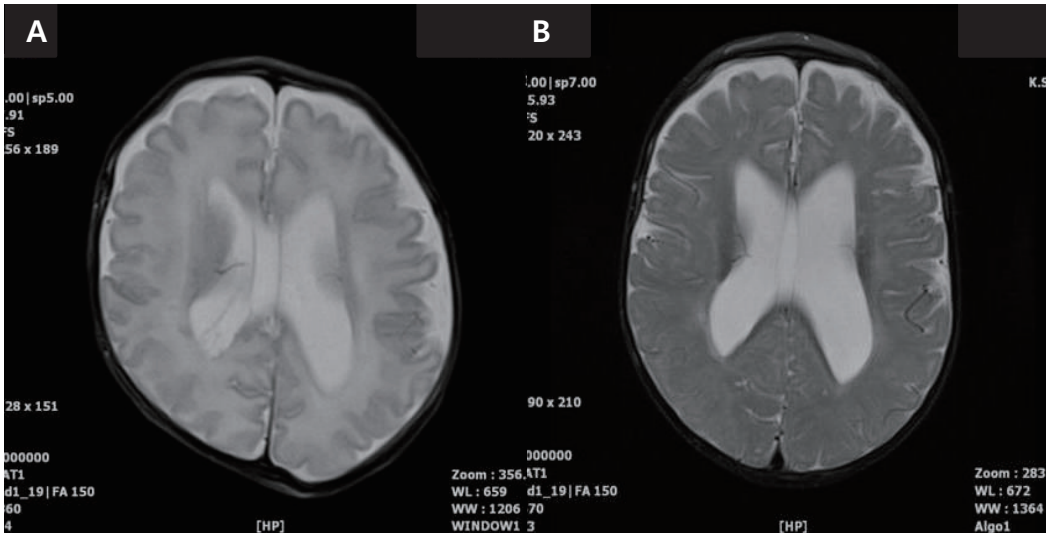

Figure 3. (A) Brain magnetic resonance imaging (T-2 weighted) of our patient at the age of 1month showed mild dilatation of left lateral ventricle without any pathological lesion. (B) Follow-up imaging at the age of 12 months yielded similar results with no interval change.

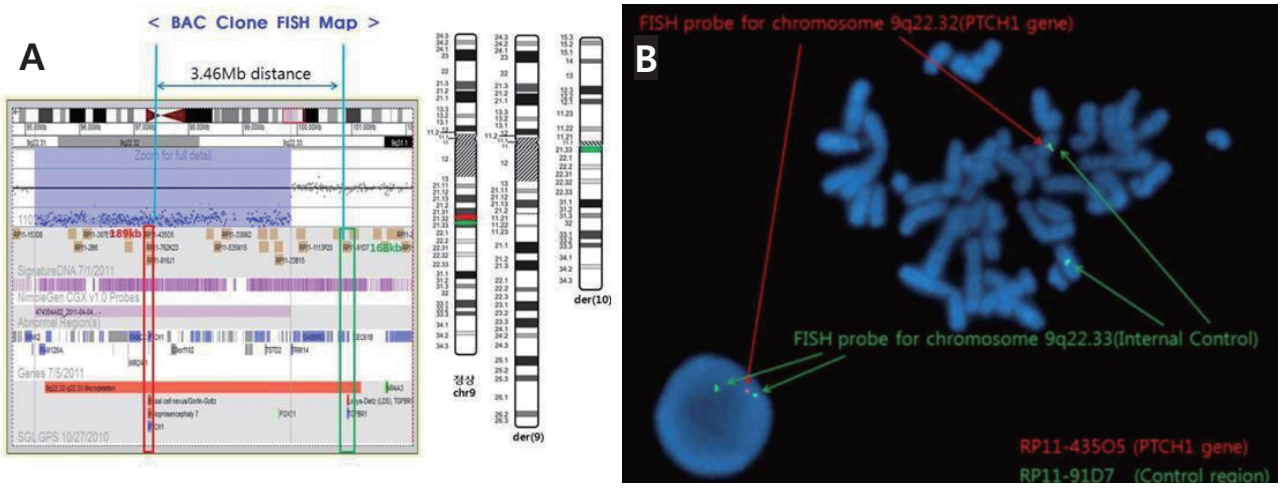

Figure 4. (A) Microarray CGH of our patient shows microdeletion of the 9q22.31-33 region spanning the *PTCH1* gene. The size of deletion is 4.7 Mb, extending from 95,174,941 to 99,879,040. (B) FISH analysis of our patient showed the deletion of *PTCH1* on 9q22.3 in all 100 cells analyzed by using the following BAC clones: RP11-435O5 (target region: red signal) at 9q22.32 and RP11-91D7 (control region: green signal) at 9q22.33. One red signal and two green signals were identified, which confirmed the deletion of Z*PTCH1*. Abbreviations: CGH, comparative genomic hybridization; *PTCH1*, *patched homolog 1*; FISH, fluorescence in situ hybridization.

drome, symptomatic PDA, muscle hypotonia, and poor sucking. Oxygen therapy was needed for 6 weeks. Additionally, neurodevelopmental treatment and oral stimulation therapy were needed to treat hypotonia and poor sucking, respectively, during the neonatal period. Prenatal onset of macrosomia, dysmorphism, and hypotonia were evaluated via CGH using human 135-K whole genome arrays (Roche NimbleGen, Germany) at 1 month of age; this analysis revealed a 4.7-Mb microdeletion in the 9q22.31-q22.33 regions, which included *PTCH1* (Figure 4A). Genes within the deleted regions included *fanconi anemia, complementation group C (FANC-C)* and *patched homolog 1 (PTCH1)*. FISH analysis at the age of 7.5 months confirmed the deletion of PTCH in all 100 cells analyzed using the BAC clones, RP11-435O5 (target region: red signal) and RP11-91D7 (control region: green signal) at 9q22.32 and 9q22.33 (Figure 4B). Global developmental delay in motor, language, and cognition was

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recorded during the infantile period using the Denver Developmental Screening Test and Bayley Scales of Infant and Toddler Development III.

Physical, occupational, and speech therapies continued until the early childhood. The patient's developmental milestones were as follows: complete head control at the age of 10 months, independent sitting at the age of 18 months, articulation of one syllable and creeping at the age of 23 months, gait with two-hand support at the age of 27 months, articulation of three or four syllables at the age of 35 months, and independent gait at the age of 38 months. The postnatal overgrowth lasted until quite recently. The patient's height was 80.0 cm (>95th percentile), weight was 11.2 kg (>90th percentile), and head circumference was 50.6 cm (>97th percentile) at the age of 12 month. The patient's height was 93.0 cm (>95th percentile), weight was 14.1 kg (>90th percentile), and head circumference was 53.3 cm (>97th percentile) at the age of 24 months. The patient's height was 99.8 cm (>95th percentile), weight was 16.0 kg (>90th percentile), and head circumference was 54.0 cm (>97th percentile) at the age of 36 months.

When the patient was 3 years and 6 months, we re-evaluated her for possible basal cell nevus syndrome (BCNS). Facial dysmorphic features including pit of the nasal dorsum and macrocephaly were still noted (Figure 1C). According to the Greulich and Pyle method[7], the bone age was appropriate at the age of 36-42 months. No major findings supporting BCNS were noted.

Table 1. Diagnostic Criteria for Basal Cell Nevus Syndrome Proposed by Kimonis, et al.[5,8]

Major criteria
Lamellar calcification of the falxcerebri prior to 20 years of age
More than five basal cell carcinoma or carcinoma before the age of 30 years
Odontogenic keratocysts of the jaw confirmed by histology
Palmar or plantar pits
First-degree relative with nevoid basal cell carcinoma syndrome
Minor criteria (Any one of the following features)
Cleft lip and/or cleft palate
Pre- or postaxial polydactyly
Macrocephaly (occipital-frontal circumference of >97th percentile
Ocular anomalies (including microphthalmia, cataract, retinal anomalies, developmental defects)
Rib and/or vertebral anomalies
Cardiac and ovarian fibromas
Childhood medulloblastoma
Lymphomesenteric or pleural cysts

Positive diagnosis is established by two major or one major and two minor criteria.

DISCUSSION

The 9q22.3 microdeletion is a very rare phenomenon. Until now, only 42 cases of 9q22.3 microdeletion have been reported[5], however, to the best of our knowledge, there are no such cases reported in Korean literature. A 9q22.3 microdeletion includes the deletion of *PTCH1* that is responsible for basal cell nevus syndrome or the Gorlin syndrome, the diagnostic criteria of which was proposed by Kimonis, et al.[5,8] (Table 1). BCNS was first clearly defined in 1960 by Gorlin and Goltz[9]. This syndrome is due to the mutation of *PTCH1* and is characterized by clinical complications such as lamellar calcification of the falx cerebri, basal cell carcinoma, jaw keratocyst, and the palmar or plantar pits[5]. This syndrome is considered to be a precancerous condition for tumors such as medulloblastoma, Wilms' tumor, cardiac, and ovarian fibromas[5,10]. Our patient met only two minor criteria of BCNS[5,8] including macrocephaly and vertebral abnormalities. Because several major signs of BCNS[5,8] are agedependent and our patient was too young to exhibit the diagnostic features of BCNS, further monitoring to check up for the possible onset of these features is required.

Clinical phenotypes vary with the size of the deleted region, which is inclusive of genes other than *PTCH1*[5]. Approximately 20% of the cases with 9q22.3 microdeletion presented prenatal onset of macrosomia that is characterized by a birth length and weight above the 90th percentile[5]. Moreover, macrosomia persisted postnatally in these patients[5]. Our patient with the overgrowth syndrome, which persists till date, presented with various facial dysmorphic features and global developmental delay in accordance with a previous report[5]. Shimojima, et al.[1] reported a patient who exhibited overgrowth, various facial dysmorphic features, and developmental changes within the lower limit of the normal range. The CGH of this patient revealed a 2.3-Mb deletion of 9q22.32 spanning the *PTCH1* gene, with clinical manifestations of BCNS subsequently. On the other hand, two patients reported by Redon, et al.[2] showed overgrowth, facial dysmorphic features, and developmental delay. Similarly, CGH revealed a 6.5-Mb microdeletion at the same region of 9q22.32-33 involving the *PTCH1* gene. However, none of the two cases met the criteria of BCNS. In addition, Kosaki, et al.[6] reported a

patient presenting overgrowth, various facial dysmorphisms, and developmental delay; a 2.44-Mb microdeletion at the 9q22.3 region spanning the *PTCH1* gene was identified by CGH. This patient as well the patient in our study did not meet the criteria of BCNS. Overlapping regions ranging from 2.3Mb to 4.7Mb were noted between the four previously reported cases and our case, indicating the possible deletion of growth suppressor gene(s) other than *PTCH1* in the overlapped regions[1,2,6]. Further studies to identify the targeted growth suppressor genes from among these candidate genes are required.

The 9q22.3 microdeletion is inherited in an autosomal dominant manner[5]. It appears to result from either a *de novo* event or inheritance of an unbalanced chromosome rearrangement from a parent with a balanced rearrangement[5]. Familial chromosome analysis is necessary for genetic counseling; however, we could not perform this for the parents.

Regarding clinical manifestations, cases with deletion >2 Mb exhibited delayed motor, speech, and behavioral developments[1,5,11]. More severe disability is expected with an increase in deletion size[5,11,12]. Our patient with a 4.7-Mb deletion and two other patients with 6.5-Mb deletions[2] showed severe developmental delay, which supports the notion that the severity of developmental delay is proportional to the size of deletion. Several cases with 9q22.3microdeletion have been reported to show cerebral ventricular dilatation ranging from mild and asymmetric to severe obstructive hydrocephalus in association with craniosynostosis and cerebral atrophy or space-occupying lesions such as medulloblastoma[5,11]. Our patient showed mild ventricular dilatation without any space-occupying lesion. Facial dysmorphism with 9q22.3 microdeletion tends to coarsen over a period of time; on the other hand, extremely large deletions may cause coarse features at birth[5]. Our patient exhibited facial dysmorphism at birth. Several cases with 9q22.3 microdeletion had Wilms' tumor and pelvic rhabdomyosarcoma[5]. Recently, a patient with BCNS syndrome due to 9q22.3 microdeletion which included the deletion of *PTCH1* and the FANC-C loci presented with multiple tumors of leiomyoma and Wilms' tumor[10]. Deletion of PTCH1 and FANC-C loci were identified in our patient via CGH; however, the FANC-C loci deletion was not confirmed by FISH. Close clinical observation is required to investigate potential tumors in our patient.

We could identify the cause of the unique clinical features in our patient by CGH. Microarray CGH has been developed since the late 1990s[1,13]. This technique enables submicroscopic chromosomal imbalance such as a microdeletion of <5 Mb owing to its high resolution and earlier detection of microdeletion in patients with peculiar clinical features[13]. Our case was diagnosed at 1 month of age similar to previous reports, showing early diagnosed 9q22.3 microdeletion syndrome with CGH method at 2 weeks to 1 year of age[11]. This technique is useful for the detection of chromosomal disorders with non-specific manifestations or microscopic chromosomal imbalance which has clinical importance over the conventional cytogenetic methods such as karyotyping based on banding pattern[13]. As per the guidelines of the International Standard Cytogenomic Array Consortium (ISAC)[14] and the American College of Medical Genetics (ACMG) Practice[15], CGH should be used in cases of non-specific multiple malformation and non-syndromic developmental delay or mental retardation, as was in our case.

In conclusion, we identified a 9q22.3 microdeletion in a baby with overgrowth syndrome, developmental delay, and facial dysmorphic features. The possible presence of a submicroscopic deletion of 9q22.3 should be considered when evaluating patients with multiple anomalies including overgrowth and developmental delay. Moreover, microarray CGH could be used as a method for the detection of non-specific multiple malformation and non-syndromic developmental delay or mental retardation, as shown in the present case.

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