# Clefting in Trisomy 9p Patients: Genotype-Phenotype Correlation Using Microarray Comparative Genomic Hybridization

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**Abstract:** Duplication 9p syndrome (partial trisomy 9p) is characterized by craniofacial anomalies, mental retardation, and distal phalangeal hypoplasia. Here, we present a female patient with microcephaly and incomplete bilateral cleft lip and palate, whose initial cytogenetic analysis revealed a de novo trisomy 9p. The patient, now 21 years old, has persistent microcephaly, craniofacial and hand anomalies, history of a seizure disorder, and global mental retardation. Oligonucleotide-based array comparative genomic hybridization was performed and revealed partial trisomy 9p21.1->9pter and a deletion of 9p12.1 to 9p11.2. Our case supports the utility of array comparative genomic hybridization for the precise characterization of chromosomal anomalies and for the ascertainment of genotype-phenotype correlation in patients with partial trisomy 9p.

**Key Words:** Orofacial cleft, array comparative genomic hybridization, 9p duplication, trisomy 9p

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Duplication 9p, or partial trisomy 9p, was first reported in 1970.[1] Typical features include dysmorphic craniofacial features (downslanting palpebral fissures, hypertelorism, prominent nose, downturned corners of the mouth, prominent and dysplastic ears), limb anomalies (short fingers and toes, dysplastic nails, fifth finger clinodactyly), and developmental delay. Genotype-phenotype correlations have suggested that the extent of trisomic material correlates with the degree of clinical severity.[2]

With respect to facial features, duplication of 9pter-p11 is associated with milder dysmorphia, whereas duplication of 9p21.1-q22-32 is associated with more severe craniofacial features.[3] Orofacial clefting is only present in approximately 5% of cases,[4] and the specific region associated with clefting has not yet been clearly identified. In addition, delineation of the genomic region responsible for the clefting phenotype is complicated by the presence of small deletions or duplications involving other chromosomes in most reported cases of trisomy 9p.[5] These additional genetic aberrations make it difficult to understand which anomalies are caused by trisomy 9p and which are caused by the other chromosomal changes.

9p duplications have previously been studied by various methods including conventional karyotyping, fluorescent in situ hybridization, and bacterial artificial chromosome (BAC) or yeast artificial chromosome arrays. The methods used in these earlier studies do not always accurately describe chromosomal aberrations.[6] Recently, oligonucleotide array–based comparative genomic hybridization (array CGH) has emerged as a more precise technique to measure copy number changes across the entire genome. This method allows for detailed examination of specific regions involved in the duplication and/or deletion of chromosomes and enables a fuller understanding of genotype-phenotype correlations. Here, we present the first patient with duplication 9p syndrome to be analyzed by array CGH.

## CLINICAL REPORT

The propositus was first evaluated as an infant by a geneticist at an outside hospital because of congenital anomalies, including a repaired bilateral cleft lip and palate and developmental delay. She was the second child born to a healthy, nonconsanguineous 21-year-old mother of Hispanic and Native American ancestry and 23-year-old father of Mexican, Filipino, and Native American ancestry with a noncontributory family history. The first child was then a healthy 2.5-year-old boy. The pregnancy was uneventful and culminated in a spontaneous vaginal delivery at term. Neonatal examination was reportedly notable for microcephaly with a narrow forehead, bilateral cleft lip and palate, bilateral single palmar creases, and bilateral fifth digit brachydactyly. A karyotype revealed extra chromosomal material of unknown origin translocated onto the short arm of chromosome 9 (data not shown). Parental karyotypes were reportedly normal, indicating that the translocation was de novo.

The patient exhibited slow weight gain, and thus, the initial reconstruction of her cleft lip was delayed until 6.5 months of life, when bilateral cleft lip repair with the rotation advancement closure technique was performed. At the time of reconstruction, the lip clefts extended up to the nasal sills bilaterally, and there was a full cleft of the alveolar complex. The palatal cleft, which was complete on the right, was repaired at age 10 months. Bilateral myringotomies with insertion of ventilation tubes were performed at the ages of 3 and 5 years. The patient received dental restorations at the age of 11, and her alveolar cleft was grafted at 12 years. At 15 years, she was noted to have a deficiency of the upper lip tubercle producing a "marked whistle tip" deformity. A cross-lip flap procedure was recommended but deferred because the craniofacial team thought that the patient would not tolerate the repair at that time.

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**FIGURE 1.** A, Frontal view. B, Profile. Craniofacial phenotype as described in text.

The patient had normal audiological evaluations. She required speech and total communication therapy because of a significant articulation disorder. She also developed a seizure disorder that was treated with valproic acid and resolved by 13 years.

The patient was first evaluated by our genetics team at 21 years. She had been diagnosed with global mental retardation and was able to dress, feed herself, and use the bathroom independently. Her craniofacial features were notable for microcephaly with a head circumference of 51.5 cm (less than the second percentile), bitemporal narrowing, fleshy ear lobes that are normally placed, neutral palpebral fissures, full nasal bridge with a prominent, bulbous tip, and bilateral cleft lip and palate status after repair (Fig. 1). She presented with a concave facial profile due to maxillary hypoplasia with an anterior cross bite of at least 4 mm and class 3 malocclusion, congenitally missing maxillary lateral incisors bilaterally, severe crowding of both arches, and retroclined maxillary central incisors. The maxillary first premolar was blocked out palatally, and the maxillary left canine had erupted bucally because of inadequate space (Fig. 2A). Oral hygiene was poor with decalcification of the right central incisor. A panoramic radiograph revealed the presence of all permanent teeth in the lower jaw including the third molars. The lower left third molar had erupted, whereas the lower right third molar was impacted. In the maxilla, both lateral incisors and third molars were congenitally missing (Fig. 2B). In addition to the craniofacial findings, the examination result was notable for bilateral brachydactyly and fifth finger clinodactyly, a third finger measuring 6.3 cm (less than the third percentile; Fig. 3), mildly prominent thoracic kyphosis, and 2 café au lait macules on the left trunk.

## MATERIALS AND METHODS

Oligonucleotide array CGH analysis was performed on the patient's DNA sample referenced to a normal female control DNA sample using an Oligo HDScan (Combimatrix Molecular Diagnostics, Irvine, CA). The test is composed of more than 99,000 DNA probes corresponding to genomic loci encompassing the 22 autosomes and the sex chromosomes. Results were confirmed with high-definition BAC array CGH.

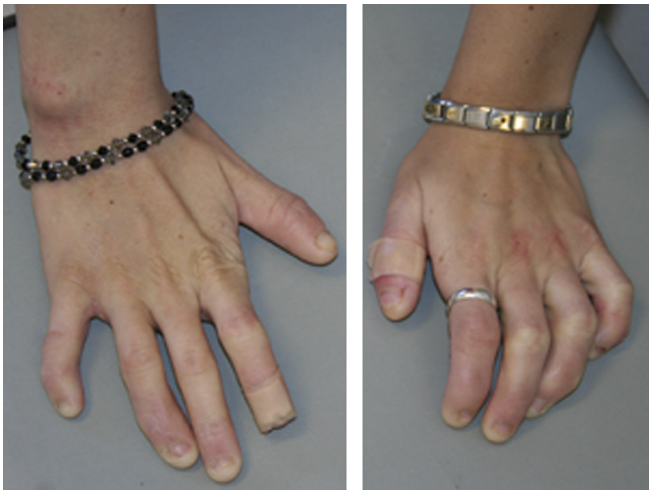

**FIGURE 3.** Hand photos showing fifth finger brachydactyly and clinodactyly. Left, Right hand. Right, Left hand.

## RESULTS

Array CGH analysis (Fig. 4) revealed a single copy number increase from 9p21.1->pter, [9p24.3p21.1 (193993-> 31363363)x3] consistent with a 31.1-Mb duplication of the short arm of chromosome 9. In addition, it identified an interstitial deletion on the short arm of chromosome 9 [9p13.1p11.2(39049699->43599644)x1], indicating a single copy number decrease of 4.5 Mb from 9p13.1 to 9p11.2 (Fig. 4). No other causal copy number alterations were detected. An array profile consistent with a female sex chromosome complement was observed.

The oligo array results were confirmed by high-definition BAC array, which showed the following aberrations: 9p24.3p21.1 (RP11-112G24->RP11-29M23)x3;9p13.1p11.2(RP11-927H19->RP11-105J4)x1.

## DISCUSSION

Here, we present the first case reported of a patient with trisomy 9p analyzed by oligonucleotide array CGH. The craniofacial findings associated with trisomy 9p are well documented in the literature. Our patient has the typical phenotypic features including microcephaly, a prominent nose, and mental retardation with significant language delay. However, in addition to these typical features, our patient also had orofacial clefting. This anomaly is present in only 5% of cases with 9p duplication syndrome.[4]

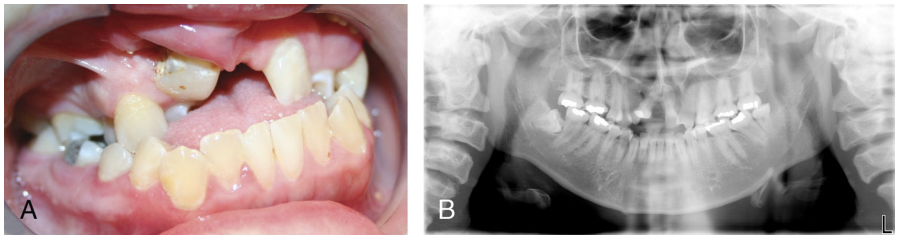


**FIGURE 2.** A, Intraoral photographs showing the missing maxillary lateral incisors, severe crowding, malocclusion, and anterior cross bite. B, Panoramic radiograph showing congenitally missing lateral incisors and third molars in the maxilla and an impacted right third molar in the mandible.

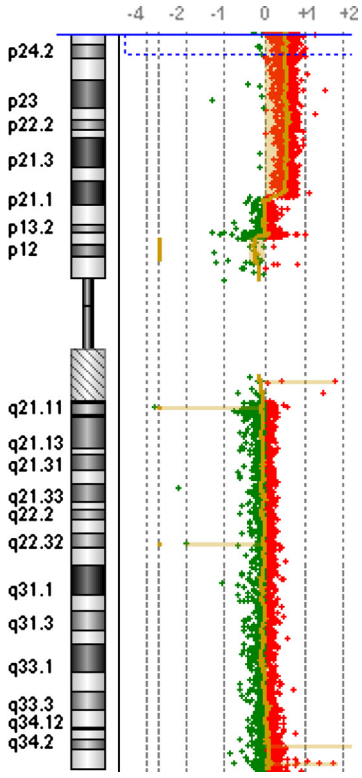


**FIGURE 4.** A, Oligonucleotide array CGH analysis showing a 31.1-Mb duplication of 9p21.1->pter, [9p24.3p21.1 (193993-> 31363363)x3] and a 4.5-Mb deletion of 9p11.2->p13.1 [(39049699->43599644)x1].

Previous attempts have been made to perform genotype-phenotype correlation in patients with clefting and duplication 9p. 9p11 was originally proposed as the critical region.[2] However, cases of clefting in 9p duplication patients that do not involve duplications of this specific region have been reported,[7] and furthermore, case reports of duplications in 9p11 that do not have any overt phenotype have been described.[8]

A number of patients with trisomy 9p have overlapping trisomic chromosomal material in the region of 9p22->9p24.[2,7,9–13] This has been proposed as a critical region for the duplication 9p phenotype.[3,14] Although not all cases with duplications of this region result in orofacial clefting, the cases in which the 9p phenotype includes an orofacial cleft have duplications in this region. Of the more than 20 known genes contained in the 9p22->9p24 interval, 2 likely candidates responsible for orofacial clefting are *CER1* and *FREM1*. *CER1* is expressed in the anterior mesendoderm of gastrula-stage embryos. The *CER1* gene product is a cytokine involved in patterning the anterior of the embryo, and mutations in this gene can induce the formation of ectopic heads.[15] It is conceivable that haploinsufficiency for this gene may lead to abnormalities in the development of the orofacial complex, which could result in clefting. This gene has been proposed to be responsible for the trigonocephaly phenotype in 9p deletion[16] and may also be involved in the phenotypic facial features of trisomy 9p. *FREM1*, another candidate gene, lies on the 9p22.3 locus and is expressed in differentiating epidermal structures. Mutations of *FREM1* have been identified in patients with a bifid nose.[17]

Precise delineation of the genotype associated with 9p clefting is complicated by the variable expression of orofacial clefting in patients with duplications of 9p22->9pter, which may be due in part to the effects of modifier genes. In addition, isolated duplications of 9p[5] are rare. Most cases involve additional chromosomal deletions or translocations. For example, in our case, the utilization of array CGH identified a cytogenetically invisible deletion in 9p11.2-9p13.1. Although clefting has not previously been associated with monosomy 9p, it is possible that small deletions in earlier cases were not identified by conventional cytogenetic methods, and thus, the contribution of this deletion to the clefting phenotype cannot be excluded. There are few genes present in the 9p11.2-9p13.1 locus. *CILP2* is located at 9p13.1 and encodes a protein expressed in joint cartilage, but disease-causing mutations have not yet been identified in this gene.[18] Another gene located in the deleted region is the SHB adaptor protein gene (9p11-9p12), but mutations in this gene have not been reported in patients to date. Thus, at this time, there are no obvious candidate genes in this region that could be responsible for a clefting phenotype.

It has been proposed that the spectrum of clinical severity of the 9p duplication syndrome correlates with the extent of trisomic chromosome material[2] rather than with an identifiable critical region of duplication.[19] This may also contribute to the variability of clefting. Interestingly, there is an increased incidence (78%) of clefting in tetrasomy 9p,[20] which suggests that additional chromosomal material from this region contributes to the clefting phenotype.

Here, we have used oligonucleotide array CGH to study a patient with clefting and 9p trisomy, and this technique is being increasingly used to detect duplications and deletions in patients with cleft lip and palate to identify critical loci as well as clefting candidate genes.[21] Our case demonstrates the utility of array CGH in establishing increasingly precise genotype-phenotype correlations. 9p duplications have previously been identified by BAC and yeast artificial chromosome arrays; however, to our knowledge, this is the first reported case of concurrent 9p duplication and 9p deletion described by oligonucleotide array. The ability to identify a critical locus for clefting is limited by the lack of technical precision in previously reported cases. As more cases of array CGH are established, the critical region, breakpoints, and correlation with

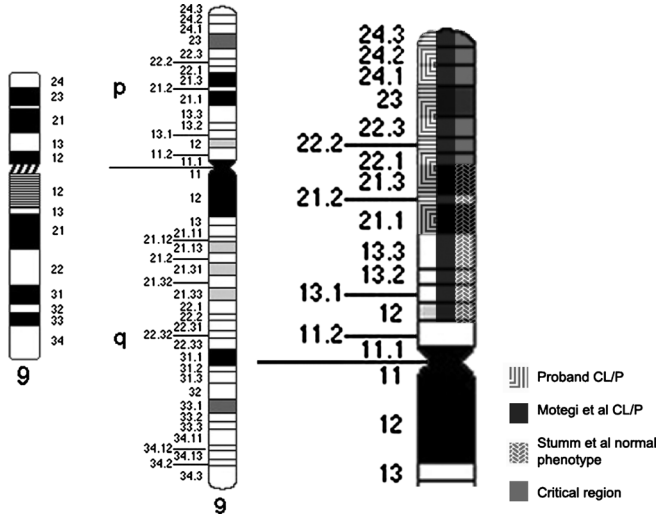


**FIGURE 5.** Ideogram of chromosome 9p highlighting duplicate material in the proband (9p21.1-pter), case report of bilateral cleft lip/palate described by Motegi et al[11] (9p12-9p24), case report with normal phenotype described by Stumm et al[22] (9p12-9p21.3), and the suspected critical region responsible for the classic phenotype (9p22-9p24). Ideogram template courtesy of National Center for Biotechnology Information.

translocations involved in clefting will be identified with increasing confidence.

In summary, our case supports the notion that 9p22-p24 is one of the critical regions responsible for the classic duplication 9p phenotype. We suggest that the 9p21.3-pter region contains genes important in the proper formation of the lip and palate (Fig. 5). This region has not, to our knowledge, previously been implicated in orofacial morphogenesis. However, we cannot discount the possible phenotypic contribution of the proband's deletion of 9p11.2-9p13.1 to the clefting phenotype. Additional investigation of the genes within chromosome 9p and corresponding dosage effects will be required for a more complete understanding of the duplication 9p phenotype.

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