Refinement of the Region for Split Hand/Foot Malformation 5 on 2q31.1

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Key Words

2q31.1 • *DLX1/DLX2* • *HOXD* • Limb anomalies • Microdeletion • SHFM5

Abstract

Background: Deletions that encompass 2q31.1 have been proposed as a microdeletion syndrome with common clinical features, including intellectual disability/developmental delay, microcephaly, cleft palate, growth delay, and hand/foot anomalies. In addition, several genes within this region have been proposed as candidates for split hand-foot malformation 5 (SHFM5). **Methods:** To delineate the genotype-phenotype correlation between deletions of this region, we identified 14 individuals with deletions at 2q31.1 detected by microarray analysis for physical and developmental disabili-

ties. **Results:** All subjects for whom detailed clinical records were available had neurological deficits of varying degree. Seven subjects with deletions encompassing the *HOXD* cluster had hand/foot anomalies of varying severity, including syndactyly, brachydactyly, and ectrodactyly. Of 7 subjects with deletions proximal to the *HOXD* cluster, 5 of which encompassed *DLX1/DLX2*, none had clinically significant hand/foot anomalies. In contrast to previous reports, the individuals in our study did not display a characteristic gestalt of dysmorphic facial features. **Conclusion:** The absence of hand/foot anomalies in any of the individuals with deletions of *DLX1/DLX2* but not the *HOXD* cluster supports the hypothesis that haploinsufficiency of the *HOXD* cluster, rather than *DLX1/DLX2*, accounts for the skeletal abnormalities in subjects with 2q31.1 microdeletions.

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Over 30 interstitial deletions of the long arm of chromosome 2 have been reported [McConnell et al., 1980; Shabtai et al., 1982; Moller et al., 1984; Ramer et al., 1989; Wamsler et al., 1991; Boles et al., 1995; Chinen et al., 1996; Courtens et al., 1997; Nixon et al., 1997; Del Campo et al., 1999; Slavotinek et al., 1999; Maas et al., 2000; Bijlsma et al., 2005; Pescucci et al., 2007; Svensson et al., 2007; Zhao et al., 2007]. Although the breakpoints and clinical features vary among reported individuals, low birth weight, postnatal growth retardation, intellectual disability (ID)/developmental delay (DD), microcephaly, mild facial dysmorphism (long eyelashes, downslanting palpebral fissures, micrognathia, low-set ears), and musculoskeletal anomalies appear to be characteristic for individuals with 2q deletions. Deletions encompassing 2q31.1 have been associated with various limb abnormalities ranging from mild digital abnormalities such as camptodactyly, 5th finger clinodactyly, syndactyly, duplicated halluces, and hypoplastic phalanges of 3rd/4th/5th finger to severe malformations such as split-hand/split-foot and monodactyly [Boles et al., 1995; Nixon et al., 1997; Goodman, 2002], although these severe malformations have been reported in only a few individuals with deletions of this region.

Disruptions of the 5'-end of the *HOXD* cluster (*HOXD11-13*) and neighboring genes on 2q31.1 have been implicated in limb malformations [Goodman et al., 2002]. The *HOXD* cluster is 1 of 4 highly conserved, homologous gene clusters coding for transcription factors with crucial roles in embryonic development. The cluster comprises 9 homologous genes: from proximal to distal they are *HOXD13*, *HOXD12*, *HOXD11*, *HOXD10*, *HOXD9*, *HOXD8*, *HOXD4*, *HOXD3*, and *HOXD1* [Goodman, 2002]. Human homeobox *EVX2* is located proximal to *HOXD13* [D'Esposito et al., 1991], and proximal to *EVX2* are 7 regulatory elements, R1-R7 [Goodman, 2002]. In mouse, expression of *Hox* genes is crucial for sonic hedgehog (Shh) transcription in the early developing limb bud. Mutations of *HOXD13* have been associated with limb abnormalities [Goodman et al., 1998; Kan et al., 2003; Zhao et al., 2007]; the most common is an expansion of a polyalanine tract in exon 1 of *HOXD13* reported to cause dominantly inherited synpolydactyly [Muragaki et al., 1996].

Based on genotype-phenotype correlations for individuals with deletions of the 2q24.3q31 region and musculoskeletal anomalies, Duijf et al. [2003] proposed the region could be divided into 3 loci for limb abnormalities: individuals with 2q24.3 deletions have flexion deformities of the fingers, individuals with proximal 2q31.1 dele-

tions have a wide gap between the 1st and 2nd toe, and distal 2q31.1 deletions that remove the *HOXD10/HOXD13* and *EVX2* genes, as well as mutations in *HOXD13*, cause synpolydactyly.

Goodman et al. [2002] identified a 117-kb microdeletion at the 5'-end of the *HOXD* cluster that removed *HOXD9-13*, *EVX2*, R1-R7, and part of a LINE element in a father and daughter with synpolydactyly. By genotype-phenotype comparison with a girl with bilateral split foot and deletion of the entire *HOXD* cluster as well as the 5-Mb region proximal to the cluster [Goodman et al., 2002], the authors suggested haploinsufficiency for the 5'-*HOXD* cluster causes synpolydactyly, whereas the 5-Mb interval proximal to *EVX2* comprised a novel split-hand/foot malformation locus, SHFM5 [Goodman et al., 2002].

The distal-less homeobox genes *DLX1/DLX2*, which are within the candidate SHFM5 region, have been proposed as candidate genes for limb malformations. Expression studies of mouse homologs in vivo showed *Dlx* family members with a possible function in osteoclast and osteoblast formation as well as in mature osteoclasts; *Dlx2* is also expressed in less-mature osteoblasts [Li et al., 2008; Lezot et al., 2010]. *Dlx2* is expressed in a subpopulation of osteoclasts in association with tooth, brain, nerve, and bone marrow volumetric growths and is also involved in the organization of cells that deposit tooth enamel in the labial region of molars during development [Lezot et al., 2008]. These data suggest a role for the *Dlx1/Dlx2* gene pair in the regulation of skeletal morphogenesis via functions within osteoclasts [Lezot et al., 2010]. However, heterozygous/homozygous knockouts of either or both genes show no limb abnormalities [Qiu et al., 1997], although null mutations of *Dlx2* are lethal [Qiu et al., 1995].

Recently, Svensson et al. [2007] reported a female with DD, microcephaly, and multiple upper- and lower-limb skeletal abnormalities including mild syndactyly of the 3rd/4th fingers, short middle phalanges, clinodactyly of the 5th digit at the distal interphalangeal joint on both hands, symphalangism of metatarsal-phalangeal joints of the 2nd/5th digits bilaterally, symphalangism of the 2nd/3rd/4th digits on both feet with fusion of the middle/distal phalanges of the 2nd/5th digits, and hallux valgus bilaterally. Oligonucleotide microarray analysis showed a 2.01-Mb deletion at 2q31.1, the distal breakpoint of which was ~361 kb proximal to the *HOXD* cluster. The authors suggested the musculoskeletal phenotype in their subject may be caused by deletion of one or more of the regulatory elements upstream of *HOXD*. Despite the absence of

limb abnormalities in mouse knockouts of either the *Dlx1/Dlx2* genes [Qiu et al., 1997], the study by Svensson et al. [2007] suggests that disruption of the region proximal to *HOXD* causes limb abnormalities of variable severity.

Several recent studies have proposed genotype-phenotype correlations for specific features associated with deletions at 2q31.1, including limb abnormalities [Mitter et al., 2010; Dimitrov et al., 2011]. In this study we report the molecular and clinical characterization of 14 individuals with deletions overlapping 2q31.1. This is the largest cohort to date of individuals with deletions of 2q31.1 characterized by microarray. Genotype-phenotype correlations among these individuals suggest regions upstream of the *HOXD* cluster can be excluded as candidates for SHFM5.

Materials and Methods

Subject Ascertainment

During the period encompassing March 2004 through February 2010, we tested 38,779 individuals submitted to Signature Genomics from the United States and abroad. The most common clinical presentations were ID, DD, or multiple congenital anomalies. Informed consent was obtained to publish photographs of the individuals described here.

Microarray-Based Comparative Genomic Hybridization

All 2q31.1 deletions were initially identified by microarray-based comparative genomic hybridization (aCGH) using various microarray platforms. Targeted BAC-based microarray analysis was originally performed on DNA from subjects 1, 2, 6, 7, 10, and 11 using previously published methods [Bejjani et al., 2005]. DNA from patients 2–5 and 7–9 was originally analyzed using whole-genome BAC-based microarray analysis as previously described [Ballif et al., 2008a]. Oligonucleotide-based microarray analysis was originally performed on DNA from patients 13 and 14, and DNA from patients 3 and 4 was re-analyzed using a 105K-feature whole-genome microarray (SignatureChip Oligo Solution v1.0, custom-designed by Signature Genomics, Spokane, Wash.; manufactured by Agilent Technologies, Santa Clara, Calif.) using previously described methods [Ballif et al., 2008b]. Oligonucleotide-based microarray analysis was originally performed on DNA from patient 12, and DNA from patients 1, 2, 5, 7, 9, and 11 was re-analyzed using a 12-plex 135K-feature whole-genome oligonucleotide microarray (SignatureChip Oligo Solution v2.0, custom-designed by Signature Genomics, manufactured by Roche NimbleGen, Madison, Wisc.) according to previously described methods [Duker et al., 2010]. Subjects 6, 8, and 10 were re-analyzed using a custom 3×720 whole-genome oligonucleotide microarray according to the manufacturer's protocol (Roche NimbleGen). Results were displayed using custom software (Genoglyphix, Signature Genomics).

Fluorescence in situ Hybridization

Fluorescence in situ hybridization (FISH) was performed using BAC clones to visualize the deletions using previously de-

scribed methods [Traylor et al., 2009]. The deletions in subjects 1–5 were confirmed by FISH using BAC probe CTD-2589O4 from the *DLX1/DLX2* locus on 2q31.1. BAC RP11-708G2 was used to confirm the deletion in subject 13, and BAC RP11-144D13 was used to confirm the deletion in subject 14. For cases 6–8 and 10–12, BAC RP11-387A1 was used to confirm the deletion.

Results

Molecular Analysis

We identified 14 individuals with microdeletions encompassing 2q31.1. The deletions ranged in size from 124.5 kb to 16.49 Mb. The deletion in subjects 1–5 encompassed the *DLX1/DLX2* loci, in 4 subjects it encompassed both the *DLX1/DLX2* and *HOXD* cluster (patients 6–9), and in 3 subjects it encompassed only the *HOXD* cluster (patients 10–12). In patients 13 and 14 the deletions fell between the *DLX* and *HOXD* loci and did not delete either. Two of the deletions, including the smallest deletion in patient 12 and the deletion in patient 9, only deleted the 5'-end of the *HOXD* cluster (fig. 1, table 1).

Thirteen of the 14 deletions were visualized by FISH which could not be performed on the DNA sample from subject 9. Parental FISH testing in 8 of the 14 subjects confirmed an apparently de novo origin (subjects 1–3, 5–7, 10, 11). Parental FISH testing for subject 13 showed the deletion was maternal in origin, and parental FISH testing for subject 14 showed the deletion was paternal in origin. Parental samples were unavailable for subjects 4, 8, and 9 (table 1).

Similar to the individuals reported by Dimitrov et al. [2011], no common breakpoints were identified in our subjects, and no mechanism of rearrangement could be delineated.

Clinical Features

Clinical characterization of the 14 individuals with deletions at 2q31.1 showed multiple common clinical features (table 1). All individuals, regardless of deletion size/interval, had neurological deficits such as moderate to severe ID/DD, speech delay, hypotonia, seizures, asymmetric reflexes, dysmetria, and/or dystonia. A majority of subjects had short stature/growth delays, and most of the individuals displayed multiple dysmorphic features previously reported in individuals with deletions at 2q31.1, including brachycephaly, plagiocephaly, ptosis, downslanted palpebral fissures, microphthalmia, low-set ears, and parietal bossing. Of 7 individuals with deletions proximal to the *HOXD* cluster, none had clinically significant hand/foot anomalies (fig. 2). The remaining 7 in-

Fig. 1. Schematic of deletions at 2q31.1 characterized by molecular cytogenetics in this and previous studies. Bars represent deletion sizes for the individuals. Green bars: deletions remove *DLX1/DLX2* but leave the *HOXD* gene cluster intact; blue bars: deletions remove *DLX1/DLX2* and the *HOXD* gene cluster; yellow bars: deletions remove the *HOXD* gene cluster but leave *DLX1/DLX2* intact; dusky pink bars: deletions leave *DLX1/DLX2* and the *HOXD* gene cluster intact. Dark blue boxes indicate genes described in the text.

dividuals, whose deletions overlapped the *HOXD* cluster, had hand/foot anomalies of varying severity, including syndactyly, brachydactyly, and ectrodactyly (fig. 3).

Discussion

Several recent studies have proposed genotype-phenotype correlations for specific features associated with deletions at 2q31.1, including limb abnormalities [Mitter et al., 2010; Dimitrov et al., 2011]. Mitter et al. [2010] characterized 8 individuals with interstitial deletions at 2q31.1. Four individuals (patients 4–6, 8) had deletions that did not encompass either the *DLX1/DLX2* or *HOXD* genes. No hand abnormalities were observed in patients 4–6, whereas mild foot anomalies were observed in patients 5, 6, and 8, such as long halluces and brachydactyly and/or syndactyly of the other toes. In addition, pa-

tient 8 had sandal gap and syndactyly of the toes. The smallest region of overlap for all 8 deletions was 88 kb and encompassed 1 known gene, *WIPF1*. The authors suggested, based on the function of *WIPF1*, that haploinsufficiency of this gene alone was unlikely to be responsible for the variable features associated with 2q31.1 deletions and that haploinsufficiency of multiple genes in the region would likely be necessary to result in the anomalies. The authors proposed that disruption of the *DLX1/DLX2* genes may be responsible for the craniofacial dysmorphism, the *HOXD* cluster for the limb and genital anomalies, *CHN1* for the ocular phenotype, *SP3* for the cardiac anomalies, and *DLX1/DLX2* and *CHN1* for the neurological features [Mitter et al., 2010].

Recently, Dimitrov et al. [2011] characterized 5 individuals with interstitial deletions at 2q31.1 using a BAC microarray. Two subjects with mild limb abnormalities had deletions that encompassed the *HOXD* cluster and

Table 1. Summary of clinical features of 14 individuals with interstitial deletions encompassing 2q31.1

Study subject:	1	2	3	4	5	6	7
Age	3 years	8 years 9 months	4 years	6 years 11 months	4 years 8 months	29 years 3 months	6 years
Array type	135K oligo	135K oligo	105K oligo	105K oligo	135K oligo	720K oligo	135K oligo
Min. deletion coordinates (chr2, hg18)	163, 190, 357–172, 706, 485	166, 519, 354–173, 745, 491	168, 702, 606–174, 842, 496	169, 823, 689–172, 870, 083	172, 190, 180–174, 860, 186	171, 777, 874–185, 147, 758	163, 942, 689–182, 115, 376
<i>DLX1/2</i> deleted	+	+	+	+	+	+	+
<i>HOXD</i> deleted	–	–	–	–	–	+	+
Inheritance	dn	dn	dn	unknown	dn	dn	dn
Height	89 cm (<5th centile)	NA	<3rd centile	NA	100 cm (5th centile)	Progressive short stature	NA
Weight	12.5 kg (<5th centile)	NA	<3rd centile	NA	14.5 kg (3rd centile)	NA	NA
Head circumference	46.5 cm (<5th centile)	NA	10–25th centile	NA	48.5 cm (2nd centile)	Microcephalic	NA
Neurological	DD, intractable seizures, severe ID, hypotonia, atrophy, poor myelination	Epilepsy	Hypotonia, DD, speech delay	Hypotonia, severe DD, speech delay	DD, speech delay, inattention, hypotonia	DD, seizure disorder	Seizures, DD
Eyes	Abnormal VER, cortical visual loss	NL	NL	Small angle esotropia s/p repair, nystagmus	NL	NL	Coloboma
Dysmorphic features	Hypertelorism, downslanted palpebral fissures, left ear pits	NL	Ptoxis, short nose, short midface	Plagiocephaly, underbite	Prominent forehead, short downslanting palpebral fissures, thin alar base, bilateral renal enlargement	NL	Microphthalmia
Musculoskeletal	NL	NL	NL	Wears AFOs	Possible hip dysplasia	NL	NL
Hands/feet	NL	NL	NL	Overlapping toes, flat arches, slender long fingers/toes, mild/moderate hyperextensibility of most joints	NL	Oligodactyly, 2 digits on right hand, 2 or 3 ulnar sides of metacarpals are missing, 5th finger clinodactyly on left hand, cutaneous syndactyly between 2nd and 3rd toes bilaterally	Ectrodactyly
Cardiovascular	NL	NA	NA	NL	NA	NL	NA
Genitourinary	NL	NL	NL	NL	NL	NL	NL
Other	Autonomic dysfunction with neurogenic bladder and GI dysmotility			Severe bruxism			Teeth missing, hearing loss, G-tube

AFOs = Ankle-foot orthotics; dn = de novo; GD = growth delay; GI = gastrointestinal; ID = intellectual disability; mat. = maternally inherited; MCP = metacarpophalangeal; NA = not available; NL = normal; pat. = paternally inherited; ROM = range of motion; VER = visual evoked response.

8	9	10	11	12	13	14
17 years 3 months	6 months	7 years 6 months	19 months	14 years 8 months	3 years 4 months	14 years 8 months
720K oligo	135K oligo	720K oligo	135K oligo	135K oligo	105K oligo	105K oligo
169, 521, 432–186, 004, 985	166, 422, 383–176, 872, 179	175, 712, 131–183, 736, 599	176, 578, 119–180, 521, 091	176, 556, 102–176, 681, 541	174, 988, 738–176, 362, 909	174, 366, 202–174, 971, 985
+	+	–	–	–	–	–
+	partial	+	+	partial	–	–
unknown	unknown	dn	dn	unknown	mat.	pat.
Short stature	63 cm (10–25th centile)	115.6 cm (3rd centile)	75.6 cm (15th centile)	Short stature	NA	170.6 cm (63rd centile)
NA	5.92 kg (5–10th centile)	32.1 kg (75th–90th centile)	10.2 kg (20th centile)	NA	NA	76.6 kg (93rd centile)
Microcephalic	41 cm (25th centile)	48.25 cm (<2nd centile)	43.2 cm (<3rd centile)	NA	NA	54 cm (50th centile)
Severe DD, increased tone in lower extremities with brisk deep tendon reflexes	Hypotonia, DD	DD, delayed speech/ speech apraxia, his- tory of febrile seizures, hypotonia, asymmet- ric reflexes, dysmetria, poor balance	Hypotonia	DD, dystonia, dysmetria	NA	DD, slightly clumsy, mild hypotonia (now resolved), mild cerebellar hypoplasia prenatally (not con- firmed postnatally)
Significant blepharo- phimosis, difficulty abducting both eyes	NL	Nystagmus, photo- phobia, esotropia/ exotropia, poor visual motor coordination	NL	NL	NL	NL
Distinctive facial features	Sparse hair, brachycephaly, parietal bossing	Mild hypotelorism, narrow inner canthal distance, synophrys, bilateral ptosis, down- slanting palpebral fissures, low-set ears	Protuberant eyes	NL	NL	Deep-set eyes, prominent supra orbital ridges
Significant kyphosis	Subluxation of 1st MCP joints bilaterally	Leg length discrep- ancy, mild scoliosis	NL	Joint contractures, decreased ROM at MCP bilaterally	NL	NL
Camptodactyly of all digits with syndactyly of toes 2 + 3 and short 4th and 5th toes bilaterally	4th toes overlapping 3rd/5th toes, partial syndactyly of 2nd/3rd toes	Brachydactyly, mild ectrodactyly, contracted thumbs	Bilateral clinodactyly with bridge or deep- set single palmar creases, 2–3 syndac- tyly, widening at distal toes	Syndactyly of right 4th/5th toes, brachy- dactyly of left 5th toe, abnormal X-rays of left/right feet	NL	Small persistent fetal pads and slight increase in mobility
NA	NA	Bicuspid valves, mild hypertension	NA	NA	NA	NL
NL	Hypoplasia of labia minora, proximally inserted anus	NL	NL	NL	NL	NL
	Cleft palate	Chronic constipation

Fig. 2. Hand and foot images of individuals with deletions at 2q31.1 that do not encompass the *HOXD* gene cluster. **a, b** Subject 2. **c, d** Subject 4. Note long, slender fingers and overlapping toes. **e, f** Subject 5.

DLX1/DLX2, 1 subject with mild limb abnormalities had a deletion that left *DLX1/DLX2* intact, and in 2 subjects with no limb abnormalities, the deletion left *HOXD* intact. The authors suggested a critical limb abnormality locus spanning from 1.5 Mb centromeric to 1 Mb telomeric of the *HOXD* gene cluster.

Although the 5-Mb region proximal to *EVX2* has been proposed as a locus for SHFM5, our results suggest otherwise. In our cohort, only the 7 individuals with deletions that encompassed at least the 5'-end of the *HOXD* cluster had hand/foot anomalies of varying severity, including syndactyly, brachydactyly, and ectrodactyly. Of the 7 individuals with deletions proximal to the *HOXD* cluster, none had clinically significant hand/foot anomalies. Subject 1 (distal breakpoint: 172.7 Mb), subject 3 (distal breakpoint: 174.8 Mb), and subject 5 (distal breakpoint: 174.9 Mb) have normal hands/feet, and subject 4 (distal breakpoint: 172.9 Mb) was reported to have only overlapping toes, flat arches, slender and long fingers/toes, and mild/moderate hyperextensibility. Therefore,

Fig. 3. Hand and foot images of individuals with deletions at 2q31.1 that encompass the *HOXD* gene cluster. Left hand (**a**) and feet (**b**) of subject 8. Note camptodactyly of all digits with syndactyly of toes 2 and 3 and short 4th and 5th toes bilaterally. Right hand (**c**) and left foot (**d**) of subject 9. Note 4th toe overlapping 3rd/5th toes and partial syndactyly of 2nd/3rd toes. Left hand (**e**) and left foot (**f**) of subject 10. Note brachydactyly, mild ectrodactyly, and contracted thumbs. **g** Left foot of subject 11. Note syndactyly of toes 2 and 3 and widening at distal toes.

the region 5' of the *HOXD* cluster, including *DLX1/DLX2*, is unlikely to be the SHFM5 locus. Furthermore, 2 of the individuals, subjects 13 and 14, have microdeletions that lie entirely between *DLX1/DLX2* and the *HOXD* cluster; the distal breakpoints are 290.2 kb and ~1.68 Mb proximal of *EVX2*, respectively. Both deletions were inherited from reportedly normal parents, the deletion in subject 13 from the mother and the deletion in subject 14 from the father. Neither subject nor parent (by report only in

the case of subject 14) had any hand or foot abnormalities. The presence of these microdeletions in phenotypically normal parents suggests deletion of genes in this interval may not have clinical consequences; at the very least it suggests deletion of the region immediately upstream of the *HOXD* cluster plays no role in the hand/foot abnormalities. This is in contrast to the report by Svensson et al. [2007] in which the proband had upper- and lower-limb skeletal anomalies and a deletion of the interval proximal to the *HOXD* cluster. However, the deletion in subject 12 in our study, who has 4th/5th toe syndactyly on the right foot, 5th toe brachydactyly on the left foot, and abnormal foot X-rays, supports that deletion of *EVX2* and the 5'-end of the *HOXD* cluster is sufficient to cause at least mild limb anomalies [Goodman et al., 2002]. Thus, our results support the suggestion by Dimitrov et al. [2011] and others that haploinsufficiency of the *HOXD* cluster, rather than *DLX1/DLX2*, accounts for the skeletal abnormalities in individuals with 2q31.1 microdeletions.

All probands for whom records were available had a diverse spectrum of neurological deficits, including moderate/severe ID/DD, speech delay, hypotonia, seizures, dysmetria, and/or dystonia. Interestingly, despite the association of hand/foot anomalies with deletions of the region, a comparison of the individuals in our study to those in the literature suggests neurological features and growth delay are the characteristic features associated with 2q31.1 deletions that do not encompass the *HOXD* cluster. Because these neurological deficits were present in multiple individuals with non-overlapping deletions, regardless of deletion size or interval, multiple genes in the region may play a role in neurodevelopmental processes. This is in contrast to the findings of Mitter et al. [2010] who suggested haploinsufficiency of *DLX1/DLX2* and *CHN1* may result in the neurological features associated with 2q31.1 deletions. However, this may also represent an ascertainment bias, because we tested individuals referred for ID/DD.

Although neither *DLX1/DLX2* are likely to play a role in the limb abnormalities of individuals with 2q31.1 deletions, these genes may play some role in the neurological deficits in these patients. The *Dlx* genes act as modulators of neuron versus oligodendrocyte development in the ventral embryonic forebrain [Petryniak et al., 2007], and both genes are implicated in differentiation and migration of neurons [Cobos et al., 2007; Le et al., 2007]. For example, differentiation and migration of striatal neurons is blocked in *Dlx1*-null mice, which have late-onset epilepsy [Cobos et al., 2005]. *DLX1/DLX2* have also been associated with autism [Laroche et al., 2008; Liu et al.,

2009]. Three individuals with deletions of *DLX1/DLX2* in this study (subjects 1, 6, and 7) experience seizures and 1 individual has epilepsy (subject 2), but seizures/epilepsy have not been reported in the remaining 5 individuals (subjects 3–5, 8, 9) whose deletions overlap *DLX1/DLX2*. Furthermore, the deletion in subject 1 extends proximal into 2q24.3 and removes *SCN1A*, deletions of which are associated with severe myoclonic epilepsy in infancy [Dimova et al., 2010; Meisler et al., 2010]. Interestingly, the deletion in subject 9, who does not have seizures, encompasses *DLX1/DLX2* and *SCN1A*, but at 6 months of age this individual may be too young to have developed this feature. In contrast to the findings of Mitter et al. [2010], who suggested haploinsufficiency of *DLX1/DLX2* may be responsible for seizures associated with 2q31.1 deletions, the absence of seizure-related phenotypes in 5 of the 7 individuals in our study with deletions of the *DLX1/DLX2* cluster but not *SCN1A* suggests haploinsufficiency of *DLX1/DLX2* may not result in seizures/epilepsy.

Based on common facial features in 3 of 5 subjects in their study, Dimitrov et al. [2011] suggested a facial gestalt that includes narrow forehead, prominent metopic suture, a small nose with bulbous tip, long and smooth philtrum, downslanting palpebral fissures, thin upper lip, thick and everted lower lip, low-set and dysplastic ears, and micrognathia. The authors proposed a 2.4-Mb critical region for the facial gestalt that begins just distal to *DLX1/DLX2* and ends just distal to *WIPF1*. In contrast, Mitter et al. [2010] suggested haploinsufficiency of *DLX1/DLX2* may result in craniofacial dysmorphism associated with 2q31.1 deletions. The individuals in our study have variable dysmorphic features. For example, subject 6, whose deletion encompasses this region, has no reported dysmorphic features, and subject 7 has only microphthalmia. Meanwhile, subject 10, whose deletion lies distal to the proposed critical region, has mild hypotelorism, narrow inner canthal distance, synophrys, bilateral ptosis, downslanting palpebral fissures, and low-set ears. Thus, our study population suggests there may not be a recognizable facial gestalt associated with deletion 2q31.1 as proposed by Mitter et al. [2010] and Dimitrov et al. [2011].

Likewise, our results suggest that proposed critical regions for several other features present in some individuals with 2q31.1 deletions may not account for the phenotypic variability associated with deletions of this region. For example, Mitter et al. [2010] proposed that haploinsufficiency of *SP3* was a promising candidate for cardiac defects, which are occasionally associated with 2q31.1 deletions. In our study, only 1 individual, subject 10, was reported to have cardiac anomalies. The deletion in this

subject lies distal to *SP3*. Likewise, Mitter et al. [2010] suggested disruption of *CHN1* as a candidate for the ocular anomalies associated with deletions at 2q31.1. In our study, 5 subjects (1, 4, 7, 8, and 10) were reported to have variable ocular anomalies. Interestingly, there is a 2.8-Mb gap between the distal breakpoint of the deletion in subject 4 and the proximal breakpoint of the deletion in subject 10, and *CHN1* lies within this gap. Thus, our results suggest that disruption of multiple genes within 2q31.1 may be responsible for the variable clinical features associated with deletions of that region.

Our results refine the model proposed by Goodman [2002] and supported by Mitter et al. [2010] and Dimitrov et al. [2011] and suggest that deletions encompassing the *HOXD* cluster result in hand/foot anomalies of varying

severity, including syndactyly, brachydactyly, and ectrodactyly; however, deletions proximal to the *HOXD* cluster do not appear to result in clinically significant hand/foot anomalies. Specifically, the absence of hand/foot anomalies in any of the individuals with deletions of *DLX1/ DLX2* but not the *HOXD* cluster excludes the region proximal to *EVX2/HOXD* and implicates the *EVX2/HOXD* region as a candidate locus for SHFM5.

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