RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Deletions and Duplications of Developmental Pathway Genes in 5q31 Contribute to Abnormal Phenotypes

Jill A. Rosenfeld,[1] Joanne Milisa Drautz,[2] Carol L. Clericuzio,[2] Tom Cushing,[2] Salmo Raskin,[3] Judith Martin,[4] Raymond C. Tervo,[5] Jose A. Pitarque,[6] Dorota M. Nowak,[7] Justyna A. Karolak,[7] Allen N. Lamb,[1] Roger A. Schultz,[1] Blake C. Ballif,[1] Bassem A. Bejjani,[1] Marzena Gajecka,[7] and Lisa G. Shaffer[1]*

[1]Signature Genomic Laboratories, Spokane, Washington

[2]Pediatric Genetics Division, Department of Pediatrics, University of New Mexico, Albuquerque, New Mexico

[3]GENETIKA—Centro de Aconselhamento e Laboratorio de Genetica, Curitiba, Parana, Brazil

[4]Providence Genetics Clinic, Spokane, Washington

[5]Gillette Children's Specialty Healthcare, St. Paul, Minnesota

[6]Department of Ophthalmology, Hospital Metropolitano, Quito, Ecuador

[7]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland

Received 26 January 2011; Accepted 15 April 2011

Although copy number changes of 5q31 have been rarely reported, deletions have been associated with some common characteristics, such as short stature, failure to thrive, developmental delay (DD)/intellectual disability (ID), club feet, dislocated hips, and dysmorphic features. We report on three individuals with deletions and two individuals with duplications at 5q31, ranging from 3.6 Mb to 8.1 Mb and 830 kb to 3.4 Mb in size, respectively. All five copy number changes are apparently de novo and involve several genes that are important in developmental pathways, including *PITX1*, *SMAD5*, and *WNT8A*. The individuals with deletions have characteristic features including DD, short stature, club feet, cleft or high palate, dysmorphic features, and skeletal anomalies. Haploinsufficiency of *PITX1*, a transcription factor important for limb development, is likely the cause for the club feet, skeletal anomalies, and cleft/high palate, while additional genes, including *SMAD5* and *WNT8A*, may also contribute to additional phenotypic features. Two patients with deletions also presented with corneal anomalies. To identify a causative gene for the corneal anomalies, we sequenced candidate genes in a family with apparent autosomal dominant keratoconus with suggestive linkage to 5q31, but no mutations in candidate genes were found. The duplications are smaller than the deletions, and the patients with duplications have nonspecific features. Although development is likely affected by increased dosage of the genes in the region, the developmental disruption appears less severe than that seen with deletion.

Key words: deletion; duplication; 5q31; aCGH; keratoconus; corneal abnormality

How to Cite this Article:
Rosenfeld JA, Drautz JM, Clericuzio CL, Cushing T, Raskin S, Martin J, Tervo RC, Pitarque JA, Nowak DM, Karolak J, Lamb AN, Schultz RA, Ballif BC, Bejjani BA, Gajecka M, Shaffer LG. 2011. Deletions and duplications of developmental pathway genes in 5q31 contribute to abnormal phenotypes.
Am J Med Genet Part A 155:1906–1916.

INTRODUCTION

There have been only 12 reports of interstitial deletions including 5q31 and six reports of duplications [Felding and Kristoffersson, 1980; Osztovics and Kiss, 1982; Harprecht-Beato et al., 1983; Martin et al., 1985; Rivera et al., 1987; de Michelena et al., 1990; Rivera et al., 1990; Kobayashi et al., 1991; Lindgren et al., 1992;

Additional supporting information may be found in the online version of this article.

Grant sponsor: Ministry of Education and Science, Poland; Grant number: NN402097837.

*Correspondence to:
Lisa G. Shaffer, PhD, Signature Genomic Laboratories, 2820 North Astor Street, Spokane, WA 99207. E-mail: lisa.shaffer@perkinelmer.com

Published online 8 July 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.34100

Courtens et al., 1998; Kramer et al., 1999; Sanchez-Garcia et al., 2001; Martin et al., 2003; Arens et al., 2004; Giardino et al., 2004; Tzschach et al., 2006; Mosca et al., 2007]. Despite the small number of reported patients, some characteristic features have been observed among individuals with interstitial deletions that encompass 5q31, including short stature, failure to thrive, developmental delay (DD)/intellectual disability (ID), hypotonia, club feet, dislocated hips, and characteristic dysmorphic features including short neck, prominent forehead, downslanting palpebral fissures, telecanthus/hypertelorism, depressed nasal bridge, anteverted nostrils, cleft or high palate, micro/retrognathia, and ear abnormalities [Felding and Kristoffersson, 1980; Harprecht-Beato et al., 1983; Rivera et al., 1987; de Michelena et al., 1990; Rivera et al., 1990; Kobayashi et al., 1991; Lindgren et al., 1992; Courtens et al., 1998; Kramer et al., 1999; Arens et al., 2004; Tzschach et al., 2006; Mosca et al., 2007]. Even fewer individuals with 5q31 duplications have been reported, and a characteristic phenotype is not obvious among those individuals, though microcephaly is a frequent finding, and several individuals have been described with heart defects, including one with an interrupted aortic arch [Osztovics and Kiss, 1982; Martin et al., 1985; Sanchez-Garcia et al., 2001; Martin et al., 2003; Arens et al., 2004; Giardino et al., 2004]. However, in many of these reports of deletions and duplications, the abnormalities were characterized by low-resolution, traditional cytogenetic banding techniques, which can complicate genotype–phenotype correlations.

To identify previously uncharacterized copy-number imbalances that may be associated with neurodevelopmental or other congenital anomalies, we constructed whole-genome microarrays with enhanced coverage of over 500 functionally significant genes including transcription factors and other developmentally important genes. Several of these genes are in 5q31, such as *PITX1*, *SMAD5*, and *WNT8A*, which are part of the PITX/TBX, BMP/SMAD, and WNT/beta-catenin signaling pathways, respectively. Alterations in these pathways and/or related genes are associated with a variety of diseases, including autosomal dominant conditions such as Axenfeld–Rieger syndrome [Semina et al., 1996; Al-Qattan, 2010; Liu and Millar, 2010; Ryan et al., 2010; Walsh et al., 2010]. We report on three individuals with deletion and two with duplication within 5q31 identified using this microarray. We propose that dosage imbalances of the developmentally important genes within 5q31 are responsible for these individuals' abnormal phenotypes. Additionally, two individuals with 5q31 deletions showed ophthalmologic abnormalities. We therefore pursued candidate gene sequencing in a previously reported family with apparently autosomal dominant keratoconus [Gajecka et al., 2009] and suggestive linkage to 5q31.

MATERIALS AND METHODS

Subject Ascertainment

Patients in this study with 5q31 copy number changes were identified after referral to Signature Genomics for microarray-based comparative genomic hybridization (aCGH) testing. Informed consent was obtained to publish photographs shown here. The study also included eight individuals from an Ecuadorian family with keratoconus (KTCN-011) who were examined in Hospital Metropolitano in Quito, Ecuador and underwent a complete ophthalmic evaluation as described elsewhere [Gajecka et al., 2009]. Pedigree is shown in Supplementary Figure 1.

Microarray-Based Comparative Genomic Hybridization (aCGH)

DNA from Patients 1–5 and from individuals KTCN-011-01 through -04 was studied using an oligonucleotide-based, 135K-feature, whole-genome microarray (SignatureChip® Oligo Solution™ version 2.0, designed by Signature Genomics, Spokane, WA and made by Roche NimbleGen, Madison, WI) according to previously described methods [Duker et al., 2010]. The parents of Patient 4 were also studied using the 135K microarray. Results were displayed using custom software (Genoglyphix®, Signature Genomics).

Fluorescence In Situ Hybridization (FISH)

Fluorescence in situ hybridization (FISH) was performed on metaphase spreads in Patients 1–5 and interphase nuclei in Patients 4 and 5 using BAC clones RP11-158M10, RP11-466C24, RP11-662J6, RP11-1067D23, and CTD-2015J11, respectively, according to previously described methods [Shaffer et al., 1994; Traylor et al., 2009]. The chromosomes of the parents of Patients 1–3 and 5 were also examined by FISH.

Linkage Analysis

A genome-wide screen was performed by genotyping the KTCN-011 family with 811 microsatellite markers, spaced at approximately 5 cM across the human genome, as previously described [Gajecka et al., 2009]. Pedstats [Wigginton and Abecasis, 2005] was used to identify potential Mendelian inconsistencies. Two-point parametric linkage analysis was performed with Superlink [Fishelson and Geiger, 2002; Silberstein et al., 2006]. An autosomal dominant mode of inheritance and a disease allele frequency of 0.0001 were applied. Two-point nonparametric and multipoint nonparametric analyses were performed with ALLEGRO [Gudbjartsson et al., 2000], using the S_{all} scoring function and the exponential allele-sharing model. Genetic map distances were derived from the Rutgers combined linkage-physical map of the human genome [Matise et al., 2007]. Haplotypes were reconstructed using the SIMWALK2 program [Weeks et al., 1995; Sobel and Lange, 1996] and illustrated with HaploPainter [Thiele and Nurnberg, 2005].

Sequencing Analyses

Oligonucleotide primers were designed to amplify all coding regions, intron-exon junctions and untranslated regions (UTRs) of the *TGFBI*, *IL9*, and *PITX1* genes (Supplementary Table I). PCR amplifications were performed using *Taq* DNA Polymerase (Fermentas Inc., Glen Burnie, MD). PCR products were purified with ExoSAP-IT (USB Corporation, Cleveland, OH) and sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc. [ABI], Foster City, CA). Sequencing was

visualized on a 3730XL DNA Analyzer (ABI) at Genomed Co. (Warsaw, Poland). Sequence reads were compared with the reference sequences of *TGFBI*, *IL9*, and *PITX1* genes (GRCh37/hg19, GenBank accession numbers for the mRNA NM_000358.2, NM_000590.1, and NM_002653.4, respectively) using Sequencher 4.10.1. software (Gene Codes Corporation, Ann Arbor, MI).

RESULTS

Molecular Analysis

Oligonucleotide-based aCGH on Patients 1–3 showed overlapping deletions within 5q31.1q31.3, 6.08, 3.6, and 8.06 Mb in size and containing 62, 33, and 101 genes, respectively. All three deletions encompass *PITX1* and *SMAD5*; *WNT8A* is deleted in Patients 1 and 3 (Fig. 1). Patient 1 also has a deletion of 5q11.2, 1.12 Mb in size and containing 14 genes (chr5:53,474,049–54,593,863, UCSC March 2006 hg18 coordinates). Oligonucleotide-based aCGH on Patients 4 and 5 showed non-overlapping duplications within 5q31.1 and 5q31.2q31.3, 830 kb and 3.4 Mb in size and containing 13 and 60 genes, respectively. Patient 4's duplication includes *PITX1*, and Patient 5's duplication includes *WNT8A* (Fig. 1). Patient 4 also has two non-contiguous duplications, minimally 67 kb and 57 kb in size, on 7q11.22 within the *AUTS2* gene [arr cgh 7q11.22 (68,972,854 × 2, 68,977,340-69,044,168 × 3, 69,052,269-69,757,882 × 2, 69,763,885-69,820,680 × 3, 69,282,229 × 2)]. The 5q31 deletions in Patients 1–3 and the 5q31 duplication in Patient 5 were confirmed by FISH. Metaphase FISH for the duplicated 5q31

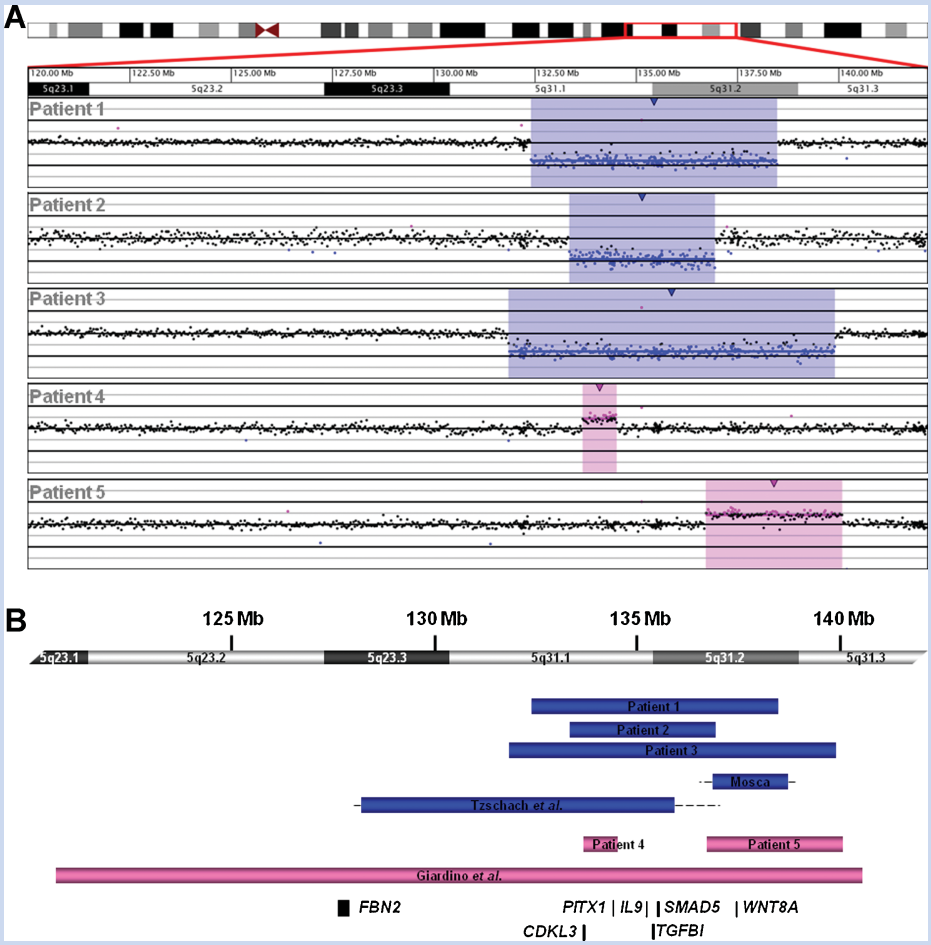

FIG. 1. Molecular cytogenetic characterization of CNVs at 5q31. A: Oligonucleotide-based microarray characterization of abnormalities at 5q31. Probes are arranged with the most proximal 5q23.1 probes on the left and the most distal 5q31.3 probes on the right. Results are visualized using custom aCGH analysis software (Genoglyphix; Signature Genomics). Analysis of Patient 1 showed a single-copy loss of 236 oligonucleotide probes, 6.08 Mb in size; Patient 2 showed a single-copy loss of 144 oligonucleotide probes, 3.59 Mb in size; Patient 3, a single-copy loss of 313 oligonucleotide probes, 8.06 Mb in size; Patient 4, a single-copy gain of 42 oligonucleotide probes, 834 kb in size; Patient 5, a single-copy gain of 120 oligonucleotide probes, 3.36 Mb in size. B: Schematic representation of abnormalities in Patients 1–5 and those reported in the literature that have been characterized by molecular cytogenetics. Deletions are shown in blue, and duplications are shown in pink. The boxes represent the minimum size of the abnormalities, and the horizontal dashed lines extend through gaps in coverage to show the maximum sizes. Genes of note within the region are represented by black boxes.

material in Patient 4 excluded an unbalanced translocation but did not reveal a duplication; interphase FISH showed three signals in 9/50 cells. Parental FISH studies in Patients 1–3 and 5 were normal. Thus, these alterations are apparently de novo. The 5q11.2 deletion in Patient 1 was also apparently de novo. Parental aCGH studies for Patient 4 demonstrated the 5q31 duplication to be apparently de novo, whereas the patient's mother carried both duplications within *AUTS2*. Neither 5q31 deletions nor 5q31 duplications of the sizes seen in our patients have been reported in control cohorts [Itsara et al., 2009; Shaikh et al., 2009]. No significant copy number changes were identified by array analysis in the four individuals studied from family KTCN-011.

Keratoconus in family KTCN-011 showed suggestive linkage to 5q31.1q35.3. Supplementary Table II displays parametric two-point LOD scores for the dominant mode of inheritance and nonparametric LOD (NPL) scores. Familial haplotypes at 5q22.1q34 are presented in Supplementary Figure 1. The proximal boundary of the proposed disease haplotype is at D5S471, defined by recombination in KTCN-011-07, and lack of a more distal recombination does not allow specification of a distal border with confidence.

In family KTCN-011, seven known sequence variants and one novel amino acid substitution c.1949C > T (Ala650Val) were detected in *TGFBI* (Supplementary Table III). This substitution did not segregate with the proposed disease haplotype. Eleven known sequence variants were identified in *PITX1*, including one synonymous substitution, one missense substitution, one intronic substitution, and eight variants in UTRs (Supplementary Table III). Sequencing of *IL9* revealed no sequence changes. Screening of *TGFBI* in Patients 1–5 revealed 14 known sequence variants, four synonymous substitutions, nine intronic variants, and one substitution in the 3' UTR. Analysis of *IL9* revealed a known missense substitution and a known intronic sequence variant in Patient 5 (Supplemental Table IV). Sequencing of *PITX1* revealed two known variants in the 3' UTR in Patients 1–5 (Supplemental Table IV).

Clinical Reports

Patient 1 is a 10-year-old male with multiple congenital anomalies, including hydrocephalus, cleft palate, bilateral hip dysplasia, neuromuscular scoliosis, a ventricular septal defect (VSD) that resolved spontaneously, pre- and postnatal growth restriction, and DD. Hydrocephalus and VSD were diagnosed prenatally at 7 months. Birth weight was 2.07 kg (<3rd centile), length was 47 cm (10th centile), and OFC was 34 cm (50th centile). Brain MRI showed normal structures other than the hydrocephalus. By 3 months his length was at −4 SD and weight at −3 SD, both of which dropped further and are currently following a curve at −5 to −6 SD. His occipitofrontal circumference (OFC) fell to <2nd centile at 8 months of age and has remained at −2.5 to −3 SD. At 2 years he had a G-tube placed for supplemental feeding due to oral aversion, and the tube was used until 10 years of age. Growth hormone (GH) deficiency was diagnosed at 1.5 years, and GH supplementation was attempted without notable results. His bone age is also delayed. He was diagnosed with mild high-frequency hearing loss and uses hearing aids. He has several musculoskeletal problems, including progressive neuromuscular scoliosis requiring rod placement for correction, bilateral hip dysplasia, bilateral metatarsus adductus, and residual flexion of his proximal interphalangeal joints. He pulled up at 23 months and walked at 3.5 years. He communicates mostly with signs but also speaks and spells some words and is able to read at a first-grade level. At 10 years of age, his height is 108.8 cm (<<3rd centile; 50th centile for a 5-year-old), weight is 16.4 kg (<<3rd centile; 50th centile for a 4-year-old), and OFC is 49 cm (<−2 SD). Dysmorphic features (Fig. 2A–C) include posteriorly rotated ears with prominent helices, downslanting palpebral fissures, epicanthal folds, mild hypertelorism, prominent and straight nasal bridge, long philtrum, peaked upper lip, slightly widely spaced teeth, small chin, two pigmented nevi on the left cheek, sternal asymmetry with right ribs more prominent than the left, digits that are broadened distally, markedly long fingers and toes, and small great toenails. Ophthalmologic findings include strabismus, which was surgically repaired; corneal clouding due to keratitis; myopia and astigmatism, for which he has worn glasses since 4 months of age; and blepharitis. He has diffuse hypotonia, though in infancy he had asymmetric, mixed hypo- and hypertonia.

Patient 2 is a 12-year-old female with seizures, growth retardation, ID, and dysmorphic features. She was born at term weighing 2.4 kg (<3rd centile). She had neonatal respiratory distress and arrest. She had difficulties gaining weight. She had surgery for tonsillar hypertrophy and ankyloglossia. At 4 years of age she had a febrile seizure, and she had another seizure episode at 8 years; she is on valproic acid to control the seizures. She is unable to read or write. At 12 years of age, her height is 136 cm (<3rd centile; 50th centile for a 9.5-year-old), and weight is 28 kg (<3rd centile; 50th centile for an 8.5-year-old). Dysmorphic features include arched eyebrows, synophrys, epicanthal folds, anteverted nostrils, high arched palate, dental crowding with large superior central incisors, and microretrognathia. Ophthalmologic problems include keratoconus and myopia. She also has scoliosis (Fig. 2D–F).

Patient 3 is a newborn male with multiple congenital anomalies including severe micrognathia, cleft palate, preaxial polydactyly, club feet, congenital heart defects, sacral anomaly, and dysmorphic features. Birth weight was 2.9 kg (10th–25th centile), and head circumference was 33 cm (10th–25th centile). At birth, severe micrognathia and an obstructed airway required intubation. He also has a large cleft palate, widely open anterior fontanelle with split sutures, short neck with redundant skin, microphallus, duplicated hallux on the left foot, club feet, scoliosis, and a skin-covered sacral bony prominence. He has bilateral moderate hydronephrosis and multiple muscular VSDs, a moderate to large atrial septal defect, and a moderate patent ductus arteriosus. A cerebral ultrasound was normal. Dysmorphic facial features include a broad forehead, possible hypertelorism, and low-set ears with reduced upper helix. He had a normal ophthalmologic examination at 4 days of age. He has significant hypotonia and joint hypermobility, with possibly dislocated hips. Fingers, thumbs, and toes are very long, and the fifth finger has a single flexion crease. His toenails are hypoplastic, and the great toes have bulbous tips. His hands are deviated ulnarly, and he has positional foot deformity bilaterally. His torso is long, and his legs appear relatively short (Fig. 2G–K).

Patient 4 is a 5-year-old male with global DD, disruptive behaviors, sacral dimple, and hypotonia. Birth weight was 2.8 kg

FIG. 2. Dysmorphic features of individuals with CNVs at 5q31. A–C: Patient 1 at 11 years of age. Note downslanting palpebral fissures, epicanthal folds, mild hypertelorism, prominent and straight nasal bridge, long philtrum, peaked upper lip, small chin, digits that are broadened distally, markedly long fingers and toes, and small great toenails. D–F: Patient 2 at 12 years of age. Note arched eyebrows, synophrys, epicanthal folds, anteverted nostrils, high palate, dental crowding with large superior central incisors, microretrognathia, and scoliosis. G–K: Patient 3. Note severe micrognathia, short neck, broad forehead, hypertelorism, low-set and abnormally shaped ears, long fingers and toes, duplicated hallux on the left foot, club feet, scoliosis, and sacral anomaly. L–M: Patient 4 at 5 years of age. Note facial asymmetry. N–O: Patient 5 at 4 years of age. Note relative microcephaly, flat occiput, triangular face with pointed chin, broad forehead, and mild flattening of the midfacial area, slight facial asymmetry with left prominence, and bilaterally rotated ears.

(10th–25th centile), and length was 48.3 cm (25th–50th centile). He reportedly failed his newborn hearing screen and has a history of multiple sets of ear tubes placed due to fluid buildup, and subsequent hearing tests were normal. He has a closed sacral dimple, and spinal MRI showed a fibrous tract contiguous with the dimple, of no clinical concern. Brain MRI at 4 years of age was normal. He has borderline short stature, with normal endocrine evaluation and short stature in both parents. He crawled at 8 months and walked at 18 months. He had some words at 18 months but at 3 years was not speaking. Testing at 3.5 years showed a full-scale IQ of 60, with a

verbal score of 58 and performance score of 73. He has attention problems and aggressive behavior, is impulsive and hyperactive, and has borderline scores in autism evaluations (Gilliam autism rating scale). At 5 years, his height is 100.8 cm (3rd centile), weight is 16.3 kg (10th centile), and OFC is 49.8 cm (−1 SD). Dysmorphic features include facial asymmetry, bilateral clinodactyly, and valgoid feet (Fig. 2L and M). He has had surgical repair of his strabismus, and he also wears glasses for astigmatism. He has joint hypermobility and is generally hypotonic. Family history is significant for learning disabilities in the patient's mother and maternal grandfather. His mother also had multiple ear tubes due to recurrent infections and had clubfoot. His mother has the two small duplications within *AUTS2* that were also identified in the patient.

Patient 5 is a 4-year-old male with global DD, hypotonia, and relative microcephaly. He had a small VSD that spontaneously closed by 4 months. He developed daily staring spells, and EEG at 3 years of age showed background slowing but no confirmed epileptiform activity; he is not on any anti-epileptic medication. Head MRI at 2 years was normal. He rolled over at 6 months and sat alone at 14 months. At 4 years, he still does not walk and does not have meaningful words. He has occasional hand flapping but is social and makes eye contact. Ophthalmologic exam at 2 years was normal. At 4 years his weight is at the 25th centile, his height is at the 50th centile, and OFC is at the 5th centile. Dysmorphic features (Fig. 2N and O) include flat occiput, a somewhat triangular face with pointed chin, broad forehead, mild flattening of the midfacial area, slight facial asymmetry with left prominence, bilaterally rotated ears, mild overbite, and moderate hypertrichosis on his back. He has generally low tone. His father's and mother's head sizes are at the 98th and 50th centiles, respectively.

DISCUSSION

We report on five patients with deletions or duplications of 5q31. Similar to other patients reported in the literature with 5q31 deletions, the three individuals with 5q31 deletions in this study have DD, short stature, cleft or high palate, and dysmorphic features (Table I). They have a variety of musculoskeletal abnormalities, which have been reported in some individuals with 5q31 deletions. They also have additional features that have not been previously reported in association with 5q31 abnormalities, such as hydrocephalus in Patient 1 and seizures in Patient 2. However, Patient 1 has an additional de novo 5q11.2 deletion, and some of his features may be a result of that deletion. The seizures in Patient 2 may be a result of neonatal hypoxia. The duplications in Patients 4 and 5 do not overlap, and their phenotypes are relatively nonspecific, including DD, behavior problems, and mild dysmorphic features (Table II). All five patients showed apparently de novo copy number changes in 5q31, which provides further support that these aberrations are causative for some of the clinical features seen in these individuals.

The copy number changes described here impact multiple genes. This cumulative effect of copy number gains and losses of multiple genes likely leads to the phenotypic expression observed in these patients. However, chromosomal band 5q31 contains several genes that are known to be part of developmental pathways, and these genes may make significant contributions to these phenotypes. *PITX1* (paired-like homeodomain transcription factor 1, OMIM 602149) encodes a bicoid-related transcription factor that is critical for proper development of the hindlimbs, anterior pituitary, and first branchial arch derivatives [Lanctot et al., 1999; Szeto et al., 1999]. Homozygous *Pitx1* knockout animals have marked hindlimb defects and pelvic changes [Lanctot et al., 1999; Logan and Tabin, 1999; Szeto et al., 1999; Minguillon et al., 2005; DeLaurier et al., 2006]. A *PITX1* missense mutation with reduced transcription activity, likely dominant-negative effects, and incomplete penetrance has been described in one family with autosomal dominant, asymmetric right-sided predominant, idiopathic clubfoot, and other limb malformations [Gurnett et al., 2008]. These observations in mouse and human suggest that *PITX1* haploinsufficiency may lead to the lower limb malformations reported in our cohort and in previously reported individuals with 5q31 deletions. Patients 1 and 3 also had markedly long toes, and arachnodactyly and camptodactyly have been seen in previously reported individuals with 5q31 deletions (Table I). These digital abnormalities have been previously hypothesized to be due to altered expression of a gene not deleted in our patients, *FBN2* at 5q23.3 [Tzschach et al., 2006], missense mutations of which cause congenital contractural arachnodactyly. We hypothesize that *PITX1* haploinsufficiency may contribute to these digital abnormalities; animal experiments have shown *Pitx1* expression in the upper limb can alter digital development [Logan and Tabin, 1999; Szeto et al., 1999; Minguillon et al., 2005; DeLaurier et al., 2006]. In the 17q23.1q23.2 microdeletion syndrome, haploinsufficiency of another hindlimb-specific transcription factor, *TBX4*, which is regulated by PITX1 [Logan and Tabin, 1999], is associated with similar long fingers and toes [Ballif et al., 2010]. Remarkably, both 17q23.1q23.2 microduplications and microdeletions have also been associated with familial isolated clubfoot [Alvarado et al., 2010].

PITX1 haploinsufficiency may also contribute to the short stature, micro/retrognathia, and cleft palates in individuals with 5q31 deletions. *Pitx1* is required for proper development of the anterior pituitary, which contains the somatotrophs that produce GH [Szeto et al., 1999]. Patient 1 has documented GH deficiency, which has also been observed in Axenfeld–Rieger syndrome, caused by mutations in the highly related gene, *PITX2* [Tumer and Bach-Holm, 2009]. *PITX2* has a similar expression pattern to *PITX1* in the developing pituitary, and it has been shown to act on some of the same promoter elements [Tremblay et al., 2000; Lamba et al., 2008]. *Pitx1* knockout mice also have abnormalities of first branchial arch derivatives, including severe micrognathia, cleft palate, bifurcate and shortened tongue, and abnormal mandibular molars [Lanctot et al., 1999; Szeto et al., 1999; Mitsiadis and Drouin, 2008]. Therefore, deletion of *PITX1* may be responsible for the orofacial anomalies seen in Patients 1–3 (Table I). Another individual with a ~9.5 Mb deletion at 5q23.3q31.2 was reported to have a branchial fistula [Tzschach et al., 2006], and this could also be a result of *PITX1* haploinsufficiency.

Two patients in our cohort with 5q31 deletions have ophthalmologic abnormalities: Patient 1 has keratitis, blepharitis, myopia, astigmatism, and strabismus, whereas Patient 2 has keratoconus and myopia. Although *PITX1* is closely related to *PITX2*, and *PITX2* mutations cause Axenfeld–Rieger anomaly and other anterior segment defects [Semina et al., 1996; Alward et al., 1998; Doward

TABLE I. Summary of Features Seen in Individuals With Deletions That Encompass 5q31.

	Patient 1	Patient 2	Patient 3	Mosca et al. [2007]	Tzschach et al. [2006]	Cytogenetically defined deletions encompassing 5q31[a]
Minimal deletion coordinates (hg18)	chr5:132,413,216–138,489,956	chr5:133,360,671–136,953,024	chr5:131,857,587–139,915,446	chr5:136,886,534–138,717,853	chr5:128,219,363–135,916,051	NA
Number of genes	62	33	101	28	69	NA
Growth parameters						
Low birth weight	+	+	–	–	–	2/10
Failure to thrive	+	+	NA	+	+	4/7
Short stature	+	+	NA	+	–	4/6
Microcephaly	+	NS	–	–	–	4/8
Neurologic features						
DD/ID	+	+	NA	+	+	8/8
Hypotonia	+	NS	+	+	–	5/8
Hydrocephalus	+	–	–	–	–	0/10
Seizures	–	+	–	–	–	0/8
Hearing loss	+	–	–	–	–	0/8
Ophthalmologic findings	+	+	–	–	–	1/8
Dysmorphic features						
Short neck	–	–	+	–	–	5/9
Prominent forehead	–	–	+	–	–	5/9
Downslanting PF	+	–	–	–	–	7/9
Hypertelorism	+	–	+	+	+	7/9
Flat nasal bridge	–	–	–	–	+	6/9
Anteverted nostrils	+	+	–	+	+	6/9
Long philtrum	+	–	–	–	+	2/9
Thin upper lip	–	–	–	–	+	2/9
Cleft or high palate	+	+	+	–	+	7/9
Micro/retrognathia	+	+	+	+	+	5/9
Abnormal ears	+	–	+	–	+	8/9
Musculoskeletal features						
Hypoplastic muscles	–	–	–	–	+	2/9
Arachno/camptodactyly	+	–	+	–	+	2/9
Dislocated hips	+	–	+	–	–	6/9
Club feet	–	–	+	–	–	5/10
Scoliosis	+	+	+	–	–	0/9
Heart defect	+	–	+	–	–	3/10
Urogenital anomalies	+	–	+	–	–	3/9

DD/ID, developmental delay and/or intellectual disability; PF, palpebral fissures; +, feature present; –, feature absent; NS, not specified; NA, not applicable.

[a]Previously reported individuals [Felding and Kristoffersson, 1980; Harprecht-Beato et al., 1983; Rivera et al., 1987; de Michelena et al., 1990; Rivera et al., 1990; Kobayashi et al., 1991; Lindgren et al., 1992; Courtens et al., 1998; Kramer et al., 1999; Arens et al., 2004].

et al., 1999; Xia et al., 2004], many of the genes' developmental roles are distinct, and a role for *PITX1* in ocular development has not been established. However, *TGFBI* (transforming growth factor beta-induced, OMIM 601692), which causes various autosomal dominant corneal dystrophies when mutated [Kim et al., 2000], is also deleted in these patients. In the corneal dystrophies, degraded mutant protein builds up on the cornea [Kannabiran and Klintworth, 2006], while decreased *TGFBI* expression has been reported in association with corneal thinning seen in keratoconus [Takacs et al., 1999]. Another candidate gene in 5q31 for keratoconus is *IL9* (interleukin 9, OMIM 156931), as there are associations between keratoconus and both increased IL6 levels [Lema et al., 2009] and *IL1B* promoter polymorphisms [Kim et al., 2008]. Haploinsufficiency for *TGFBI* or *IL9* may contribute to Patient 2's keratoconus and possibly some of the other ophthalmologic features seen in Patients 1 and 2. Although Patient 3 had a normal newborn ophthalmologic evaluation, some of these features may develop with age, and he may be at risk for similar complications as the other two patients.

We sequenced *TGFBI*, *IL9*, and *PITX1* in a family with autosomal dominant keratoconus and linkage to 5q31 to determine whether one of these genes may be associated with corneal abnormalities.

TABLE II. Summary of Features Seen in Individuals With Duplications That Encompass 5q31.

	Patient 4	Patient 5	Giardino et al. [2004]	Cytogenetically defined duplications encompassing 5q31[a]
Minimal duplication coordinates (hg18)	chr5:133,689,895–134,524,283	chr5:136,733,090–140,096,525	chr5:120,715,629–140,597,460	NA
Number of genes	13	60	177	NA
Growth parameters				
Low birth weight	–	–	+	0/4
Growth retardation	–	–	+	2/4
Microcephaly	–	+	+	4/5
Neurologic features				
DD/ID	+	+	+	3/4
Behavior problems	+	+	NA	0/2
Hypotonia	+	+	+	1/4
Seizures or abnormal EEG	–	+	–	1/5
Ophthalmologic findings	+	–	–	2/5
Dysmorphic features				
Facial or cranial asymmetry	+	+	+	1/5
Prominent forehead	–	+	–	1/5
Hypertelorism	–	–	+	1/5
Depressed nasal bridge	+	+	–	2/5
Thin upper lip	–	–	–	2/5
Cleft palate	–	–	–	0/5
Micro/retrognathia	+	–	+	1/5
Abnormal ears	+	+	+	1/5
Limb abnormalities				
Syndactyly	+	–	+	2/5
Brachydactyly	–	–	–	2/5
Hyperconvex nails	–	–	–	2/5
Club feet	–	–	+	0/5
Heart defect	–	+	+	3/5
Urogenital anomalies	+	–	+	1/5

DD/ID, developmental delay and/or intellectual disability; +, feature present; –, feature absent; NA, not applicable.

[a]Previously reported individuals [Osztovics and Kiss, 1982; Martin et al., 1985; Sanchez-Garcia et al., 2001; Martin et al., 2003; Arens et al., 2004], counting only one individual in the family reported by Arens et al. [2004].

We did not find a causative mutation, which suggests another gene or genes within the deleted region may be responsible for the ophthalmologic abnormalities in Patients 1 and 2 and the KTCN-011 family, or there may be separate genes responsible for the ophthalmologic abnormalities in Patients 1 and 2 and the KTCN-011 family. Separate etiologies would be consistent with previous reports that showed suggestive linkage peaks for keratoconus more distal on 5q than our patients region of deletion overlap [Li et al., 2006; Bisceglia et al., 2009]. Additionally, keratoconus is seen more commonly among individuals with intellectual disabilities [Hestnes et al., 1991; Haugen, 1992; Kirby et al., 2005] and has been suggested to be caused by inflammation secondary to eye rubbing [McMonnies, 2009]. Thus, the corneal anomalies in Patients 1 and 2 may also be the result of non-genetic factors.

Potentially, dosage of other developmentally important genes is altered in some of the 5q31 chromosome abnormalities in our cohort, and haploinsufficiency or overexpression of these genes may also contribute to the abnormal phenotypes. *SMAD5* (OMIM 603110) is a signal mediator for bone morphogenic proteins (BMPs), with roles in proper development of the brain [Lopez-Coviella et al., 2006; Lebeurrier et al., 2008], digits [Suzuki et al., 2008], and bones [Retting et al., 2009]. While homozygous knockout of the gene is embryonic lethal in animals [Chang et al., 1999; Chang et al., 2000], conditional knockouts show functional heart defects [Umans et al., 2007], and haploinsufficient animals show progressive hearing loss [Yang et al., 2009]. *WNT8A* (OMIM 606360) encodes a secreted signaling molecule that is a posteriorizing/caudalizing factor in neural and mesodermal tissue and a regulator of cell differentiation [Erter et al., 2001; Lekven et al., 2001; Olivera-Martinez and Storey, 2007] and formation of the otic placode [Urness et al., 2010]. *CDKL3* (cyclin-dependent kinase like 3, OMIM 608459) helps to regulate neuronal morphogenesis [Liu et al., 2010] and was shown to be disrupted and haploinsufficient in an individual with nonspecific ID and a balanced translocation [Dubos et al., 2008]. Furthermore, overexpression of *CDKL3* also alters neuronal morphogenesis [Liu et al., 2010]. The phenotypes in

the patients reported here likely result from a combinatorial effect of disruption of these several genes with roles in developmental pathways, as well as a contribution of other yet unidentified additional genetic and nongenetic factors.

The deletions and duplications reported here are all apparently de novo and likely disrupt developmental pathway(s), which suggest they are clinically relevant findings that contribute to abnormal phenotypes. Individuals with deletions share some common features, including DD, short stature, cleft or high palate, dysmorphic features, and musculoskeletal anomalies. Haploinsufficiencies of *PITX1* and *SMAD5* may cause many of these features, and other developmentally important genes in the region may also contribute. Furthermore, we report an association of 5q31 deletions and corneal anomalies, possibly due to *TGFBI* or *IL9* haploinsufficiency. Individuals with duplications have nondistinct phenotypes, and while their delays and other features are likely caused by the duplications, the nonspecific features and low number of reported patients complicate genotype–phenotype correlations. Identification of additional individuals with copy number changes in 5q31 will aid in further understanding of how dosage imbalances of specific genes may contribute to the abnormal phenotypes seen in these individuals.

ACKNOWLEDGMENTS

The authors thank Aaron Theisen for his critical reading of this manuscript. This work was supported in part by the Ministry of Education and Science, Poland, Grant NN402097837.

REFERENCES

Al-Qattan MM. 2010. Wnt pathways and upper limb anomalies. J Hand Surg Eur Vol 36:9–22.

Alvarado DM, Aferol H, McCall K, Huang JB, Techy M, Buchan J, Cady J, Gonzales PR, Dobbs MB, Gurnett CA. 2010. Familial isolated clubfoot is associated with recurrent chromosome 17q23.1q23.2 microduplications containing TBX4. Am J Hum Genet 87:154–160.

Alward WL, Semina EV, Kalenak JW, Heon E, Sheth BP, Stone EM, Murray JC. 1998. Autosomal dominant iris hypoplasia is caused by a mutation in the Rieger syndrome (RIEG/PITX2) gene. Am J Ophthalmol 125:98–100.

Arens YH, Engelen JJ, Govaerts LC, van Ravenswaay CM, Loneus WH, van Lent-Albrechts JC, van der Blij-Philipsen M, Hamers AJ, Schrander-Stumpel CT. 2004. Familial insertion (3;5)(q25.3;q22.1q31.3) with deletion or duplication of chromosome region 5q22.1-5q31.3 in ten unbalanced carriers. Am J Med Genet Part A 130A:128–133.

Ballif BC, Theisen A, Rosenfeld JA, Traylor RN, Gastier-Foster J, Thrush DL, Astbury C, Bartholomew D, McBride KL, Pyatt RE, Shane K, Smith WE, Banks V, Gallentine WB, Brock P, Rudd MK, Adam MP, Keene JA, Phillips JA 3rd, Pfotenhauer JP, Gowans GC, Stankiewicz P, Bejjani BA, Shaffer LG. 2010. Identification of a recurrent microdeletion at 17q23.1q23.2 flanked by segmental duplications associated with heart defects and limb abnormalities. Am J Hum Genet 86:454–461.

Bisceglia L, De Bonis P, Pizzicoli C, Fischetti L, Laborante A, Di Perna M, Giuliani F, Delle Noci N, Buzzonetti L, Zelante L. 2009. Linkage analysis in keratoconus: Replication of locus 5q21.2 and identification of other suggestive loci. Invest Ophthalmol Vis Sci 50:1081–1086.

Chang H, Huylebroeck D, Verschueren K, Guo Q, Matzuk MM, Zwijsen A. 1999. Smad5 knockout mice die at mid-gestation due to multiple embryonic and extraembryonic defects. Development 126:1631–1642.

Chang H, Zwijsen A, Vogel H, Huylebroeck D, Matzuk MM. 2000. Smad5 is essential for left-right asymmetry in mice. Dev Biol 219:71–78.

Courtens W, Tjalma W, Messiaen L, Vamos E, Martin JJ, Van Bogaert E, Keersmaekers G, Meulyzer P, Wauters J. 1998. Prenatal diagnosis of a constitutional interstitial deletion of chromosome 5 (q15q31.1) presenting with features of congenital contractural arachnodactyly. Am J Med Genet 77:188–197.

de Michelena MI, Villacorta J, Chavez J. 1990. Double chromosome anomaly: Interstitial deletion 5q and reciprocal translocation (1;11)(p22;q21). Am J Med Genet 36:29–32.

DeLaurier A, Schweitzer R, Logan M. 2006. Pitx1 determines the morphology of muscle, tendon, and bones of the hindlimb. Dev Biol 299:22–34.

Doward W, Perveen R, Lloyd IC, Ridgway AE, Wilson L, Black GC. 1999. A mutation in the RIEG1 gene associated with Peters' anomaly. J Med Genet 36:152–155.

Dubos A, Pannetier S, Hanauer A. 2008. Inactivation of the CDKL3 gene at 5q31.1 by a balanced t(X;5) translocation associated with nonspecific mild mental retardation. Am J Med Genet Part A 146A:1267–1279.

Duker AL, Ballif BC, Bawle EV, Person RE, Mahadevan S, Alliman S, Thompson R, Traylor R, Bejjani BA, Shaffer LG, Rosenfeld JA, Lamb AN, Sahoo T. 2010. Paternally inherited microdeletion at 15q11.2 confirms a significant role for the SNORD116C/D box snoRNA cluster in Prader-Willi syndrome. Eur J Hum Genet 18:1196–1201.

Erter CE, Wilm TP, Basler N, Wright CV, Solnica-Krezel L. 2001. Wnt8 is required in lateral mesendodermal precursors for neural posteriorization in vivo. Development 128:3571–3583.

Felding I, Kristoffersson U. 1980. A child with interstitial deletion of chromosome no. 5. Hereditas 93:337–339.

Fishelson M, Geiger D. 2002. Exact genetic linkage computations for general pedigrees. Bioinformatics 18:S189–198.

Gajecka M, Radhakrishna U, Winters D, Nath SK, Rydzanicz M, Ratnamala U, Ewing K, Molinari A, Pitarque JA, Lee K, Leal SM, Bejjani BA. 2009. Localization of a gene for keratoconus to a 5.6-Mb interval on 13q32. Invest Ophthalmol Vis Sci 50:1531–1539.

Giardino D, Finelli P, Amico FP, Gottardi G, Civa R, Corona G, Nocera G, Larizza L. 2004. Unbalanced segregation of a complex four-break 5q23-31 insertion in the 5p13 band in a malformed child. Eur J Hum Genet 12:455–459.

Gudbjartsson DF, Jonasson K, Frigge ML, Kong A. 2000. Allegro, a new computer program for multipoint linkage analysis. Nat Genet 25:12–13.

Gurnett CA, Alaee F, Kruse LM, Desruisseau DM, Hecht JT, Wise CA, Bowcock AM, Dobbs MB. 2008. Asymmetric lower-limb malformations in individuals with homeobox PITX1 gene mutation. Am J Hum Genet 83:616–622.

Harprecht-Beato W, Kaiser P, Steuber E, Reinhard W. 1983. Interstitial deletion in the long arm of chromosome no. 5. Clin Genet 23:167–171.

Haugen OH. 1992. Keratoconus in the mentally retarded. Acta Ophthalmol (Copenh) 70:111–114.

Hestnes A, Sand T, Fostad K. 1991. Ocular findings in Down's syndrome. J Ment Defic Res 35:194–203.

Itsara A, Cooper GM, Baker C, Girirajan S, Li J, Absher D, Krauss RM, Myers RM, Ridker PM, Chasman DI, Mefford H, Ying P, Nickerson DA, Eichler EE. 2009. Population analysis of large copy number variants and hotspots of human genetic disease. Am J Hum Genet 84:148–161.

Kannabiran C, Klintworth GK. 2006. TGFBI gene mutations in corneal dystrophies. Hum Mutat 27:615–625.

Kim JE, Kim SJ, Lee BH, Park RW, Kim KS, Kim IS. 2000. Identification of motifs for cell adhesion within the repeated domains of transforming growth factor-beta-induced gene, betaig-h3. J Biol Chem 275:30907–30915.

Kim SH, Mok JW, Kim HS, Joo CK. 2008. Association of -31T>C and -511 C>T polymorphisms in the interleukin 1 beta (IL1B) promoter in Korean keratoconus patients. Mol Vis 14:2109–2116.

Kirby D, Jackson AP, Karbani G, Crow YJ. 2005. Mental retardation, keratoconus, febrile seizures and sinoatrial block: A previously undescribed autosomal recessive disorder. Clin Genet 67:448–449.

Kobayashi T, Narahara K, Yokoyama Y, Ueyama S, Mohri O, Fujii T, Fujimoto M, Ohtsuki S, Tsuji K, Seino Y. 1991. Gardner syndrome in a boy with interstitial deletion of the long arm of chromosome 5. Am J Med Genet 41:460–463.

Kramer RL, Feldman B, Ebrahim SA, Kasperski SB, Johnson MP, Evans MI. 1999. Molecular cytogenetic analysis of a de novo 5q31q33 deletion associated multiple congenital anomalies: Case report. Am J Med Genet 82:143–145.

Lamba P, Khivansara V, D'Alessio AC, Santos MM, Bernard DJ. 2008. Paired-like homeodomain transcription factors 1 and 2 regulate follicle-stimulating hormone beta-subunit transcription through a conserved cis-element. Endocrinology 149:3095–3108.

Lanctot C, Moreau A, Chamberland M, Tremblay ML, Drouin J. 1999. Hindlimb patterning and mandible development require the Ptx1 gene. Development 126:1805–1810.

Lebeurrier N, Launay S, Macrez R, Maubert E, Legros H, Leclerc A, Jamin SP, Picard JY, Marret S, Laudenbach V, Berger P, Sonderegger P, Ali C, di Clemente N, Vivien D. 2008. Anti-Mullerian-hormone-dependent regulation of the brain serine-protease inhibitor neuroserpin. J Cell Sci 121:3357–3365.

Lekven AC, Thorpe CJ, Waxman JS, Moon RT. 2001. Zebrafish wnt8 encodes two wnt8 proteins on a bicistronic transcript and is required for mesoderm and neurectoderm patterning. Dev Cell 1:103–114.

Lema I, Sobrino T, Duran JA, Brea D, Diez-Feijoo E. 2009. Subclinical keratoconus and inflammatory molecules from tears. Br J Ophthalmol 93:820–824.

Li X, Rabinowitz YS, Tang YG, Picornell Y, Taylor KD, Hu M, Yang H. 2006. Two-stage genome-wide linkage scan in keratoconus sib pair families. Invest Ophthalmol Vis Sci 47:3791–3795.

Lindgren V, Bryke CR, Ozcelik T, Yang-Feng TL, Francke U. 1992. Phenotypic, cytogenetic, and molecular studies of three patients with constitutional deletions of chromosome 5 in the region of the gene for familial adenomatous polyposis. Am J Hum Genet 50:988–997.

Liu F, Millar SE. 2010. Wnt/beta-catenin signaling in oral tissue development and disease. J Dent Res 89:318–330.

Liu Z, Xu D, Zhao Y, Zheng J. 2010. Non-syndromic mild mental retardation candidate gene CDKL3 regulates neuronal morphogenesis. Neurobiol Dis 39:242–251.

Logan M, Tabin CJ. 1999. Role of Pitx1 upstream of Tbx4 in specification of hindlimb identity. Science 283:1736–1739.

Lopez-Coviella I, Mellott TM, Kovacheva VP, Berse B, Slack BE, Zemelko V, Schnitzler A, Blusztajn JK. 2006. Developmental pattern of expression of BMP receptors and Smads and activation of Smad1 and Smad5 by BMP9 in mouse basal forebrain. Brain Res 1088:49–56.

Martin DM, Mindell MH, Kwierant CA, Glover TW, Gorski JL. 2003. Interrupted aortic arch in a child with trisomy 5q31.1q35.1 due to a maternal (20;5) balanced insertion. Am J Med Genet Part A 116A: 268–271.

Martin NJ, Cartwright DW, Harvey PJ. 1985. Duplication 5q(5q22—5q33): From an intrachromosomal insertion. Am J Med Genet 20:57–62.

Matise TC, Chen F, Chen W, De La Vega FM, Hansen M, He C, Hyland FC, Kennedy GC, Kong X, Murray SS, Ziegle JS, Stewart WC, Buyske S. 2007. A second-generation combined linkage physical map of the human genome. Genome Res 17:1783–1786.

McMonnies CW. 2009. Mechanisms of rubbing-related corneal trauma in keratoconus. Cornea 28:607–615.

Minguillon C, Del Buono J, Logan MP. 2005. Tbx5 and Tbx4 are not sufficient to determine limb-specific morphologies but have common roles in initiating limb outgrowth. Dev Cell 8:75–84.

Mitsiadis TA, Drouin J. 2008. Deletion of the Pitx1 genomic locus affects mandibular tooth morphogenesis and expression of the Barx1 and Tbx1 genes. Dev Biol 313:887–896.

Mosca AL, Callier P, Leheup B, Marle N, Jalloul M, Coffinet L, Feillet F, Valduga M, Jonveaux P, Mugneret F. 2007. Fortuitous FISH diagnosis of an interstitial microdeletion (5)(q31.1q31.2) in a girl suspected to present a cri-du-chat syndrome. Am J Med Genet Part A 143A:1342–1347.

Olivera-Martinez I, Storey KG. 2007. Wnt signals provide a timing mechanism for the FGF-retinoid differentiation switch during vertebrate body axis extension. Development 134:2125–2135.

Osztovics M, Kiss P. 1982. Trisomy 5q15-q31 due to maternal insertion, ins (6; 5) (q21; q15q31). Acta Paediatr Acad Sci Hung 23:231–237.

Retting KN, Song B, Yoon BS, Lyons KM. 2009. BMP canonical Smad signaling through Smad1 and Smad5 is required for endochondral bone formation. Development 136:1093–1104.

Rivera H, Garcia-Esquivel L, Moller M, Cantu JM. 1987. Constitutional del(5)(q23.3q31.1). Ann Genet 30:91–93.

Rivera H, Simi P, Rossi S, Pardelli L, Di Paolo MC. 1990. A constitutional 5q23 deletion. J Med Genet 27:267–268.

Ryan JD, Ryan E, Fabre A, Lawless MW, Crowe J. 2010. Defective bone morphogenic protein signaling underlies hepcidin deficiency in HFE hereditary hemochromatosis. Hepatology 52:1266-L1273.

Sanchez-Garcia JF, de Die-Smulders CE, Weber JW, Jetten AG, Loneus WH, Hamers AJ, Engelen JJ. 2001. De novo duplication (5)(q31.3q33.3): Report of a patient and characterization of the duplicated region using microdissection and FISH. Am J Med Genet 100:56–61.

Semina EV, Reiter R, Leysens NJ, Alward WL, Small KW, Datson NA, Siegel-Bartelt J, Bierke-Nelson D, Bitoun P, Zabel BU, Carey JC, Murray JC. 1996. Cloning and characterization of a novel bicoid-related homeobox transcription factor gene, RIEG, involved in Rieger syndrome. Nat Genet 14:392–399.

Shaffer LG, McCaskill C, Han JY, Choo KH, Cutillo DM, Donnenfeld AE, Weiss L, Van Dyke DL. 1994. Molecular characterization of de novo secondary trisomy 13. Am J Hum Genet 55:968–974.

Shaikh TH, Gai X, Perin JC, Glessner JT, Xie H, Murphy K, O'Hara R, Casalunovo T, Conlin LK, D'Arcy M, Frackelton EC, Geiger EA, Haldeman-Englert C, Imielinski M, Kim CE, Medne L, Annaiah K, Bradfield JP, Dabaghyan E, Eckert A, Onyiah CC, Ostapenko S, Otieno FG, Santa E, Shaner JL, Skraban R, Smith RM, Elia J, Goldmuntz E, Spinner NB, Zackai EH, Chiavacci RM, Grundmeier R, Rappaport EF, Grant SF, White PS, Hakonarson H. 2009. High-resolution mapping and analysis of copy number variations in the human genome: A data resource for clinical and research applications. Genome Res 19:1682–1690.

Silberstein M, Tzemach A, Dovgolevsky N, Fishelson M, Schuster A, Geiger D. 2006. Online system for faster multipoint linkage analysis via parallel

execution on thousands of personal computers. Am J Hum Genet 78:922–935.

Sobel E, Lange K. 1996. Descent graphs in pedigree analysis: Applications to haplotyping, location scores, and marker-sharing statistics. Am J Hum Genet 58:1323–1337.

Suzuki T, Hasso SM, Fallon JF. 2008. Unique SMAD1/5/8 activity at the phalanx-forming region determines digit identity. Proc Natl Acad Sci U S A 105:4185–4190.

Szeto DP, Rodriguez-Esteban C, Ryan AK, O'Connell SM, Liu F, Kioussi C, Gleiberman AS, Izpisua-Belmonte JC, Rosenfeld MG. 1999. Role of the Bicoid-related homeodomain factor Pitx1 in specifying hindlimb morphogenesis and pituitary development. Genes Dev 13:484–494.

Takacs L, Csutak A, Balazs E, Modis L Jr, Berta A. 1999. Expression of betaig-h3 is lower than normal in keratoconus corneas but increases with scarring. Cornea 18:599–605.

Thiele H, Nurnberg P. 2005. HaploPainter: A tool for drawing pedigrees with complex haplotypes. Bioinformatics 21:1730–1732.

Traylor RN, Fan Z, Hudson B, Rosenfeld JA, Shaffer LG, Torchia BS, Ballif BC. 2009. Microdeletion of 6q16.1 encompassing EPHA7 in a child with mild neurological abnormalities and dysmorphic features: Case report. Mol Cytogenet 2:17.

Tremblay JJ, Goodyer CG, Drouin J. 2000. Transcriptional properties of Ptx1 and Ptx2 isoforms. Neuroendocrinology 71:277–286.

Tumer Z, Bach-Holm D. 2009. Axenfeld-Rieger syndrome and spectrum of PITX2 and FOXC1 mutations. Eur J Hum Genet 17:1527–1539.

Tzschach A, Krause-Plonka I, Menzel C, Kalscheuer V, Toennies H, Scherthan H, Knoblauch A, Radke M, Ropers HH, Hoeltzenbein M. 2006. Molecular cytogenetic analysis of a de novo interstitial deletion of 5q23.3q31.2 and its phenotypic consequences. Am J Med Genet Part A 140A:496–502.

Umans L, Cox L, Tjwa M, Bito V, Vermeire L, Laperre K, Sipido K, Moons L, Huylebroeck D, Zwijsen A. 2007. Inactivation of Smad5 in endothelial cells and smooth muscle cells demonstrates that Smad5 is required for cardiac homeostasis. Am J Pathol 170:1460–1472.

Urness LD, Paxton CN, Wang X, Schoenwolf GC, Mansour SL. 2010. FGF signaling regulates otic placode induction and refinement by controlling both ectodermal target genes and hindbrain Wnt8a. Dev Biol 340:595–604.

Walsh DW, Godson C, Brazil DP, Martin F. 2010. Extracellular BMP-antagonist regulation in development and disease: Tied up in knots. Trends Cell Biol 20:244–256.

Weeks DE, Sobel E, O'Connell JR, Lange K. 1995. Computer programs for multilocus haplotyping of general pedigrees. Am J Hum Genet 56:1506–1507.

Wigginton JE, Abecasis GR. 2005. PEDSTATS: Descriptive statistics, graphics and quality assessment for gene mapping data. Bioinformatics 21:3445–3447.

Xia K, Wu L, Liu X, Xi X, Liang D, Zheng D, Cai F, Pan Q, Long Z, Dai H, Hu Z, Tang B, Zhang Z, Xia J. 2004. Mutation in PITX2 is associated with ring dermoid of the cornea. J Med Genet 41:e129.

Yang SM, Guo WW, Hu YY, Sun YX, Hou ZH, Sun JH, Wang X, He DZ, Zhai SQ, Young WY, Han DY, Yang X. 2009. Smad5 haploinsufficiency leads to hair cell and hearing loss. Dev Neurobiol 69:153–161.