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Molecular (SNP) Analyses of Overlapping Hemizygous Deletions of 10q25.3 to 10qter in Four Patients: Evidence for *HMX2* and *HMX3* as Candidate Genes in Hearing and Vestibular Function

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Abstract

We report on the analyses of four unrelated patients with de novo, overlapping, hemizygous deletions of the long arm of chromosome 10. These include two small terminal deletions (10q26.2 to 10qter), a larger terminal deletion (10q26.12 to 10qter), and an interstitial deletion (10q25.3q26.13). Single nucleotide polymorphism (SNP) studies (Illumina 550 K) established that these deletions resulted in the hemizygous loss of ~6.1, ~6.1, ~12.5, and ~7.0 Mb respectively. Additionally, these data establish that Patients 1, 2, and 3 share common, distal, hemizygous deleted regions of 6.09 Mb containing 37 RefSeq genes. Patients 3 and 4 share a 2.52 Mb deleted region corresponding to the proximal deleted region of Patient 3 and the distal deleted region of Patient 4. This common, hemizygous region contains 20 RefSeq genes including two H6 family homeobox genes (*HMX2* and *HMX3*). Based on previous reports that *Hmx2/Hmx3* knockout mice have vestibular anomalies, we propose that hemizygous deletions of *HMX2* and *HMX3* are responsible for the inner ear malformations observed from CT images, vestibular dysfunction, and congenital sensorineural hearing loss found in Patients 3 and 4.

Keywords

10q deletion; hearing loss; vestibular dysfunction; *HMX2*; *HMX3*; SNP microarray

INTRODUCTION

Deletions of chromosome 10qter were first reported by Lewandowski et al. in 1978. Subsequently, Wulfsberg et al. [1989] reported three new patients and reviewed 15 previous

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

patients and proposed a 10qter deletion syndrome. Cytogenetically, these patients had deletions of bands 10q25 or 10q26 to the terminus, as well as interstitial deletions and translocations within this region. Additionally, both familial and de novo patients have been reported [Irving et al., 2003]. Irving et al. [2003] reported 15 new patients involving interstitial and terminal deletions of chromosome bands 10q25.2–26.3, and reviewed 17 patients from the literature, supporting wide clinical variability for deletions in this region yet common features including strabismus, facial asymmetry, prominent chin, down-slanting palpebral fissures, hypertelorism, malformed or large ears, and a thin upper lip. Kehrer-Sawatzki et al. [2005] also described the phenotype of a patient with an interstitial del(10)(q25.2q25.3~q26.11) and reviewed four previously described interstitial deletion patients involving bands 10q25.2–q26.1. Courtens et al. [2006] reported a subterminal deletion del(10)(q26.2) and reviewed the literature, describing the phenotype of subterminal deletions. Subterminal patients were noted to commonly have low birth weight, microcephaly at birth, short stature in childhood/adulthood, genital anomalies in males, and behavioral problems. Their craniofacial anomalies included broad/prominent nasal bridges, prominent nose, strabismus, thin upper lip, and fifth finger clinodactyly. Overall, there is variability in the sizes and positions of distal 10q deletions, presumably causing haploinsufficiency of many different genes in this large region and therefore contributing to significant variation in phenotype.

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The majority of patients noted in the literature as having interstitial or terminal deletions of distal 10q have been characterized only with standard Giemsa staining (G-banding). In 20 of the approximately 60 patients in the literature, techniques more sensitive than G-banding have been employed [Lukusa and Fryns, 2000; McCandless et al., 2000; Ogata et al., 2000; Irving et al., 2003; Scigliano et al., 2004; Chen et al., 2005; Kehrer-Sawatzki et al., 2005; Courtens et al., 2006; Joshi et al., 2006]. These techniques include subtelomeric fluorescence in situ hybridization (FISH), FISH with BAC probes spanning the deletion region, genotyping with microsatellite markers, and array comparative genome hybridization. Although they refine the breakpoints, these techniques have limited accuracy and/or precision that is important for localizing the breakpoint in these individuals to the resolution needed for genotype–phenotype correlations.

With the resolution of prior cytogenetic techniques, many distal 10q deletion patients were described together without an understanding of how their unique deletions related to their different phenotypes. Now with higher resolution, we can compare and contrast presumably similar cytogenetic anomalies to narrow down to individual genes potentially responsible for specific phenotypes. We report on the analyses of four 10q deletion patients using single nucleotide polymorphism (SNP) microarrays. Three patients have terminal deletions and one has an interstitial deletion. All these probands were extensively phenotyped, and for two probands, we obtained SNP data from their unaffected parents. Such trio data permit additional analyses of genetic mechanisms and inheritance underlying the deletions. Among our findings, we identify a common deleted area shared by two of the four patients and propose candidate genes for vestibular and/or hearing dysfunction.

MATERIALS AND METHODS

Four patients and their parents (available for two of the patients) were studied following the guidelines of the Institutional Review Board at the Johns Hopkins School of Medicine.

Chromosome and FISH Analysis

Chromosome and FISH analyses were performed on thymidine synchronized peripheral blood lymphocyte cultures. At least 20 cells were analyzed from two independent cultures after standard G-banding at the 550 band level or higher. FISH was performed on interphase and metaphase spreads with a commercial probe for the subtelomeric region of 10q (Abott Molecular/Vysis, Des Plaines, IL). FISH was also performed with probes prepared from BAC DNA clones (BacPac, Oakland, CA) mapped to 10q. For FISH probes at least 20 metaphase cells were analyzed. Information regarding BAC clones position and band designation was obtained from NCBI and the Ensembl Genome Browser.

SNP Genotyping

Allelic composition and signal intensity of 550,000 human SNPs were performed with the HumanHap550 Genotyping Beadchip v.1.0 (Illumina, San Diego, CA) at the Johns Hopkins University Genetic Resources Core Facility SNP Center. Samples were processed using the Illumina Infinium II Assay protocol. SNPs were detected using a two-color single base extension reaction [Gunderson et al., 2006]. Approximately 1,125 ng of genomic DNA (15 μ l at 75 ng/ μ l) was used as input for the Infinium II whole genome amplification reaction. After a 20- to 24-hr incubation at 37°C, amplified DNA was fragmented, precipitated, and resuspended according to the manufacturer's protocol except for the robotic platform used, which was the Perkin Elmer® Multiprobe® II HT PLUS robot. Denatured samples were hybridized on prepared HumanHap550 beadchips for 16–24 hr at 48°C. Following hybridization, a single-base extension reaction was performed followed by signal amplification utilizing Tecan instruments. Beadchips were then imaged on Illumina BeadArray Readers. After each batch of chips was processed, the intensity data for all samples were loaded into BeadStudio Genotyping Module v.2.3.41 and analyzed with the Illumina-provided standard definition cluster-file for the Human-Hap500 v.1.0 product. Data analysis was done by examination of signal intensity (log R ratio) and allelic composition (allelic frequency) with the BeadStudio 2.3.41 Loss of Heterozygosity Plus software module (Illumina).

SNP Analysis

With trios of the proband and his or her parents, SNP trio analysis was done using the default settings on the website (<http://pevsnerlab.kennedykrieger.org/SNP trio.htm>) [Ting et al., 2007]. Copy number variants were detected using SNPcnv and SNPlog R but were not verified with FISH. SNPcnv is a copy number analysis tool designed to detect deletions or duplications (including small deletions or duplications, involving ~5 to 10 SNPs for Illumina 550 K SNP data). SNPcnv identifies blocks of consecutive homozygous SNP calls. Its output includes the number and identity of RefSeq genes in a deletion region (based on links to the UCSC Genome Browser) as well as the copy number (log R ratio). For consecutive homozygous calls or NoCalls, we set SNPcnv to only show blocks with a minimum $\log_2(R \text{ ratio})$ delta of 0.4. We allowed three consecutive SNPs with low $\log_2(R \text{ ratio})$ delta of 0.1 in a block. Results were limited to a minimum window size of seven SNPs, a setting that was optimal in minimizing false positive and false negative results. All chromosomes were analyzed. A synthetic data set was generated to simulate hemizygous deletions. With these settings and a minimum size of 100 kb, 1 out of 20 findings were false positives and 3% of deletions were not detected (false negatives). The SNPlog R algorithm indicates a deletion if three or more SNPs have log R values less than -0.7 and are 10 kb or less from each other. For insertions, a log R threshold of greater than 0.5 is used. Visual inspection is used to determine the copy number state for

regions with SNPs spaced further apart, up to 50 kb from each other. Deletions and duplications were reported if any of the algorithms detected them. If they had a single copy number change to 1 or 3, the deletion or duplication minimum size was set at 100 kb to minimize false positives. Homozygous deletions were reported regardless as these are detected more easily.

RESULTS

Patient 1

Patient 1 is a female born at term by vaginal delivery after a pregnancy complicated by decreased fetal movement and maternal anemia. In infancy, there was temperature instability and poor growth. The physical exam was significant for frontal bossing, widow's peak, strabismus, stellate irides and bilateral fifth finger clinodactyly. Milestones were delayed with walking at 20 months and unsteady gait by 3 years. Speech was most significantly delayed with only four words at 3 years. Audiologic testing and brain MRI were normal (Table I).

G-banded cytogenetic analysis of cultured lymphocytes revealed a hemizygous deletion of the terminal end of chromosome 10q, interpreted as 46,XX,del(10)(q26.2) (Fig. 1). Subtelomere FISH analysis confirmed a telomeric deletion (data not shown). Cytogenetic studies of the parents found the abnormality to be de novo in the proband.

A 6.1 Mb deletion (Figs. 2 and 3, Table II) involving 37 RefSeq genes, with an additional RefSeq gene likely being hemizygous at the terminus (supporting information Table I may be found in the online version of this article), was detected with the Illumina HumanHap550 SNP microarray platform and a suite of analysis software used to determine copy number variations (CNVs) [see Materials and Methods Section and Ting et al., 2007]. (The gene near the terminus is distal to the most distal SNP on 10q. This gene could be present in two copies, but is likely to be deleted based on the cytogenetic findings consistent with a terminal deletion.) SNP trio analysis of the data from the patient and her parents established a de novo 10q deletion of the proband occurred on the paternal chromosome (supporting information Fig. 1 may be found in the online version of this article). Deletions that overlapped with two previously reported CNVs (Database of Genomic Variants <http://projects.tcag.ca/variation/> [Iafrate et al., 2004; Sebat et al., 2004; Sharp et al., 2005; Tuzun et al., 2005; Conrad et al., 2006; Hinds et al., 2006; McCarroll et al., 2006; Redon et al., 2006]) were also detected on chromosomes 14 and 22 in the proband (supporting information Table II may be found in the online version of this article), the former inherited from the mother and the latter de novo.

Patient 2

Patient 2, a female, was born at term after an uncomplicated pregnancy. She presented at 14 months with strabismus, mild epicanthal folds, a mildly recessed columella, tapered fingers and axial hypotonia (Table I). By 14 months of age, she was not walking independently, babbling, or speaking. There was no clinical record of hearing loss.

Cytogenetic analysis of G-banded blood lymphocyte cultures showed a terminal hemizygous deletion of chromosome 10 in all metaphases examined, determined to be 46,XX,del(10)(q26.2) (Fig. 1).

SNP analysis showed the hemizygous terminal deletion to be approximately 6.1 Mb in length (Figs. 2 and 3, Table II). This deletion was 57 kb larger than that of Patient 1, a difference undetectable by G-band karyotyping. This larger deletion possibly encompasses neuropeptide S (*NPS*) in addition to the 37 (likely 38) genes that were hemizygous in both Patients 1 and 2 (supporting information Table I may be found in the online version of this article). One deletion and one duplication were also detected elsewhere in the genome in Patient 2 (supporting

information Table II may be found in the online version of this article). The former overlapped with a previously reported CNV. Parental samples were not available for study.

Patient 3

Patient 3 is a male who was small for gestational age (3rd centile) after a full term pregnancy. At age 8 months, he exhibited failure to thrive, with weight and height below the 5th percentile. Head circumference was at the 25th centile for age. Dysmorphic features included a triangular face, brachycephaly, a long broad forehead, strabismus, hypotelorism, a small nose with a prominent nasal bridge, and hypoplastic toenails. Later, low-set, abnormally “simple” shaped ears were appreciated (Table I). Other physical findings included a short sternum, contractures of the elbows, 5th finger clinodactyly, and hypotonia. Diagnostic testing revealed a small ventriculo-septal defect, bilateral hydronephrosis which resolved spontaneously, a right undescended testicle, umbilical and inguinal hernias, and a patent urachus. A brain MRI and ophthalmology exam were normal. He was diagnosed with congenital bilateral sensorineural hearing loss with aided pure tone average (PTA) levels of 50–60 dB and unaided levels of 75 dB at five months of age. Due to chronic middle ear effusions, he was treated with myringotomy and placement of middle ear ventilation tubes, first at 1 year of age and replaced multiple times during early childhood. He had moderate to severe developmental delay with no words at 3 years of age and walking with a wide-based gait at 4 years 7 months.

MRI and temporal bone computed tomography (CT) studies revealed inner ear malformations, classified as Mondini type, although they involved both the cochlear and vestibular organs. Specifically, fine cut CT of temporal bones revealed both cochlear and vestibular abnormalities bilaterally, being more severe on the left side (Fig. 4A). On the right, the internal auditory canal (IAC) was narrow, the cochlea having only 1–1.5 turns with a dilated and rudimentary shape, while the cochlear aqueduct was enlarged. The vestibule was massively enlarged and abnormally shaped. While all three semi-circular canals (SCCs) appeared abnormally dilated, the superior SCC was the largest, and the vestibular aqueduct was mildly prominent. On the left, the IAC was very small and narrow while the cochlea was devoid of distinct structure without normal partitioning. The vestibule, as well as the utricle and saccule, were replaced with a 1 cm cyst. The horizontal and posterior SCCs were absent while the superior SCC was abnormally thickened. The vestibular aqueduct could not be identified. Though independent ambulation has been present for greater than 1 year, the wide-based gait persists and is thought to be related to his vestibular anomalies.

Chromosome analysis of G-banded blood lymphocyte cultures from this patient revealed a large, hemizygous terminal deletion of the long arm of chromosome 10, that is, 46, XY,del(10)(q26.12) in all metaphases examined (Fig. 1). FISH analysis using BAC probe RP11–162A23 (124,795,486–124,978,916 bp) confirmed that the deletion included a region in common with Patient 4.

SNP analysis of DNA isolated from the patient's cultured lymphoblast cells confirmed that the above deletion resulted in the hemizygous loss of a minimum of 12.4 Mb (Figs. 2 and 3, Table II). This region encompassed 78 genes and likely a 79th gene at the terminus (supporting information Tables I, III, and IV may be found in the online version of this article). In addition to the major 10q deletion, one homozygous deletion on chromosome 11 and one duplication on chromosome 16, both previously reported CNVs, were found (supporting information Table II may be found in the online version of this article).

Patient 4

Patient 4 is a male, born at term after an uncomplicated pregnancy (Table I). At birth, he was diagnosed with ambiguous genitalia with bilateral cryptorchidism, a large phallus held down

by chordee, a large labial-scrotal fold on the right, and a smaller fold on the left. Ultrasound examination showed the absence of Müllerian ducts. No uterus was seen by genitogram, but there was a large utriculovaginal pouch. At 7 months of age, hearing loss was documented (see below) and physical examination revealed failure to thrive with height, weight and head circumference below the 5th centile, prominent metopic suture, bitemporal narrowing, prominent eyes, bilateral epicanthal folds, down-slanting palpebral fissures, prominent ears, and mild micrognathia with a high arched palate. Other features included a long philtrum, thin upper lip, left single palmar crease and mild 5th finger clinodactyly. At 15 months, the patient underwent left abdominal orchiectomy, right abdominal orchiopexy, and male reconstructive surgery of the external genitalia [Mardo et al., 2008]. The patient returned to our facility at 32 months for hypospadias repair. At that time, his metopic suture was more prominent and a head CT demonstrated metopic and sagittal craniosynostosis; anterior and middle cranial fossa expansion surgery was performed. Height, weight and head circumference remained symmetrically below the 5th centile. New features noted were a depressed nasal tip and crease in the inferior ridge of the columella, flat philtrum, bilateral and symmetric skin dimples at the superolateral scapular border, and hypotonia. Parents described generalized unsteadiness as delayed motor milestones were attained, particularly while reaching for objects and walking (achieved at 31 months). He had no words at the 32-month visit.

Similar to Patient 3, Patient 4 also had congenital sensorineural hearing loss first documented at seven months of age by the absence of otoacoustic emissions. Auditory brainstem response indicated bilateral sensorineural hearing loss (mild/moderate low frequency on the right and mild to severe up-sloping on the left). He was prescribed bilateral hearing aids at 15 months of age. Head CT revealed metopic and sagittal craniosynostosis as well as slightly dysplastic cochlea bilaterally with an enlarged vestibular chamber on the right and a slightly enlarged vestibular chamber on the left (Fig. 4B,C). This was determined by measuring the ratio of the transverse dimension of the membranous vestibule to the inner diameter of the lateral SCC (see Davidson et al. [1999] for analysis method).

In contrast to the previous three patients with terminal deletions, chromosome analysis of this patient revealed a hemizygous interstitial deletion of the long arm of chromosome 10 that was interpreted as 46, XY,del(10)(q25.2q26.11 or q25.3q26.13) in all metaphases examined (Fig. 1). While the precise break points could not be determined by cytogenetic analysis, FISH using BAC probe RP11-162A23 (124,795,486–124,978,916 bp) confirmed that the deletion included band 10q26.13. While the distal breakpoint of this interstitial deletion did not overlap the terminal deletions of Patients 1 and 2, it did overlap the proximal breakpoint of the larger terminal deletion in Patient 3 (Figs. 2 and 3). Chromosome analyses of the parents were normal.

SNP analysis confirmed the patient's interstitial deletion and indicated a 7.0 Mb deletion with physical coordinates approximately 118–125 Mb, refining the karyotype to be 46,XY,del(10)-(q25.3q26.13). This region encompassed 45, possibly 46 genes depending on where the exact breakpoint is located (Fig. 2, Table II, and see supporting information Tables III and V which may be found in the online version of this article). Twenty-one of these genes were in common with the hemizygous deletion in Patient 3, the other patient with an otologic pathology (Supplemental Table III). As with Patient 1, analysis of the family SNP data established that while the deletion was de novo, it involved the paternal chromosome (supporting information Fig. 1 may be found in the online version of this article). Additionally, four duplications and/or deletions were detected, all of which were inherited (supporting information Table II may be found in the online version of this article). One of these is a small deletion in the intron and possibly an exon of the *DMD* gene. Deletions in exons of this gene can cause Duchenne Muscular Dystrophy (DMD); however, a creatine kinase test was performed on this patient to rule out DMD and was normal.

DISCUSSION

In this study we mapped the breakpoints of four 10q hemizygous deletion patients to within 2.3–25.1 kb by SNP analysis (Figs. 2 and 3, Table II). Through the use of a UCSC Genome Browser BED file (supporting information Table VI may be found in the online version of this article), we displayed the deletions in our patients and selected patients from the literature on a map of chromosome 10 (Fig. 3). This approach established that Patients 1, 2, and 3 have 10q terminal hemizygous deletions of 6.1, 6.1, and 12.4 Mb respectively, thus sharing in common the smallest (6.1 Mb) deletion found in Patient 1 (see Fig. 2 and Table II). In addition, Patients 2 and 3 have a common, small (57 kb) deleted region, not shared with Patient 1 (see the region at position 129 Mb on Fig. 3A, marked ^a). The proximal end of the deletion present in Patient 3 was also found to overlap the distal end of the interstitial deletion of Patient 4 resulting in a common deletion of 2.5 Mb in these two individuals (Fig. 3 and Table II).

These four patients had a number of common clinical findings. They all shared delayed speech development and had strabismus. While these features have been reported as being common in other patients with deletions in 10q (Table III), the resolution of this study shows that their genetic causes are not in common. Because the hemizygous deletions of Patients 1 and 2 do not overlap with the hemizygous deletion of Patient 4, these findings likely have distinct genetic and environmental causes. The patients also had a number of unique clinical findings. With two entirely separate deletions (Patients 1 and 2 vs. Patient 4), the possibility exists of further refining a broad 10q phenotype into more specific deletion phenotypes, such as a 10q26.3 phenotype. Unfortunately, the few clear differences between these two genetically distinct groups (Table I) do not allow us to further refine a 10q phenotype. Three of the four patients (2, 3, and 4) were <10%ile for weight and height for their age and had some degree of hypotonia. Of note, Patient 4 was the only one of the two males in this group that had ambiguous genitalia. It is possible that a gene important in genital development is present in the 4.5 Mb deleted region at the centromeric end of 10q that is unique to this patient.

The common deleted region in Patients 3 and 4 is of particular interest as these two patients have congenital bilateral sensorineural hearing loss and abnormal balance. In Patient 3, the hearing loss is associated with bilateral inner ear malformations that were classified as Mondini malformations, though both the cochlear and vestibular organs were severely affected (Fig. 4A). For Patient 4, axial head CT images show evidence of abnormal vestibular enlargement (Fig. 4B,C), which often occurs in the setting of congenital hearing loss.

The wide-based gait and delayed development of milestones involved in balance, such as sitting, standing, and walking are clinically recognized features seen with vestibular loss or malfunction [Brown, 2005; Salem-Hartshorne and Jacob, 2005]. Thus, the absence or malformation of vestibular organs in the inner ear such as the SCCs have a profound impact upon the ability to stand up and walk comfortably. Additionally, we have identified four patients from the literature with wide-based gait, poor balance, or poor coordination out of 22 10q deletion patients that were examined for these physical features/findings (Table III). Additionally, seven of these patients were reported with delayed walking. We also note seven with hearing loss (Table III). These 22 patients are depicted in Figure 3 (see supporting information Table VI in the online version of this article for further information on displaying the data).

The hemizygous deleted 10q region shared by Patients 3 and 4 resulted in a single copy of 21 genes. These include two contiguous genes, H6 family homeobox 2 (*HMX2*) and H6 homeobox family 3 (*HMX3*), shown in null knockout mice to have key roles in cell fate determination and morphogenesis of vestibular components of the inner ear. These structures include the SCCs, utricle, saccule, and their sensory epithelia as well as the endolymphatic duct and sac.

The inner ear, which mediates both hearing and balance, develops from a placode of ectoderm known as the otic placode. *HMX2* and *HMX3* are coexpressed in the dorsolateral otic epithelium to temporally and spatially signal the embryonic transition of the pars superior of the otocyst to a fully developed vestibular system [Wang et al., 2001]. They are also expressed in the developing central nervous system, including the neural tube and hypothalamus [Wang et al., 2001].

In view of our patient's difficulties with balance, it is of note that Wang et al. [2004] reported complete loss of balance in *HMX2/3* null mice with progressive degeneration of the entire vestibular system among other phenotypes. Inner ear imaging revealed that *HMX2/3* double knockout mice had altered vestibular development starting from disoriented otic vesicle orientation to absence of SCCs, utricle-sacculus fusion, degeneration of the vestibule, and lack of normal sensory epithelium. Cochlea were found to be of normal shape with the normal three turns in these mice. Mice with heterozygous mutations in *HMX2* or *HMX3* were not found to have this vestibular phenotype and were described as normal.

We suggest that the phenotype of Patients 3 and 4 may represent a human correlate to the phenotypes described in *HMX2/3* knockout mice. The large 1 cm cyst replacing the vestibule in our Patient 3 correlates with utricle-sacculus fusion seen in the knockout mice (Fig. 4A). Additionally, the enlarged abnormally shaped vestibule in Patient 3 parallels the degenerate vestibules seen in *HMX2/3* knockout mice. The human phenotype of dilated, bilateral SCC abnormalities along with the absence of left horizontal and posterior SCCs is comparable to the phenotype of absent SCCs in *HMX2/3* knockout mice. Patient 4 also has abnormally large vestibules bilaterally (Fig. 4B,C).

A third gene in the common hemizygous deleted region of Patients 3 and 4 is fibroblast growth factor receptor 2 (*FGFR2*). Fibroblast growth factors (FGF) and fibroblast growth factor receptors (FGFR) have a vital function in the osteogenesis of long and calvarial bones [Ornitz and Marie, 2002]. In humans, "gain of function" point mutations in *FGFR2* are known to induce craniosynostosis (i.e., premature fusion of cranial sutures [Yang et al., 2008]). In mice, the *FGFR2* knockout is lethal and the hemizygous model is apparently normal. Patient 4 had metopic and sagittal craniosynostosis which was corrected surgically. This is the first report of a hemizygous deletion of *FGFR2* being present in a patient with craniosynostosis. Based on the established gain of function mechanism for point mutations in *FGFR2* and lethality in the mouse knockout, the relationship between *FGFR2* hemizygosity in humans and craniosynostosis is unclear. FGF are also known signals of induction in the otic placode [Ohyama et al., 2007]. *FGFR2* mice knockouts also have various organ abnormalities including small otic vesicles (<http://www.informatics.jax.org>), a phenotype related to the vestibular defects in Patients 3 and 4.

The deletion in Patient 4, but not Patient 3, results in hemizygosity of the transcription factor empty spiracles homeobox 2 (*EMX2*). *EMX2* has been implicated, along with orthodenticle homeobox 2 (*OTX2*) (chr14q), in anterior-posterior position signaling in the forebrain [Suda et al., 2001]. *OTX2* helps to determine cell fate during otic invagination. In mutant mouse investigations, *EMX2* has been reported as necessary for the development of structures in the middle ear and the development and organization of the sensory hair cells of the cochlea. Null mice with *EMX2* mutations were found to have sensorineural hearing loss [Rhodes et al., 2003]. Additionally, *EMX2* is necessary for mammalian urogenital tract development [Troy et al., 2003]. The hemizygous deletion of *EMX2* in Patient 4, discussed further in Mardo et al. [2008], could account for the genito-urinary pathology in that patient.

In conclusion, detailed analyses of the breakpoints in four chromosome 10q deletions patients permit a description of genes that are hemizygous within the region. The 2.5 Mb common

deletion region (10q26.12 to 10q26.13) in Patients 3 and 4 is associated with inner ear malformation, vestibular dysfunction, and hearing loss in two of our patients with congenital sensorineural hearing loss. In light of these findings, *HMX2* and *HMX3* are strong candidate genes for human inner ear vestibular development and have a role in sensorineural hearing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

Karyograms of the 10q deletions. Partial karyograms showing normal and deleted chromosomes of three patients (1, 2, and 3) with terminal deletions and Patient 4 with an overlapping interstitial deletion of the long arm of chromosome 10. ^aThe karyogram for Patient 4 indicated a possible karyotype of del(10)(q25.2q26.11 or q26.11q26.13); analysis with SNP microarrays refined the karyotype to del(10)(q25.3q26.13).

FIG. 2.

10q Deletions in BeadStudio. Analysis of genotype and copy number data for four 10q deletion patients based on SNP genotyping. Results are shown for Patients 1–4 using BeadStudio software (Illumina). For each patient, results are shown for log R ratio (a measure of chromosomal copy number; y-axis) versus genomic position on chromosome 10. A horizontal smoothing line indicates that the copy number for each is at euploid levels across most of the chromosome but is present in one copy in the deletion region (oval). The B allele frequency panel indicates the presence of BB genotype calls (B allele frequency of 1.0), AB calls (frequency of 0.5), and AA calls (frequency of 0.0); in the deletion region (oval) only homozygous calls are evident. An ideogram illustrates chromosome 10, and the lower panels

for each patient provide a detailed view of each deletion region. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

FIG. 3. Overview of 10q deletion patients and hearing loss. Deletions of 10q for each patient are plotted. Thick bars indicate the minimum deletion. Thin bars indicate the maximum deletion. Diagonal lines (in two of the cases) indicate conductive hearing loss; diamond patterns (in six of the cases) indicate sensorineural hearing loss; and solid gray (in the remaining cases) indicates no hearing loss. A: Positions of the four deletions presented in this paper. B: Patients from the literature that have a pure 10q deletion and report on status of hearing loss. The row labels refer to the first author of the relevant publication and the patient number therein. ^aThe deletion in Patient 2 is 57 kb larger than in Patient 1. ^bIrving, Patients 1–6 are familial patients and thus depicted together [Teyssier et al., 1992; Petersen et al., 1998; Leonard et al., 1999; Tanabe et al., 1999]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

FIG. 4.

CT imaging of inner ear abnormalities, Patients 3 and 4. CT images showing the inner ear abnormalities in Patients 3 and 4. A: Axial CT of Patient 3 at the level of the inner ear bilaterally. Right: Abnormally enlarged cystic vestibule (asterisk) and mildly prominent vestibular aqueduct (white arrow) are shown. Left: Abnormally large 1 cm cyst replacing the vestibule (asterisk), and small, narrowed IAC (black arrow) are shown. B: Axial CT of Patient 4 at the level of the right inner ear. The ratio of the transverse dimension of membranous vestibule (white arrow) to the inner diameter of the lateral SCC (black arrow) is increased. Bracket ({}) indicates slightly dysplastic cochlea. C: Axial CT of Patient 4 at the level of the left inner ear. The ratio of the transverse dimension of membranous vestibule to the inner diameter of the

lateral SCC is slightly increased (arrows same as in panel B). Bracket ({} indicates slightly dysplastic cochlea. Abbreviations: L, patient's left; R, patient's right.

TABLE I

Clinical Findings

Clinical feature	Patient 1	Patient 2	Patient 3	Patient 4
Karyotype	4G,XXdel(10)(q26.2)	4G,XXdel(10)(q26.2)	4G,XYdel(10)(q26.12)	4G,XY,del(10)(q25.2q26.11 OR q26.11q26.13
Age at last visit	5 years	14 months	4.5 years	32 months
Weight	10–25th%ile	10th%ile	<3rd%ile	<3rd%ile
Height	25–50th%ile	<3rd%ile	<3rd%ile	<3rd%ile
Head circumference	50th%ile	50th%ile	25th%ile	<3rd%ile
Frontal bossing	+	–	–	+
Brachycephaly	–	–	+	–
Prominent forehead	+	–	+	+
Craniosynostosis	ND	ND	–	+, Metopic, sagittal
Widow's peak	+	–	–	–
Hypotelorism	–	–	+	–
Strabismus	+	+, BL	+	+
Stellate irides	+, BL	–	–	–
Broad nasal bridge	ND	–	+	+
Prominent nose	ND	–	+	–
Abnormal columella	ND	Recessed	Long	Crease inferior ridge
Flat philtrum	ND	–	–	+
High arched palate	–	–	–	+
Micrognathia	–	–	–	+
Hearing loss	–	–	+, BL SN	+, BL SN
Temporal bone anomaly	ND	ND	+, BL, Mondini	+, BL vestibule enlarged
Heart anomalies	ND	ND	Small VSD	–
Kidney anomalies	ND	ND	BL hydronephrosis	–
Genital anomalies	–	–	Unilateral undesc. testicle	Ambiguous genitalia
Single transverse palmar crease	–	–	ND	+, Unilateral
Tapering fingers	–	+	ND	–
5th finger clinodactyly	+	–	+	–
Pes planus	ND	ND	+	ND
Overlapping toes	ND	ND	+	ND
Hypoplastic toe nails	ND	ND	+	ND
Feeding difficulties	–	ND	+	–
Hypotonia	–	+, Axial	+, Global	+, Global
Gait	Mild unsteadiness	NA	Wide-based	Wide-based
Age at walking	20 months	Standing at 14 months	4 years ? months	31 months
Age at first words	3 years	None at 14 months	No words at 3 years	One word at 15 months
Cognitive level	Mild MR	NA	Mod to severe MR	Mod MR
Mother's karyotype	NL	NL	NA	NL
Father's karyotype	NL	NL	NA	NL
Other normal tests	FXs, PAA, UOA, Williams FISH	UOA, PAA, T4, TSH	PAA, UOA, T4, TSH	FISH SRY+, NL subtelomeres, CK

Clinical features of the four patients. BL, bilateral; ND, not described; NL, normal; NA, not assessed, MR, mental retardation; SN, sensorineural; VSD, ventriculo-septal defect; uni, unilateral; SRY, sex-determining region Y; T4, thyroxine-4; TSH, thyroid stimulating hormone; UOA, urine organic acids; PAA, plasma amino acids; FISH fluorescence in situ hybridization; CK creatine phosphokinase.

TABLE II
Breakpoints of the 10q Deletions Determined by SNP Microarray

Patient	10q Deletion	Normal SNPs		Aberrant SNPs		Min. size of deletion	Number of altered SNPs	RefSeq genes
		Proximal Location	Distal Location	Proximal Location	Distal Location			
1	q26.2	129,325,323	Not present ^a	129,327,663	135,323,184	6,085,966	1,583	37
2	q26.2	129,263,981	Not present ^a	129,270,851	135,323,184	6,142,778	1,605	37
3	q26.12	122,956,563	Not present ^a	122,960,206	135,323,184	12,453,423	3,418	78
4	q25.3q26.13	118,483,053	125,493,454	118,496,745	125,482,095	6,985,351	1,773	45

The physical locations are specified in base pairs and based on the May 2004 assembly of the human genome.

^a Distal normal SNPs were not present because the deletions were terminal (as determined by subtelomeric fish).

TABLE III

Literature Review of Vestibular-Related Findings in Selected Cohort of 10q Deletion Patients

Patient information			Deletion characteristics				Symptomatology			
Refs.	Patient #	Sex	10q deletion	cent-q26.12	26.12–26.13	26.13-ter	Hearing loss	Vestib. disfxn	Strab ismus	Speech/language
This study	1	F	26.2ter	D	D	HZ	–	MU	+	SD
This study	2	F	26.2ter	D	D	HZ	–	DW	+	SD
This study	3	M	26.12ter	D	HZ	HZ	SNHL	DW, WBG	+	SD
This study	4	M	25.3–26.13	HZ	HZ	D	SNHL	DW, WBG	–	SD
McCandless et al. [2000]	1	M	25.1–25.3	HZ	D	D	SNHL	NT	–	NS
Irving et al. [2003]	13	F	25.2–26.1	HZ	U	D	–		–	SD
Irving et al. [2003]	12	F	25.2–26.1	HZ	U	D	SNHL	PC	+	SD
Petersen et al. [1998]	1	F	25.3-ter	HZ	HZ	HZ	SNHL	WBG	+	SD
Irving et al. [2003]	15	F	26.1ter	U	HZ	HZ	–	NT	–	
Irving et al. [2003]	14	M	26.13–26.3	D	HZ	HZ	CHL	DW	+	SD
Wulfsberg et al. [1989]	1	F	26.1	D	U	HZ	SNHL	NT	+	
Teyssier et al. [1992]	1	M	26	U	HZ	HZ	SNHL	NT	–	
Tanabe et al. [1999]	1	M	26.11	D	HZ	HZ	–	NT	+	SD
Leonard et al. [1999]	1	M	26.1-ter	D	U	HZ	–	WBG	+	SD
Irving et al. [2003]	11	F	26.1ter	U	HZ	HZ	–	DW	–	SD
Joshi et al. [2006]	1	M	26.13	D	U	HZ	CHL		+	
Courtens et al. [2006]	1	F	26.2	D	D	U	–	DW	+	SD
Irving et al. [2003]	9	F	26.2ter	D	D	HZ	–	PB	+	Normal
Irving et al. [2003]	8	F	26.2ter	D	D	HZ	–	DW	–	SD

Refs.	Patient information		Deletion characteristics				Symptomatology			
	Patient #	Sex	10q deletion	cent-q26.12	26.12-26.13	26.13-ter	Hearing loss	Vestib. disfxn	Strabismus	Speech/language
Irving et al. [2003]	7	F	26.2ter	D	D	HZ	-	-	-	Normal
Irving et al. [2003]	6	F	26.2ter	D	D	HZ	-	-	+	SD
Irving et al. [2003]	5	F	26.2ter	D	D	HZ	-	-	-	SD
Irving et al. [2003]	4	F	26.2ter	D	D	HZ	-	DW	-	SD
Irving et al. [2003]	3	F	26.2ter	D	D	HZ	-	DW	+	Normal
Irving et al. [2003]	2	F	26.2ter	D	D	HZ	-	DW	-	Normal
Irving et al. [2003]	1	F	26.2ter	D	D	HZ	-	-	+	Normal

The patient information refers to the first author of the relevant publication and the patient number therein. F, female; M, male; HZ, heterozygous; U, uncertain; D, diploid; SNHL, sensorineural hearing loss; NT, not tested; MU, mildly unstable; DW, delayed walking; PC, poor coordination; PB, poor balance; WBG, wide-based gait; SD, speech delay; AS, absent speech; CHL, conductive hearing loss; +, symptom present; -, symptom absent.