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X-linked Deafness-2 (DFNX2) Phenotype Associated with a Paracentric Inversion Upstream of *POU3F4*

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Abstract

Purpose—The authors report a seven-year-old male, designated FR, with severe sensorineural hearing loss. Features include round face, hypertelorism, epicanthal folds, and flat nasal root. Although there were early developmental concerns, all but his speech delay resolved when he was placed in an educational program that accommodated his hearing loss. To investigate genetic causes for his hearing loss, genetic studies were performed.

Methods—History, physical examination, audiologic assessment, and imaging were performed according to usual practice. *FMR1*, *GJB2*, *GJB6*, and *POU3F4* genes were sequenced. Chromosomal studies consisted of karyotyping and breakpoint analysis by fluorescence in situ hybridization (FISH).

Results—Results from *FMR1*, *GJB2*, *GJB6*, and *POU3F4* sequencing and echocardiography, ECG, and abdominal ultrasound were normal. CT revealed a large fundus of the internal auditory canals and absence of the bony partition between the fundus and the adjacent cochlear turns with a widened modiolus bilaterally. CT findings are consistent with those described in persons with DFNX2 hereditary deafness. His karyotype was 46,inv(X)(q13q24),Y,ish inv(X)(XIST+)mat. FISH refined the breakpoints to inv(X)(q21.1q22.3). The Xq21.1 breakpoint was narrowed to a 25 kb region 450 kb centromeric to the DFNX2 gene, *POU3F4*. There are rare case reports of DFNX2 patients with chromosomal rearrangements positioned centromeric to *POU3F4* and no mutations within the gene.

Conclusions—We hypothesize that FR's hearing loss is caused by dysregulation of *POU3F4* due to separation from regulatory elements affected by the inversion.

Keywords

DFNX2; hereditary deafness; sensorineural hearing loss; *POU3F4*

Introduction

We report a seven-year-old Caucasian male patient from Ontario, Canada, with a history of severe bilateral sensorineural hearing loss. He was delivered at 41 weeks gestation with a birth weight of 3.155 kg after an uncomplicated pregnancy. There were no prenatal or perinatal risk factors for hearing loss, including evidence of congenital infection, hypoxic insult, jaundice, or exposure to ototoxic drugs. The child was designated FR.

His mother noted an absence of response to sounds at two months of age, which became more conspicuous with age. At 8 months of age, auditory brainstem response (ABR) test results indicated 50 dB null in the left ear and 70 dB null in the right ear. These results were consistent with mild to moderate hearing difficulty and moderate to moderate-severe hearing loss, respectively. Distortion-product otoacoustic emission (DPOAE) test emissions were poor across all frequencies. At 1 year and 1 month of age, ABR test results were 60 dB null in the left ear and 70 dB in the right ear. At 2 years of age, audiogram results indicated a moderate-severe to severe mixed hearing loss in at least one ear. Also at two years of age, myringotomy tubes were inserted due to recurrent episodes of otitis media that complicated his clinical course. This did not result in hearing improvement. At 2 years and eight months of age, audiogram results indicated moderately severe to possibly flat hearing loss in the left ear. Only one response could be obtained in the right ear and was suggestive of a possible severe hearing loss at 2000 Hz. At 3 years and 11 months of age, hearing assessment using play audiometry indicated a severe, essentially flat hearing loss bilaterally. Bone conduction threshold suggested the hearing loss had a conductive component. Eardrum mobility was within normal limits. The results of pure tone audiometry conducted at 4 years and 8 months are presented in figure 1. By 5 years and 8 months of age, FR presented with profound, broad, U-shaped (cookie bite) hearing loss bilaterally. Immittance measurements indicated normal eardrum mobility and middle ear pressure.

He had no visual, cardiac, or renal abnormalities, no significant illnesses, and was not taking any chronic medications. The child's height, weight, and occipital-frontal circumference were well above the 97th percentile at six years of age. His ECG revealed an incomplete right bundle branch block. Other features included macrocephaly, round face, hypertelorism, epicanthal folds, flat nasal root, downturned corners of the mouth, ridged metopic suture, and simple helix of the left ear (Fig. 2). There were no additional external otic anomalies.

A CT scan of the head revealed a large fundus of the internal auditory canals and absence of the bony partition between the fundus and the adjacent cochlear turns with a widened modiolus bilaterally. He was, therefore, at risk for a fistulous communication between the subarachnoid space and inner ear, which could result in a perilymphatic gusher upon stapes manipulation.

During an initial physical examination, some neurodevelopmental issues were noted, including mild incoordination, hypotonia, and impaired social interaction. Incoordination and hypotonia resolved over time but there was no specific therapy utilized for this. The child was enrolled in a school for the deaf that provided education in ASL. As the child began to communicate with ASL, the teachers determined that his cognitive development

was in the normal range although his speech remained delayed. Early concerns about attention resolved over time and were attributed to his difficulties with communication.

His parents were not related and there was no family history of hearing impairment, blindness, or developmental delay. His mother was healthy except for short stature, relative to familial heights. A maternal aunt died at 21 days of age with an unspecified cardiac defect and cleft lip.

To determine if there was a genetic basis for this child's phenotype, chromosomal studies were performed.

Materials and Methods

Karyotyping

Metaphase chromosome preparations were obtained from phytohemagglutinin stimulated lymphocytes cultured for 72-96 hours in RPMI 1640 growth medium supplemented with fetal bovine serum (Gibco). Mid-resolution chromosomes were obtained by adding 5-bromo-2'-deoxyuridine (BrdU), at a final concentration of 0.2 mg/mL, to each culture 16-17 hours before thymidine release (Sigma-Aldrich). The cultures were incubated an additional 5 hours prior to colcemid treatment and harvested using standard cytogenetic procedures. Chromosomes were stained using standard GTG staining.

Fluorescence in situ hybridization (FISH)

The presence of the X-inactivation locus (XIST), localized to chromosome band Xq13, was confirmed by fluorescence in situ hybridization (FISH) analysis using the XIST probe (ONCOR, Gaithersburg, MD, USA). FISH is a cytogenetic technique that allows for specific DNA sequences to be localized on chromosomes. Further FISH experiments were performed by the Developmental Genome Anatomy Project (DGAP) group, using 17 BAC probes localized to Xq21.1 and 10 BAC probes localized within Xq22.3, to define better the breakpoints (Table 1). Genomic locations of BAC probes were confirmed using eFISH, an electronic database of genomic variants (<http://projects.tcag.ca/cgi-bin/efish/index.cgi>). The child was designated FR.

POU3F4 sequencing

POU3F4 is a gene that is located on the X-chromosome and mutations in and around this gene have been linked to deafness (Bitner-Glindzicz et al., 1995; de Kok, Merks, et al., 1995; Friedman et al., 1997; Hagiwara, Tamagawa, Kitamura, & Kodera, 1998; Vore et al., 2005; Wang et al., 2006). Sequencing of the *POU3F4* coding sequence and surrounding DNA was performed at the University of Iowa's Department of Otolaryngology.

Additional genetic testing

Fragile X testing (*FMRI* sequencing) and *GJB2/GJB6* sequencing were performed using standard molecular techniques.

Results

FR was found to have a male karyotype, with a paracentric inversion of the long arm of the X chromosome. Paracentric inversions are chromosomal rearrangements that avoid the centromere and occur in the chromosome arm. Breakpoints for the inversion were initially estimated by G-banding analysis to involve q13q24. The XIST locus, which is a gene on the X chromosome and a major effector of the X inactivation process, was examined by FISH in order to determine if it were affected by this inversion and no abnormality was noted. Breakpoints were subsequently localized to Xq21.1 and Xq22.3 by FISH (Fig. 3). Sequencing of *POU3F4*, which is located near these breakpoints, as well as approximately 1200 kilobases (kb) of upstream genomic DNA, failed to identify mutations or deletions.

Cytogenetic analysis of FR's mother found that she had mosaicism consisting of 19/30 cells with the 46,inv(X)(q21.1q22.3),X karyotype and 11/30 cells with a 45,X karyotype. The maternal grandparents had normal karyotypes, indicating that the inversion arose *de novo* in FR's mother.

Fragile X testing revealed 19 CGG repeats in FR, which is within the normal range. No mutations were detected in the coding region of *GJB2* or *GJB6*.

Discussion

Hearing loss occurs in approximately 1 in 1000 infants (Friedman, et al., 1997). Fifty percent of these cases are inherited, either as a non-syndromic isolated trait or as part of a cluster of congenital anomalies. Approximately 80% of inherited cases are autosomal recessive (*e.g.*, largely *GJB2* mutations), 18% autosomal dominant, and 2% X-linked.

Although the breakpoints of the inv(X) did not disrupt any known genes, there were several genes identified in the vicinity. The most intriguing candidate near the breakpoints was *POU3F4* (also known as brain-4). *POU3F4* is associated with an X-linked deafness phenotype designated DFNX2 by Petersen and colleagues in 2008 (Petersen, Wang, & Willems, 2008), formerly known as DFN3 (de Kok, van der Maarel, et al., 1995). The Xq21.1 breakpoint was approximately 450 kb proximal to this gene. The DFNX2 phenotype is associated with characteristic anatomical features of the middle and inner ear (Friedman, et al., 1997). These abnormal features include: dilation of the internal acoustic canal, an abnormally wide communication between the internal and acoustic canal and inner ear, a small cochlea, a reduced diameter of the semicircular canals, and an abnormality of the cochlear modiolus resulting in communication between the subarachnoid and perilymphatic spaces. These anatomic features are unique and help define the DFNX2 disorder in a patient with isolated hearing loss. A CT scan of our patient's head showed this unique configuration of the acoustic structures, which strongly supports a diagnosis of DFNX2 in FR.

The typical features of DFNX2 consist of bilateral, progressive, mixed hearing loss affecting all frequencies, as was seen with FR, and anatomical abnormalities, also as seen with FR. Although most patients with DFNX2 demonstrate mixed hearing loss, there have been descriptions of isolated sensorineural hearing loss (Friedman, et al., 1997). The anatomical anomalies found in patients with DFNX2 predispose them to developing perilymphatic

gushers following stapedectomy surgery (Cremers, 1985; Cremers & Huygen, 1983). In fact, diagnosis of DFNX2 is often made following the development of a perilymphatic gusher during surgery to ameliorate the conductive component of the deafness (Cremers, 1985). Additionally, there have been some reports of vestibular dysfunction and motor delay with DFNX2, which correlate with the early presentation seen in our patient (Vore, et al., 2005). The overall clinical presentation of our patient is highly consistent with DFNX2, indicating that the phenotype is due to inactivity of *POU3F4*.

POU3F4 encodes a transcription factor that binds DNA through two specific subdomains: a POU-specific domain and a POU homeodomain. At least five POU domain genes are expressed in the developing inner ear (Ryan, Crenshaw, & Simmons, 1991). Murine and rat gene homologues to *POU3F4* are expressed in the brain, neural tube, and otic vesicles during embryonic development (Douville, Atanasoski, Tobler, Fontana, & Schwab, 1994; Friedman, et al., 1997; Hagiwara, et al., 1998; LeMoine & Young, 1992). Disruption of *POU3F4* was first proposed as the genetic etiology for DFNX2 in 1995, when several mutations within this gene were identified in affected individuals (de Kok, van der Maarel, et al., 1995). Since 1995, several more mutations located within the gene have been described (Bitner-Glindzicz, et al., 1995; Friedman, et al., 1997; Hagiwara, et al., 1998; Vore, et al., 2005; Wang, et al., 2006). These mutations primarily affect the POU domain and are believed to disrupt DNA binding ability.

In addition to reports of mutations within *POU3F4*, there have been descriptions of individuals with DFNX2 who have an abnormality positioned upstream, but not within, *POU3F4* (de Kok, Merkx, et al., 1995; de Kok et al., 1996; Song et al., 2010; Vore, et al., 2005). One case of particular relevance to FR is that of de Kok and colleagues (de Kok, Merkx, et al., 1995). This group described a patient with DFNX2 associated with a duplication and paracentric inversion affecting the X chromosome with a breakpoint positioned approximately 400 kb upstream of *POU3F4*. Several explanations were offered to account for the appearance of the DFNX2 phenotype in association with upstream chromosomal rearrangements, including transcriptional silencing due to a positional effect. de Kok and colleagues concluded that the DFNX2 features were due to separation of *POU3F4* from an upstream enhancer element. This conclusion has been further supported by the finding of additional patients with alterations of the DNA sequence proximal to *POU3F4* (de Kok, Merkx, et al., 1995; Song, et al., 2010; Vore, et al., 2005). One group reported a deletion involving a segment 530 kb upstream (Vore, et al., 2005) and another reported a deletion 90 kb upstream (Song, et al., 2010) of *POU3F4*. There is also a report of six patients with microdeletions in a small region 900 kb upstream of *POU3F4* (de Kok, et al., 1996).

FR had severe sensorineural hearing loss and an X chromosome inversion of unknown clinical significance. Normal hearing in the mother is consistent with DFNX2, as it is an X-linked recessive disorder. Furthermore, FR has the classic anatomical anomalies associated with the X-linked nonsyndromic deafness condition DFNX2, also known as DFN3. The typical phenotype and the close spatial association between *POU3F4* and the proximal breakpoint of the inversion strongly suggest that the inversion is responsible for the deafness in FR.

This case adds further support to the previously reported hypothesis that the region upstream of *POU3F4* plays an important role in *POU3F4* transcriptional regulation. We suggest that, in patients with a typical DFNX2 presentation, the upstream sequence should be evaluated in addition to the coding sequence of *POU3F4*. Identification of individuals with DFNX2 prior to surgery has the potential to optimize clinical management, including increasing awareness of the risk for a perilymphatic gusher and providing appropriate genetic counseling.

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Fig. 1. Results of pure tone audiometry for FR at 4 years and 8 months of age
Audiogram results indicated broad hearing loss bilaterally. **A)** Audiogram for right ear. **B)** Audiogram for left ear.

Fig. 2. Front and lateral views of FR at 1 year and 4 months of age

Facial features include macrocephaly, round face, hypertelorism, epicanthal folds, flat nasal root, downturned corners of the mouth, ridged metopic suture, and simple helix of the left ear. Other features included clinodactyly of the fifth fingers bilaterally and bridged palmar creases (not shown).

Fig. 3. FISH analysis

A) Ideogram of the X chromosome with breakpoint regions at Xq22.1 and Xq22.3 indicated by red lines. The *POU3F4* locus is depicted to the right of the Xq21.1 breakpoint region. **B)** FISH results for BAC RP11-842n12. This BAC probe maps to Xq21.1 and shows a split hybridization signal indicating that the Xq21.1 breakpoint occurs within the sequence of this BAC. **C)** FISH results for BAC RP11-105f23. This BAC probe maps to Xq22.3 and shows a split hybridization signal indicating that the Xq22.3 breakpoint occurs within the sequence of this BAC.

Table 1

Results of the FISH experiments performed to define the inv(X) breakpoints.

BAC	FISH Result	Base position on ChrX [*]
Xq21.1 BACs		
RP13-216e22	Centromeric	73,036,433-73,204,100
RP11-141m9	Centromeric	79,611,117-79,760,003
RP11-54m14	Centromeric	81,727,122-81,893,907
RP11-2d7	Centromeric	81,874,722-82,036,214
CTD-2369k7	Centromeric	82,008,341-82,100,965
RP11-359h11	Centromeric	82,045,266-82,243,535
CTD-3155e14	Centromeric	82,243,552-82,284,802
RP11-842n12	Splits	82,214,877-82,402,106
CTD-2314c18	Splits	82,253,439-82,334,072
RP11-453c8	Splits	82,262,442-82,428,935
RP11-58h8	Telomeric	82,309,990-82,499,466
RP11-125n23	Telomeric	82,791,281-82,945,939
RP11-703i22	Telomeric	83,391,052-83,553,536
RP11-70i5	Telomeric	83,888,924-84,042,951
RP11-33m9	Telomeric	84,236,945-84,413,546
RP11-246g22	Telomeric	82,736,909-82,910,526
RP11-223k4	Telomeric	102,731,932-102,782,375
Xq22.3 BACs		
CTD-2237j10	Centromeric	105,700,173-105,779,967
RP11-575k24	Centromeric	105,635,707-105,787,175
CTD-3015p22	Centromeric	108,058,035-108,136,325
RP11-199f23	Splits	108,056,941-108,220,427
RP11-105f23	Splits	108,088,637-108,232,952
CTD-2116f15	Telomeric	108,120,765-108,277,184
RP11-471a8	Telomeric	109,216,071-109,373,232
RP11-1112f19	Telomeric	109,252,061-109,413,393
RP11-79n19	Telomeric	110,820,409-110,970,563
RP11-22c20	Telomeric	120,927,280-121,076,573

* Based on February 2009 genome assembly (GRCh37/hg19).