MYO1F as a Candidate Gene for Nonsyndromic Deafness, DFNB15

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Background: Earlier studies have mapped the autosomal recessive nonsyndromic deafness locus, DFNB15, to chromosomes 3q21.3-q25.2 and 19p13.3-13.1, identifying one of these chromosomal regions (or possibly both) as the site of a deafness-causing gene. Mutations in unconventional myosins cause deafness in mice and humans. One unconventional myosin, myosin 1F (MYO1F), is expressed in the cochlea and maps to chromosome 19p13.3-13.2.

Objective: To evaluate MYO1F as a candidate gene for deafness at the DFNB15 locus by determining its genomic structure and screening each exon for deafness-causing mutations to identify possible allele variants of MYO1F segregating in the DFNB15 family.

Methods: We used radiation hybrid mapping to localize MYO1F on chromosome arm 19p. We next determined its genomic structure using multiple long-range polymerase chain reaction experiments. Using these data, we completed mutation screening using single-stranded conformational polymorphism analysis and direct sequencing of affected and nonaffected persons in the original DFNB15 family.

Results: Radiation hybrid mapping placed MYO1F in the DFNB15 interval, establishing it as a positional candidate gene. Its genomic structure consists of 24 coding exons. No mutations or genomic rearrangements were found in the original DFNB15 family, making it unlikely that MYO1F is the disease-causing gene in this kindred.

Conclusions: Although we did not find MYO1F allele variants in one family with autosomal recessive nonsyndromic hearing loss, the gene remains an excellent candidate for hereditary hearing impairment. Given its wide tissue expression, MYO1F might cause syndromic deafness.


Phenotypic traits are determined by the inheritance of genes. Diseases caused by single genes (monogenic) that segregate in a family may be localized to a specific chromosome by linkage analysis (see Ott,1 Jorde et al,2 Terwillinger and Ott,3 and Conneally and Rivas4 for a discussion of linkage analysis). However, because the linked chromosomal region will contain many genes, identification of the specific disease-causing gene can be difficult and time-consuming. Prioritizing genes in the linked interval for further study is facilitated if their function is known. This approach, the selection of a gene for in-depth analysis based on its chromosomal location and purported function, is known as the “positional candidate gene” approach to gene identification.

In general, if complementary DNA (cDNA) is available and the cDNA sequence of a candidate gene is known, rapid mutation screening is possible. However, because RNA is unstable and the presence of a given gene depends on the RNA source, cDNA screening is not always possible. Genomic DNA has the benefit of being much easier to isolate and very stable; however, mutation screening requires knowledge of the candidate gene’s genomic structure.

In an earlier study, Chen et al5 used a small consanguineous family from India in which several children had prilongual autosomal recessive nonsyndromic hearing loss to map the DFNB15 locus to chromosomes 3q21.3-q25.2 and 19p13.3-13.1 (Figure 1). Because the gene responsible for deafness at this locus has not been identified, we have been using a candidate gene approach to select genes for mutation screening. One interesting candidate is MYO1F, an unconventional myosin that is expressed in the cochlea and maps to chromosome 19p13.3-13.2.6,7 Unconventional myosins play a variety of

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MATERIALS AND METHODS

MAPPING OF MYO1F

To verify that MYO1F is a positional DFNB15 candidate gene, we used primer pairs that amplified exons 6, 13, and 21 and a radiation hybrid chromosome mapping panel (Genebridge 4; Research Genetics, Huntsville, Ala.) to place MYO1F on the framework map (Table). Data were analyzed using RHMAPPER, provided by the Whitehead Institute for Biomedical Research/MIT Center for Genome Research, Cambridge, Mass.

5’ AND 3’ RAPID AMPLIFICATION OF cDNA ENDS

We next determined the complete cDNA sequence of MYO1F using 5’ and 3’ rapid amplification of cDNA ends (RACE) using the Marathon RACE Kit and, as template DNA, a fetal brain Marathon-ready cDNA library (Clontech Laboratories Inc, Palo Alto, Calif).

DETERMINATION OF INTRON-EXON BOUNDARIES

To determine the genomic structure of MYO1F, we used exon-specific primers designed from the MYO1F cDNA sequence (GenBank accession numbers X98411 and U57053). Possible splice sites were determined by homology to consensus splice sites and by modeling the genomic structure of MYO1F. Polymerase chain reaction (PCR) amplified products longer than predicted by the cDNA sequence were assumed to include intron sequence.

Polymerase chain reactions were performed in a 25-µL reaction mixture containing 50 ng of genomic DNA, 2.5 µL of 10× PCR buffer (Bioline USA Inc, Reno, Nev), 2.25 µL of 50mM magnesium chloride (Bioline), 0.4 U of Biolase polymerase (Bioline); 1 µL of 50% glycerol; 0.9 µL of 30mM magnesium chloride (Bioline); 0.4 U of Biolase polymerase (Bioline); 10 pmol of forward and reverse primers; 0.12 µL of [35S]deoxyadenosine triphosphate ([35S]dATP); 200 nmol each of deoxyguanosine triphosphate ([35S]dGTP), deoxythymidine triphosphate ([35S]dTTP), and deoxycytidine triphosphate ([35S]dCTP); 2 nmol of cold dATP; and enough sterile double-distilled water to bring the volume to 10 µL. Each reaction was overlaid with mineral oil. Amplification was carried out for 44 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 35 seconds. Each labeled PCR reaction was mixed with 2 µL of formamide loading dye (99% formamide, 20mM EDTA, 0.03% bromophenyl blue, and 0.05% xylene cyanol). Samples were denatured for 5 minutes at 94°C and electrophoresed at 20 W on a 6% polyacrylamide gel (49:1 acrylamide:bis, 5% glycerol, and 1× Tris-EDTA (TE)). Total electrophoresis time was proportional to product size. Constant temperature was maintained with a cooling fan. Gels were transferred to 3M Whatman paper (Whatman International, Maidstone, England) and dried. Kodak X-OMAT film (Kodak, Rochester, NY) was used for autoradiography. Band shifts were assessed by visual inspection. Sequence data were compared with published cDNA sequences for MYO1F using a software package (Sequencer 3.1; Gene Codes Corp Inc, Ann Arbor, Mich).

RESULTS

Using the Genebridge 4 radiation hybrid screening panel (Research Genetics), we mapped MYO1F to chromosome 19p13.3-13.2 within the interval flanked by D19S216 and D19S221 (Figure 2). Mapping data were concordant for primer pairs that amplified exons 6, 13, and 21 (Table).

The complete coding sequence of mouse Myo1f, as well as the 5’ and part of the 3’ untranslated regions performed in 50-µL reactions using the Takara Long Range PCR Kit (LA PCR Kit; Takara Shuzo Co, available through Panvera Corporation, Madison, Wis), with 1.0 µg of genomic DNA as a template. Conditions of PCR were described in the Takara touch down PCR directions. Products of PCR were purified from 1% agarose gels using Amicon Ultrafree-DNA spin columns (Millipore Corporation, Bedford, Mass). Each PCR product was bidirectionally sequenced by dye primer using an automated sequencer (model 373; Applied Biosystems, Norwalk, Conn).

MUTATION SCREENING

We screened the entire MYO1F coding region and splice sites using single-stranded conformational polymorphism (SSCP) and direct sequencing in the DFNB15 family. Labeled PCRs for SSCP analysis were performed in a 10-µL reaction mixture containing 20 ng of genomic DNA; 1 µL of 10× PCR buffer (Bioline); 1 µL of 50% glycerol; 0.9 µL of 30mM magnesium chloride (Bioline); 0.4 U of Biolase polymerase (Bioline); 10 pmol of forward and reverse primers; 0.12 µL of [35S]deoxyadenosine triphosphate ([35S]dATP); 200 nmol each of deoxyguanosine triphosphate ([35S]dGTP), deoxythymidine triphosphate ([35S]dTTP), and deoxycytidine triphosphate ([35S]dCTP); 2 nmol of cold dATP; and enough sterile double-distilled water to bring the volume to 10 µL. Each reaction was overlaid with mineral oil. Amplification was carried out for 44 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 35 seconds. Each labeled PCR reaction was mixed with 2 µL of formamide loading dye (99% formamide, 20mM EDTA, 0.03% bromophenyl blue, and 0.05% xylene cyanol). Samples were denatured for 5 minutes at 94°C and electrophoresed at 20 W on a 6% polyacrylamide gel (49:1 acrylamide:bis, 3% glycerol, and 1× Tris-EDTA (TE)). Total electrophoresis time was proportional to product size. Constant temperature was maintained with a cooling fan. Gels were transferred to 3M Whatman paper (Whatman International, Maidstone, England) and dried. Kodak X-OMAT film (Kodak, Rochester, NY) was used for autoradiography. Band shifts were assessed by visual inspection. Sequence data were compared with published cDNA sequences for MYO1F using a software package (Sequencer 3.1; Gene Codes Corp Inc, Ann Arbor, Mich).

Figure 1. Pedigree of the DFNB15 family (see Chen et al3). Open square indicates male; open circle, female; and solid symbols, affected.

(UTRs), has been reported by Crozet et al.8 The encoded protein is 1099 amino acids in length and structurally similar to other unconventional myosins. However, the cDNA sequence of the human orthologue, MYO1F, has been reported only in part (GenBank accession numbers U57053...
and X98411).6,7 We determined the complete 5’ coding region of MYO1F using 5’ RACE to identify a start codon (ATG) that corresponds with that described in murine Myo1f. We then used 3’ RACE to determine the unre-ported portion of the 3’ UTR and the poly-A tail (Figure 3). In most respects, MYO1F shows good homology to Myo1f, but the head domain of MYO1F is length-ened by 52 amino acids encoded by a 156-base pair (bp) stretch of cDNA sequence that is not homologous to Myo1f.

The genomic structure of MYO1F was determined by a series of conventional and long-range PCR reactions using exon-specific primers generated from a combi-nation of published MYO1F cDNA sequences (Gen-Bank accession numbers X98411 and U57053) and cDNA sequences determined using 3’ and 5’ RACE. Polymerase chain reaction primers were chosen using homology to consensus splice site sequences and modeling of MYO1F genomic structure. The PCR product sizes were compared with the sizes predicted by the cDNA sequence. Products that were longer than predicted were assumed to contain an intron and were bidirectionally sequenced. No mutations were found, making it unlikely that MYO1F is the cause of DFNB15-related hearing impairment.

### Table: Mutation Screening Polymerase Chain Reaction Primers, Splice Donor and Acceptor Sites, and Exon Sizes

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![Figure 2](https://example.com/fig2.png)

**Figure 2.** Mapping of MYO1F within the DFNB15 interval on chromosome arm 19p.

Severe hearing impairment affects approximately 1 in 1000 newborns, making it the most common sensory disor-der.13,14 Hearing impairment might be inherited with a con-stellation of other phenotypic features as a recognizable syn-drome or in isolation as nonsyndromic hearing loss. Nonsyndromic hearing loss can be inherited in an auto-somal dominant, autosomal recessive, or X-linked fash-
ison. By convention, **DFNA** denotes a nonsyndromic, dominant deafness locus, with the numerical suffix reflecting the order of locus discovery (ie, **DFNA1**, **DFNA2**, etc). Autosomal recessive nonsyndromic deafness loci and X-linked deafness loci are designated **DFNB** and **DFN**, respectively.

The most common form of prelingual inherited hearing impairment is autosomal recessive nonsyndromic hearing loss. It is highly heterogeneous and almost exclusively monogenic. In a previous study, Chen et al5 mapped **DFNB15** to chromosomes 3q21.3-q25.2 and 19p13.3-13.1, identifying one or possibly both regions as the site of a deafness gene. In this study, using a radiation hybrid mapping panel, we placed **MYO1F**, an unconventional myosin gene expressed in the cochlea, within the **DFNB15** interval.

Unconventional myosins differ from conventional myosins. They do not form bipolar filaments but instead function as intracellular motors that move along actin filaments, generating force through the hydrolysis of adenosine triphosphate. They share a conserved head domain that contains adenosine triphosphate and actin binding sites, a neck regulatory domain that can contain 1 or more IQ motifs that bind calmodulin or calmodulin-like light chains,15 and a tail domain that varies from one unconventional myosin to another. It is this last domain that determines the function of each unconventional myosin.

Unconventional myosins play a variety of roles necessary for cell locomotion, phagocytosis, organelle transport, and mechanoregulation of membrane protein function.8 They are important constituents of the cytoskeletal framework of the inner ear, the integrity of which is essential for cochlear mechanoelectrical transduction. Three unconventional myosins—myosins VI, VIIA, and XV—have been demonstrated to be essential for normal hearing.9-12 Mutations in murine **Myo6** are found in the Snell waltzer mutant, which has a phenotype characterized by deafness and vestibular dysfunction; the protein is concentrated in the cuticular plate.10 Mutations of **Myo7a** are found in the shaker-1 mutant, which also presents with hearing impairment and vestibular dysfunction.9

![Figure 3. Human myosin 1F (MYO1F) and mouse Myo1f complementary DNA (cDNA). A, Dashed area on the mouse cDNA represents a 156-base pair interval present in human **MYO1F** but not in mouse **Myo1f**. B, Previously unpublished 5’ and 3’ cDNA sequences and the interval of cDNA that is present in human **MYO1F** but absent in mouse **Myo1f**. RACE indicates rapid amplification of cDNA ends; ATP, adenosine triphosphate.](https://jamanetwork.com/)

![Figure 4. Genomic structure of MYO1F with intron and exon sizes (kb indicates kilobase).](https://jamanetwork.com/)
sin VIIA has been implicated in formation of the cytoskel-
etal network and in intracellular vesicular transport.16 It is
expressed in the inner ear and retina and is the cause of
nonsyndromic deafness (DFNB2 and DFNA11) and
syndromic deafness (USH2B—characterized by hearing
loss, vestibular dysfunction, and retinitis pigmentosa).17-10
Mutations in Myo15 give rise to the shaker-2 mutant,
which, similar to the other 2 mouse mutants, has audi-
tory and vestibular impairment20; mutations in MYO15
result in DFNB3.21

Based on these findings and the map location of
MYO1F, we considered MYO1F an attractive functional
and positional DFNB15-causing candidate gene. Ge-
nomic DNA was used for mutation screening because
there was no readily available source of RNA. To deter-
mine the genomic structure of MYO1F for mutation
screening, we used a series of conventional and long-
range PCR experiments.

MYO1F was found to span 25.45 kb and to consist of
24 exons. The average size of a human nuclear gene, in-
cluding introns, is approximately 10 kb,22 although there is
enormous variability, with gene size ranging from a few
hundred nucleotides to several megabases. The average num-
ber of exons per gene also has wide variability but roughly
correlates with gene size. Small genes might have only 1
exon, whereas large genes have numerous exons. MYO1F
follows these trends; however, with an average size of 176
bp, MYO1F exons are slightly smaller than the average
nuclear gene exon size of 200 bp. Exon size is inde-
pendent of gene size, whereas intron size is directly corre-
Table 1. Mutational Analysis

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<th>Description</th>
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<tr>
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<td>DFNB3</td>
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The candidate gene approach to determining the disease-
causing gene for a specific hereditary condition is only one
method among many. However, selection of a putative dis-
ease-causing gene for mutation screening based on chro-
mosome location, tissue expression, and purported func-
tion will become an even more important approach as
sequencing of the human genome is completed.

Although we did not find MYO1F allele variants in one
family with autosomal recessive nonsyndromic hear-
ing loss, the gene remains an excellent candidate for he-
reditary hearing impairment. MYO1F is expressed in liver,
kidney, spleen, eye, brain, lung, and small intestine (Crozet et al8). Given its wide range of tissue expres-
sion, we believe that MYO1F might be a candidate for a
syndromic hearing loss.

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