Original Article

Myosin–7A Mutation Screening in Patients with Prelingual Non–Syndromic Hearing Impairment

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Abstract
Objective To screen for mutations in myosin-7A gene (MYO7A) in patients with prelingual nonsyndromic hearing impairment. Methods 31 sporadic patients with congenital hearing impairment and 65 patients from 34 families with prelingual hereditary hearing impairment in China were tested in this study, and 100 hearing normal individuals were used as control. Genomic DNA isolated from whole blood of all subjects was subjected to polymerase chain reaction (PCR) to amplify selected exons of MYO7A gene. The PCR products were subsequently screened using single strand conformational polymorphism analysis (SSCP) and direct sequencing when the fragments showed an abnormal electrophoretic pattern. Results GgA transition at position 617 in exon 7, which would produce an A206G amino acid substitution, was detected in two patients but in none of the unaffected members in the families. This heterozygous missense mutation happened within a highly conserved heptapeptide sequence of MYO7A protein, and is closely relevant to prelingual nonsyndromic deafness. Conclusions The A206G substitution is possibly a new mutation to cause prelingual nonsyndromic hearing impairment. Our results provide evidence that exon 7 of MYO7A is a mutational hotspot in genetic hearing impairment.

Key Words gene; mutation; prelingual hearing impairment; myosin

Introduction

Hereditary deafness is the most common hereditary disease in Otolaryngology. It is estimated that about 60% of the prelingual non-syndromic hearing loss cases are caused by genetic factors. To date, 18 genes have been reported to cause autosomal recessive deafness (DFNB), 15 genes are involved in autosomal dominant deafness (DFNA) and 1 gene causes X-linked deafness (DFN).

Myosins belong to the superfamily of molecular motor proteins (18 classes identified so far) and are present in probably all eukaryotic cells. The proteins bind to actin and use the energy from ATP hydrolysis to generate force to pull itself along actin filaments. They are involved in muscular contraction, cytokinesis, short-range membrane-vesicle transport and a host of other cellular processes.

So far, 6 classes of myosins, including MYO7A, MYO15A, MYO6, MYO3A, MYO1A, MYH9 (nonmuscle myosin heavy chain 9) and MYH14 (nonmuscle myosin heavy chain 14)¹,²,³, have proved to be associated with genetic deafness disorders in mammals. For example, mutations in MYO7A gene are responsible for DFNB2, DFNA11 and Ush 1B syndrome.

To ascertain the frequency and characteristics of gene mutations in Chinese patients with prelingual nonsyndromic hearing impairment, we screened for mutations of MYO7A in 31 sporadic patients with congenital hearing impairment and 65 patients from 34 families with prelingual hereditary hearing impairment in China.

Materials and methods

31 sporadic patients and 65 patients from 34 families in China were investigated in this study. The
affected patients in this study had congenital sensorineural hearing impairment without any other associated symptoms. All the individuals included in this study gave a complete history and underwent otoscopic examination and pure tone audiometry or auditory brainstem response (ABR) test, as well as general physical examination. In addition, vestibular function was evaluated via bithermal caloric testing.

Peripheral blood samples were drawn from all the patients and some family members, as well as 100 control individuals, after obtaining formal consent. Genomic DNA (gDNA) samples were isolated from the blood in these subjects.

**SSCP Analysis**

The human MYO7A gene spans 120 kb and comprises 49 exons (Genebank accession number: U39226). Since 4 mutations have been reported to cause nonsyndromic hearing loss[4, 5], the primers were designed to amplified the coding region and flanking intronic sequences of 4 exons (exon 4, 7, 15 and 28) of the MYO7A gene. PCR was performed in 20 μl volume containing 1 μl diluted gDNA and 1 μM of oligonucleotide primers targeted to the 4 exons under different calibrated conditions.

The products were subjected to single strand conformational polymorphism analysis (SSCP) by electrophoresis on 6% polyacrylamide gel (contain 5% glycerol) at 8W for 24 hours and result images were scanned and analyzed using a Genescan scanner (Bio Rad, USA).

**DNA Sequencing**

To define mutation/sequence changes, DNA fragments that exhibited abnormal mobility shifts in SSCP analysis, as well as normal fragments, were reamplified, purified with Qiagen gel extraction kit and then sequenced through an ABI377 sequencer. All result PCR products were sequenced completely on both strands, and analyzed with DNASTar software in comparison to the sequence in Genebank.

**Results**

SSCP analysis showed abnormal mobility shifts in PCR products for exon 7 of MYO7A in two patients but not in the unaffected members in their families (Figure 1). One of the cases was a boy who showed a prelingual bilateral profound sensorineural hearing loss without any other associated symptoms, and his mother also suffered from the same symptom. His grandfather was said to be postlingually deaf also but no gDNA sample was available from him because he had deceased (Figure 2). Mutation screening for Cx26

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**Table 1** Primers designed for the four exons in MYO7A

<table>
<thead>
<tr>
<th>exon</th>
<th>Forward primer (5’ -3’ )</th>
<th>Reverse primer (5’ -3’ )</th>
<th>Products (bp)</th>
<th>Tm (℃ )</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>CCTGAAGTGCGCAGCCTGG</td>
<td>CCGGACGGGCACCGG</td>
<td>242</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>ACCAGAGTCCGAGGTTG</td>
<td>AGGGGCCTGGGCTATT</td>
<td>261</td>
<td>58</td>
</tr>
<tr>
<td>15</td>
<td>TCCTGGGACACTGGATG</td>
<td>CATCCTTCACCTCCCACCTA</td>
<td>289</td>
<td>60</td>
</tr>
<tr>
<td>28</td>
<td>ACTGGCTGCTAGGAGGA</td>
<td>ATTGCTCTCCCACAGTG</td>
<td>278</td>
<td>56</td>
</tr>
</tbody>
</table>
and Cx31 genes were applied to the family and revealed no mutation in the two genes. The mutation was not detected in the 100 unaffected individuals involved in the study.

From sequencing the DNA fragments, a heterozygous nucleotide transition GgA at position 617 in exon 7 was detected (Fig3). This mutation causes Arg206Gln substitution within a highly conserved heptapeptide sequence of protein MYO7A, and is closely relevant to the preligual nonsyndromic hearing impairment.

**Discussion**

Myosin-7A(MYO7A) belongs to the unconventional myosin family. Unconventional myosins are motor molecules with structurally conserved heads that move along actin filaments using their actin-activated ATPase activity. Mutations in some myosins result in abnormalities in stereocilia in the sensory cells in the inner ear. For example, when MYO7A is missing, stereocilia become disorganized, and they grow short with the loss of myosin XV. Scanning electron microscopy shows that stereocilia are malformed when the MYO7A, MYO15A, or MYO6 genes are defective, suggesting that myosins are essential for maintaining the structure of stereocilia [1]. It has been shown that mutations in the MYO7A gene are responsible for DFNB2, DFNA11 and Ush1B syndrome [5-8].

Exon 7 encodes the ATP-binding site of the motor head of the protein. Several lines of evidence have led us to suggest that the resultant amido-acid change may impair the ATP-binding property of the protein. The mutation results in the A206G substitution in exon 7, which encodes the highly conserved heptapeptide sequence that is invariant in all myosins of all classes. This mutation resides in C-terminal to the ATP-binding loop, at the base of the ATP binding pocket. In light of the absolute evolutionary conservation of residues within this region, it is expected that the A206G mutation would severely impair the ATP-binding function of the protein. In our study this mutation was found to be segregated in the patients and there was no mutation found in unaffected members in the family.

**Table. 2** Clustal W amino acid alignments of different myosins around the location of the mutation.

<table>
<thead>
<tr>
<th>Human myosin VIIA</th>
<th>LEATPILEAFNAKTIRNDNSSRFGBKYMIDFKNKRGAEKIEQYLYLLEK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse myosin VIIa</td>
<td>LEATPILEAFNAKTIRNDNSSRFGBKYMIDFKNKRGAEKIEQYLLSLK</td>
</tr>
<tr>
<td>Rat myosin I heavy chain</td>
<td>LQSNPVELAFNAKTIRNDNSSRFGBKYMDFVDGBKAPVGHILSYLLEK</td>
</tr>
<tr>
<td>human myosin I beta</td>
<td>LQSNPVELAFNAKTIRNDNSSRFGBKYMDFDKAPVGHILSYLLEK</td>
</tr>
<tr>
<td>chicken myosin I</td>
<td>LQSNPVLEAFNAKTIRNDNSSRFGBKYMDFDKAPVGHILSYLLEK</td>
</tr>
<tr>
<td>rat myosin I</td>
<td>LKSNCVLEAFNAKTIRNDNSSRFGBKYMDFDKAPVGHILSYLLEK</td>
</tr>
<tr>
<td>Human myosin IB heavy chain</td>
<td>L------EAFNAKTIRNNSRFGBKYMIEQFDLKGDPVGGGRISNYLLEK</td>
</tr>
<tr>
<td>human myosin ID</td>
<td>LQSNPLLEAFNAKTIRVRRNNSRFGBKYMFEIQFSRGEPDGKSGKISNFLLEK</td>
</tr>
<tr>
<td>human myosin VA</td>
<td>LTSNPMESIGNAKTRRNNSRFGBKYMIEQFDKRYRIIGANMRTYLYLLEK</td>
</tr>
<tr>
<td>mouse myosin VI</td>
<td>VEANPLLEAFNAKTIRVRRNNSRFGBKVFIEIHNFENKSSVVGGFVSHYLYLLEK</td>
</tr>
</tbody>
</table>

The 206 arginine (indicated by arrows) is highly conserved in most of myosin classes. Genebank accession numbers: human myosin VIIA: u34277, mouse myosin VIIa: u81453, rat myosin I heavy chain: x74800, human myosin I beta: x98507, chicken myosin I: x58479, rat myosin I: x71997, myosin IB heavy chain: J02974, human myosin ID: u57053, human myosin VA: a53016, mouse myosin VI: u49739.
We conclude that this mutation is a possible cause for the prelingual nonsyndromic deafness.

The MYO7A mutation observed here was heterozygotic, and the pattern of the hearing loss in this family was autosomal dominant with nonpenetrance. Even though exons of the MYO7A gene other than exon 7 were not studied in this family, we speculate that mutation on other allele in unscreened regions of the gene is still possible. Weston (1996) screened a panel of 189 genetically independent cases of Usher syndrome type I for the presence of mutations in the motor domain of MYO7A by analyzing 14 exons. They found 23 mutations segregated with the disease, and 2 mutations (R212H and R212C) accounting for the greatest percentage of observed mutant alleles (31% alleles). They found some USH1B mutations present in heterozygous state and presumed that the mutation on the other allele was present in the unscreened regions of the gene \[^9\].

Although the MYO7A gene consists of 49 exons, most of the deafness-causing mutations reported previously are located in the first 14 exons, which encode N-terminal coding portion of the motor domain of MYO7A[7,9]. Previous report have shown that exon 7 of MYO7A is a mutational hotspot in genetic hearing loss because some mutations in exon 7 have been detected to be segregating with the hereditary hearing impairment \[^{10,11}\].

Due to its limitations, it is not possible to detect all mutations in MYO7A using the SSCP technique. Some mutations may exist in unscreened regions of the gene. With regard to previous reports, we conclude that exon 7 of MYO7A is a hotspot of deafness-causing mutation. We expect that further investigations will reveal other mutations in the myosins and their phenotype of prelingual hearing impairment.

**Summary**

The current study identified an A206G mutation in a prelingual nonsyndromic hearing loss family. Sequence analysis suggests that the mutation was responsible for the symptom in the family. With regard to previous reports, we conclude that exon7 of MYO7A is a hotspot of deafness-causing mutation.

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**References**