Mutation at exon 39 of myosin 15 is rare or absent among people with profound sensorineural deafness in Kerala

Hearing impairment is a serious ailment that can also affect other faculties of an individual such as learning, speech, etc. Globally it is estimated that one in thousand newborns and 4 in 100 people below 45 years of age are affected by deafness. More than 50% of the total deafness cases are estimated to be due to genetic factors⁷. Out of these, 80% are nonsyndromic recessive deafness which is estimated to be caused by approximately 20 different loci that have been mapped by segregation analysis in resource families².

People in Kerala suffer from different forms of hereditary deafness. However no data are available on the frequency and distribution of these hereditary defects in the population. Owing to the high rate of consanguinity existing among the different communities of Kerala, it is worthwhile to assess the frequency of mutations causing deafness and the pattern of inheritance in this population, which may facilitate genetic counselling and prenatal diagnosis.

One of the loci identified, that segregates with profound deafness is DFNB3 mapped on chromosome 17p11.2 (ref. 4). Positional cloning has enabled the identification of a gene at this locus that belongs to the unconventional myosins, nomenclatured as MYO15 (ref. 5). Myosin 15 gene, which is presumed to code for a mechano enzyme, has 50 exons and mutations have been identified in two of the exons associated with hearing impairments (exon 28 and exon 39). These mutations have been identified in Bengkala kindreds in Bali and in a few Indian families⁶. Mutation in exon 39 is a nonsense mutation that results in a truncated product or no product at all⁶. This mutation could be easily detected by PCR–RFLP, using XmnI restriction enzyme, as the restriction site for this enzyme is eliminated by the point mutation.

A representative sample of 25 affected families from Kerala with one or two affected children was screened by PCR–RFLP for the nonsense mutation in exon 39 of MYO 15 gene.

DNA samples were obtained from patients reporting to National Institute of Speech and Hearing (NISI) for audiotherapy. A total of 85 individuals comprising 25 families having at least one affected child have been selected for the study. Twenty five random DNA samples from individuals who were phenotypically normal were also screened. Sample population encompasses all the major religious and socio-economic groups of Kerala. No previous linkage assessment has been done on these families. All the clinical data were collected in a pre-designed format including audiometry index, familial history, post-natal developments, etc. All non-genetic factors were eliminated by history.

DNA was isolated from 10 ml of venous blood as per the standard protocol⁷. The purity and concentration was determined by spectrophotometry. The mutation-specific region of the exon 39 of MYO15 was amplified from genomic DNA using a pair of primers⁵. The primer sequences are:

Forward – 5’ TCT CCC TGG ATT CTC ATT A 3’
Reverse – 5’ TCT TTG TCT TTG TCT GTT CCA CC 3’.

100 ng of genomic DNA was amplified in a reaction mix containing 25 ppm of each primer, 200 µg of each dNTP, 50 mM KCl, 10 mM HCl, 1.5 mM MgCl₂ and 1 unit of Taq polymerase in a reaction volume of 25 µl. The cyclic condition included an initial denaturation of 1 min at 95°C followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s with a final extension at 72°C for 7 min.

5 µl of the PCR product was digested with 5 units of XmnI in a reaction volume of 20 µl as per manufacturer’s recommended conditions at 37°C for 1 h. The reactions were stopped by adding 1 µl of 0.5 mM EDTA.

The digested products were resolved on 6% non-denaturing conventional PAGE at 20 mA for 3 h. HaeIII digest of φX 174 DNA was used as molecular size marker. The fragments were visualized under UV illuminator after staining with ethidium bromide for 20 min.

The sample population selected for the investigation had hearing impairment exclusively due to genetic factors as all other factors could be ruled out. The primer pairs could amplify a fragment of 279 bp from all the samples screened as well as the control group (Figure 1 a). The PCR product digested with XmnI

![Figure 1](image_url)

Figure 1. a. PCR amplification of exon 39 of myosin 15 gene from affected and normal individuals. Lane 1: Affected; Lane 2: Normal; Lane 3: 100 bp ladder. b. Polyacrylamide gel analysis of exon 39 of myosin 15 gene digested with XmnI restriction enzyme. Lane 1: φX174/HaeIII digest; Lane 2: Uncut PCR product; Lanes 3–6: Affected samples; Lanes 6–9: Normal samples.
resulted in two co-migrating fragments of approximate size 143 bp and 136 bp, respectively (Figure 1b). Out of the total 85 samples from 25 affected families and 25 random samples, none of them exhibited the reported mutation. Unconventional myosin 15 is presumed to play a major role in auditory hair cell function. The role of this gene was confirmed by correcting shaker-2 mouse mutation, the homologue of human DFNB3 in mice using a BAC transgene. The nonsense mutation in exon 39 of myosin 15 is presumed to result in either a truncated protein or no protein at all.

Though this is one of the mutations identified in DFNB3 loci and detected in one Indian family, the frequency of this mutation is not available for any population. Hence, it is not surprising that the sample of affected families screened did not show the mutation as it could be a very rare one. Since there are more than 20 loci identified for hearing impairment with a number of mutations in many of these loci, the probability of detecting a specific point mutation in a random sample is low.

This study shows that the Xml1 site mutation of MYO15 may be less prevalent or absent in this population.


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