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Prevalence of the *CDH23* mutation p.D990N among South Indian Hearing Impaired Individuals

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A B S T R A C T

Cadherin-23, encoded by the gene *CDH23*, forms the upper part of the tip links of inner ear hair cells, and are crucial for normal hearing. Mutations in *CDH23* are known to cause hearing loss that is often autosomal recessive and non-syndromic (DFNB12). The mutation p.D990N (c.2968G>A) occurring in the Ca^{2+} binding motif on extracellular domain 9 of Cadherin-23 is known to cause DFNB12. This mutation has been previously reported in Indian and Pakistani families; however, its prevalence in South India is unknown. In order to study its occurrence and prevalence, we screened for the *CDH23* mutation p.D990N using a rapid and cost effective PCR-RFLP approach, among a cohort of 77 South Indian hearing impaired individuals recruited from deaf schools. One individual, who had parental consanguinity, was found to be homozygous for the mutation accounting to a frequency of 1.3% in our study cohort, thus, suggesting its common occurrence among South Indian HI; no heterozygous individuals were identified. The p.D990N mutation positive proband had profound hearing loss, without any other associated abnormalities. The gene *GJB2* is known to be the most common causative factor in genetic deafness among South Indians (~20%); other genes account to 1-5% each. In such diseases with high genetic heterogeneity, identifying and screening for prevalent, population specific mutations would be a cost effective strategy to implement in genetic diagnostics.

Introduction

Hearing loss is the most common sensory disorder affecting one in 500 newborns, worldwide (Morton & Nance, 2006) and 50% of the cases are genetic (Smith, 2014). More than 100 genes have been reported to be associated with autosomal recessive non-syndromic hearing loss (ARNSHL) (Hereditary hearing loss homepage). The most common deafness causing gene is GJB2, which accounts for about 25% of the cases (Mani *et al.*, 2008). The gene *CDH23*

(69 exons) encodes Cadherin-23, which forms the upper part of the tip links. Tip links play a crucial role in mechanoelectrical transduction and hence in hearing (Mueller *et al.,* 2008). Missense mutations in *CDH23* are known to cause autosomal recessive sensorineural hearing loss, also called DFNB12 (Bork *et al.,* 2001). *CDH23*has been frequently reported to cause ARNSHL in various populations, and one study on Indian population reported a frequency of 1.8% (Ganapathy *et al.,* 2014). The *CDH23* mutation p.D990N was reported to cause DFNB12 in Indian and Pakistani families (Bork *et al.,* 2001; Schultz *et al.,* 2011; Ganapathy *et al.,* 2014). The mutation occurs on extracellular domain 9 of Cadherin-23, disrupting its Ca^{2+} binding motif, thus affecting the structural configuration of the protein.

However, there are no reports till date about the prevalence of *CDH23* mutations in South Indian population. Here, we screened for the *CDH23* mutation p.D990N to determine its prevalence among South Indian hearing impaired population.

Materials and Methods

Subjects

Hearing impaired (HI) probands for the study were ascertained from various schools for childhood HI in and around Chennai. The school administration was approached for permission to meet the parents, teachers and students to elaborate them about screening for genetic hearing loss. A total of 77 HI were recruited, who conformed to the selection criteria were included. The criteria for selecting the study participants included- familial hearing loss (with a family history of HI), prelingual, profound and non-syndromic hearing loss, and the probands must be South Indian. The parents were explained about the genetic testing and after obtaining a written informed consent, peripheral blood (~5-10 ml) was collected from the probands using sterile vacutainer. For children who were below the age of 18, the informed consent was obtained from the parent or guardian, on their behalf.

Mutation screening

Genomic DNA isolation was carried out by PCI method. The *CDH23* exon 25 was amplified by polymerase chain reaction using primers - Forward: 5'-GGG GAA CAA CTG TGT CTA-3', Reverse: 5'-GGA GGG CAG CTC AGA AAG-3'. The amplified PCR product was subjected to restriction fragment length polymorphism analysis using the enzyme *Hpy99I*. The wildtype *CDH23* exon 25 has two sites specific for restriction digestion by *Hpy99I*, however, in the presence of the mutation p.D990N, one site is lost, thus resulting in two fragments, instead of the four fragments seen with the wildtype allele. The digested products were resolved on a 2.5% agarose gel.

Results and Discussion

Subjects

The 77 hearing impaired (HI) recruited, comprised of Tamil, Telugu, Kannada, and Malayalam-speaking, South Indians. Of the 77, 50 had parental consanguinity, while 27 did not.

All the probands had familial hearing loss which was profound, bilateral and prelingual; they did not have any other associated abnormalities other than hearing loss. The average age of the probands ranged from 10 to 22 years (Mean age = 15.9). These probands were previously screened for *GJB2* mutations (that cause DFNB1) and were found to be negative.

Frequency of the mutation p.D990N

One proband of the 77 screened, was found to be homozygous for the *CDH23* mutation p.D990N (c.2968G>A). None of the 27 probands who had non-consanguineously married parents tested positive for the mutation. No heterozygous individuals were identified in the cohort.

A mutation frequency of 1.3% was observed in the hearing impaired individuals recruited from deaf schools.

The individual LFC-41, that tested positive, was a 16 year old female, with profound, prelingual hearing loss, and no symptoms other than hearing loss were observed. Her parents were consanguineously married, with it being an Uncle-Niece union. The family had a history of hearing loss, where three of the proband's mother's siblings were also affected. The proband has three older siblings, who were all normal hearing. However, mutation testing in the other affected and unaffected family members could not be carried out due to non-consent.

Fig.1 Representative gel picture showing PCR-RFLP analysis in screening for the *CDH23* mutation p.D990N. The gel shows resolved bands that were restricted with the enzyme *Hpy99I* (Lane1: Marker; Lanes 2&3: Undigested PCR product; Lane 4: Homozygous p.D990N mutation; Lanes 5, 6 & 7: wild type or absence of p.D990N mutation)

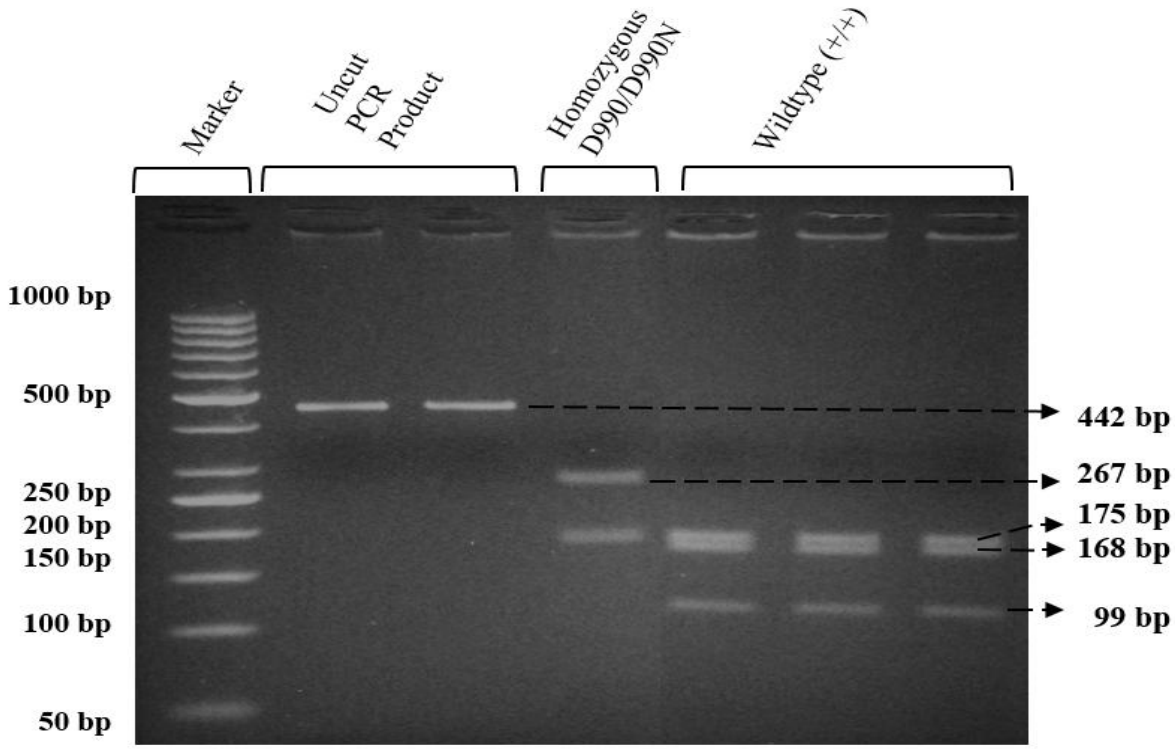
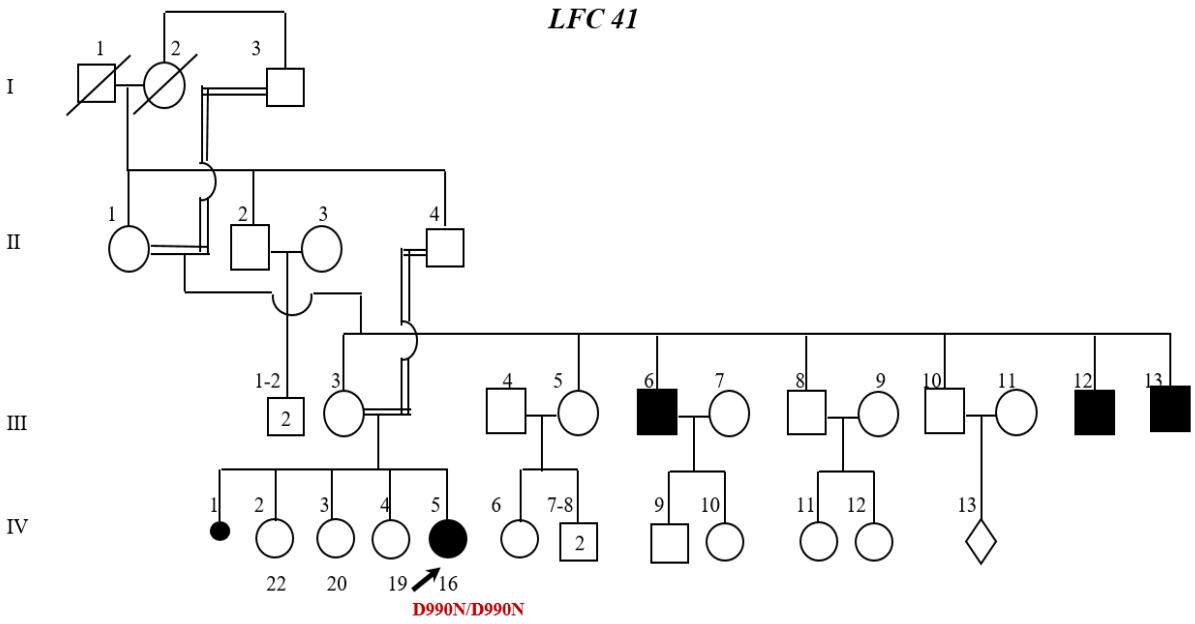


Fig.2 Family pedigree of the p.D990N mutation positive proband LFC-41



Hearing loss is known to cause deafness in 50% of the cases and around 70% of them are autosomal recessive and non-syndromic in nature (Smith, 2014). Although several genes have been identified, *GJB2* is the most common deafness causing gene in India (Mani *et al.,* 2009).

Most of the other genes are known to have a prevalence ranging from 1-5% (Smith, 2014). *CDH23* is yet another deafness causing gene that has a high frequency in Asian populations. It is a large gene with 70 exons which encodes the protein Cadherin-23 with 27 extracellular domains, a transmembrane domain and a cytoplasmic domain. Cadherin-23 forms the upper part of the inner ear tip links, which play a major role in efficient mechanotransduction. The extracellular domains have Ca^{2+} binding motifs that are crucial for proper structure and function of the proteinhearing (Mueller *et al.,* 2008).

The mutation p.D990N is known to cause DFNB12 by disrupting a Ca^{2+} binding motif on extracellular domain 9 in Cadherin-23. Since this mutation has been reported in Indian and Pakistani families(Bork *et al.,* 2001; Schultz *et al.,* 2011; Ganapathy *et al.,* 2014), we screened for its occurrence in South India. One HI individual was found to be homozygous for the mutation, in all the 77 HI screened, thus occurring at a frequency of 1.3% in the study cohort.

The mutation positive proband had parental consanguinity (Uncle-Niece marriage). Consanguinity is known as a major contributor in recessive disorders, and has been evidently established in genetic deafness (Bener *et al.,* 2005). The occurrence of this mutation in the consanguineously married family suggest that consanguinity might play a role in the occurrence of this mutation in South India.

The previous Indian population study reported an overall allelic frequency of 1.8% for *CDH23* mutations, with whole gene screening. The mutation p.D990N was also reported in the study (Ganapathy *et al.,*

2014). The frequency of 1.3% for the mutation p.D990N in our cohort suggests the probability that this might be a prevalent mutation among South Indian HI. However, this might be established with screening for this mutation in a larger cohort.

Additionally, due to its large gene size, a detailed analysis for mutations in *CDH23* is cost wise demanding. Even with the advent of Next-generation sequencing technologies, routine diagnostics for genetic deafness is distant in the Indian scenario. Therefore, identifying population-specific mutations that can be preliminarily screened for genetic diagnosis, before going in for whole gene screening is a cost effective alternative.

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