ORIGINAL ARTICLE

Genetic Mapping of Candidates of Deafness Genes in Pakistani Families

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ABSTRACT

Objective: DNA analysis for the Genetic Mapping of Candidates of Deafness Genes in Pakistani Families.
Study Design: It was a cross sectional study.
Place and Duration of the Study: Department of Biochemistry/Molecular Biology, Quaid I Azam University, Islamabad Pakistan. The Clinical examination, biochemical tests, interpretation of results and preparation of results completed in approximately one year, 2006 2007.
Materials and Methods: Study was conducted on two Pakistani families.
Subjects (Families) selected for the study:
Two Pakistani families labeled as family A and B were selected for the study. Family A comprises of three normal and three affected (Deaf) individuals. Family B comprises of two normal and four affected (Deaf) individuals. The blood samples were immediately dispatched to Molecular genetic laboratory, Quaid I Azam University, Islamabad for analysis 2006 2007.
Results: In family A, linkage was established to DFNB47 locus on the chromosome 2p25.1-p24.3. In family B, linkage to DFNB1 locus was excluded first by genotyping polymorphic microsatellite markers linked to the candidate region and then by sequencing GJB2 gene
Conclusion: The genetic mapping of candidates of deafness genes brings greater understanding of molecular basis of deafness and would modify the preventive and curative methods.

Key words: *DNF, DNA, GJB, PCR and Electrophoresis*

Introduction

Hearing impairment is the most common sensory disorder worldwide.[1] It is clinically and genetically very heterogeneous and auditory genes are discovered at very rapid pace. Genetic factors are probably responsible for more than 50% of the cases of early onset H1. [2] Where as in most of the late onset H1 a combination of genetic as well as environmental factors is involved.[3] Studies of the epidemiology of hearing impairment have suggested that approximately 1 in 1000 to 1 in 2000 children show a profound hearing loss at birth or in early childhood.[4, 6]

Most frequently hearing impairment, is classified as syndromic or non syndromic, or according to its transmission via as autosomal dominant, autosomal recessive, X-Chromosomal recessive, or maternal trait.[7]

X-Chromosomal dominant and Y linked transmission are rare. Syndromic hearing impairment is associated with malformation of the external ear or other organs with medical problems involving other organ systems. More than 70% of the hereditary hearing loss is non syndromic.[8]

Of the 30,000 50,000 human genes, 1% i.e. 300 500 genes, are estimated to be necessary for hearing.[9] Gap junctions are clusters of intercellular channels, vital of intercellular communication. The following connexins expressed in the auditory system have been implicated in hereditary deafness, GJB2, GJB3, GJB6 and GJA1.[11, 14, 16] Mutation in the Alpha tectorin gene on chromosome 11q has been found in families with both autosomal dominant and autosomal recessive having prelingual hearing loss.[15] Mutations in the Trans membrane inner ear (TMIE), Trans membrane channel like 1 (TMC1), MY06

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gene, MY015 gene, transcription regulators, POU3F4, POU4F3, ICERE-1, COCH, KCNQ4, COL11A2 and mitochondrial genes (12 SrRNA gene) have been found to be involved in different types of deafness in many studies.[17, 30]

Materials and Methods

A cross sectional study was conducted on two Pakistani families at Department of Biochemistry/Molecular Biology, Quaid I Azam University, Islamabad Pakistan.

The Clinical examination, biochemical tests, interpretation of results and preparation of thesis completed in approximately one year 2006-2007.

Families Studied

Two families labeled as family A and B were selected for the study. Family A comprises of three normal and three affected (Deaf) individuals. Family B comprises of two normal and four affected (Deaf) individuals. After detailed discussion with the elders of these families, genetic pedigrees were drawn by following standard method.[37] Mode of inheritance was inferred through pedigree analysis.

Blood Sampling

Blood samples from both normal as well as affected individuals including their parents were collected by 10 cc syringes (08×38 mm 21G×11/2) in standard potassium EDTA tubes. The blood samples were immediately dispatched to Molecular genetic laboratory, Quaid I Azam University, Islamabad for analysis 2006-2007.

Extraction and Purification of Genomic DNA from Blood

Genomic DNA was extracted from blood by phenol / chloroform method.

DNA Dilution and Micro Pipetting Polymerase Chain Reaction (PCR)

PCR was performed using gene Amp PCR System 2400 and 9600 thermo cycler (Perkin Elimer USA).

Agarose gel Electrophoresis

Agarose gel Electrophoresis was carried out to analyze the amplified DNA samples. After Electrophoresis amplified product was detected by placing the gel on UV Trans illuminators (Life Technology, USA).

Polyacrylamide gel Electrophoresis

Gel was photographed by using Digital Camera DC 120 (Kodak, USA).

Genotyping and Primer Database Analysis

Microsatellite markers mapped by Cooperative Human Linkage Centre (CHLC), were obtained from research genetics, Inc. (USA). The cytogenetic location of these markers as well as the length of the amplified product was obtained from genome data base homepage (www.gdb.org) and Marshfield Medical Center(www.marshmed.org.gov/genetics)

Linkage studies

Linkage studies were performed, Automated Genetic Analyzer ABI Prism 310 (Applied Bio System, USA).

Results

In the present study family A was first tested for mapping to several known loci by using polymorphic microsatellite markers from their candidate linkage intervals. The family A was found to be linked to DFNB47 locus on chromosomal region 2p25.1-p24.3. Two loci for ARNSH1 have previously been localized to chromosome.[2]

In family BDNFB1 and several other loci were tested for linkage. Electropherograms obtained by genotyping the microsatellite linked to the candidate linkage gene interval revealed that the affected individuals were heterozygous for different combinations of

parental alleles, thus indicating exclusion of family B from linkage to DFNB1 and several other known autosomal recessive non syndromic hearing loss loci. Linkages to DFNB1 locus were also excluded by sequencing the coding region of exon 2 of GJB2 gene. The novel locus harboring the disease gene in family B can be located by a genome wide search by using polymorphic markers spaced at 10 cM apart on all the autosomes.

Discussion

To date 23 known genes lie in the 5.3 Mb-region that contains DFNB 47. One of the genes in this region, KCNFI, is a strong candidate for DFNB47. This gene codes for potassium voltage-gated channel. Potassium ion channels are a diverse family of plasma member's proteins that play an essential role in various cellular processes, including maintenance of membrane potential and cell signaling.[31] KCNQ4 is a voltage gated K+ channel gene expressed in the cochlea. Voltage-gated K+ channel genes have been shown to be responsible for various hereditary diseases. For instance, mutation in the KVLQTI gene (a voltage-gated K+ channel gene) result in Jervell and Lange-Nielsen syndrome (JLNS) and Long QT syndrome, which are inherited AR disease, with congenital HI being one of their characteristics.[32] JLNS can also result from mutations in another voltage-gated K+ channel gene, KCNEI.

Another good candidate gene is inhibitor of DNA binding 2 (ID2), which is a member of the ID family genes that promotes cell proliferation. In embryonic mouse, ID2 expression was detected in the vestibular and acoustic ganglia, and also in the epithelium of the otic vesicle and surrounding mesenchyme[33]. Other genes that are expressed in the inner ear include: (1) cleavage and polyadenylation specific factor 3 2004); (2) tyrosine 3/ tryptophan 5-monooxygenase (YWHAQ), which is also expressed in the spinal cord of patients with amyotrophic lateral sclerosis.[35] And ornithine decarboxylase 1 (ODCI), the rate limiting enzyme in polyamine synthesis.

The recent identification of several deafness genes by molecular genetic studies has enabled the molecular basis of normal and pathological auditory function. In the coming years, further deafness genes are sure to be identified and mouse models for the human disease will be constructed as start in the long process of understanding the pathological processes involved in deafness. The rate of discovery of deafness genes by positional cloning in human will be accelerated by the freely available human genome sequence and by a catalogue of Expressed Sequence Tags (ESTs) within genetic intervals known to contain locus for human hereditary hearing loss. To assist in the identification of deafness genes cDNA library has been synthesized, partially sequenced and many ESTs assigned map position.[36]

Conclusion

The genetic mapping of candidates of deafness genes brings greater understanding of molecular basis of deafness and would modify the preventive and curative methods.

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