STUDY OF RECESSIVE DEAFNESS LOCUS (DFNB1) BY LINKAGE ANALYSIS IN SOME FAMILIES FROM BALOCHISTAN

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by

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# CONTENTS

<table>
<thead>
<tr>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>Review of Literature</td>
<td>4</td>
</tr>
<tr>
<td>Objectives</td>
<td>6</td>
</tr>
<tr>
<td>Plan of Work and Methodology</td>
<td>7</td>
</tr>
<tr>
<td>Reference</td>
<td>9</td>
</tr>
</tbody>
</table>
INTRODUCTION

Profound hearing loss affects almost one in 1000 newborns, and more than 50% of these are caused by genetic factors (Lalwani and Castelein, 1999). It is estimated that the prevalence of profound bilateral hearing loss is 1.6 per 1000 in Pakistan and 70% of hearing loss arises in consanguineous families (Elahi et al., 1998; Jaber et al., 1998). Hereditary hearing loss may be syndromic or non-syndromic; about 30% of deafness cases are syndromic, while 70% is nonsyndromic. The main pattern of inheritance of deafness in Pakistani population is autosomal recessive. The common deafness syndromic is Usher, Pandred and Waarenburg syndrome. The non-syndromic deafness may be Autosomal dominant, Autosomal Recessive, X-linked, Y-linked and mitochondrial. Approximately 75% cases of inherited deafness are autosomal recessive, 12-24% autosomal dominant and 1-3% is X-linked (MaraZita et al., 1993). To date more than 67 loci and 21 genes have been identified for nonsyndromic recessive deafness. \textit{GJB2} gene on chromosome 13q12-13 that codes for a gap junction protein called connexin 26 is responsible for the majority of genetic nonsyndromic hearing losses. This transmembrane protein forms connexons in the cochlea that functions in potassium recycling in the hair cells. (Hereditary hearing loci home page http://dnalab.www.via.ac.be/dnalab/hhh).

Mutations in the \textit{GJB2} gene that cause abnormal connexin synthesis result in impaired potassium cycle, and in turn hearing loss. There are numerous \textit{GJB2} mutations, the frequencies of which vary among different populations. The most common mutations are 35delG, 167delT, and 235delC, which are frequent in the Caucasian, Jewish and Asian populations, respectively (Morell \textit{et al.}, 1998; Abe \textit{et al.}, 2000). The 35delG mutation constitutes almost 50% of all \textit{GJB2} mutations in the Caucasians (Zelante \textit{et al.}, 1997; Estivill \textit{et al.}, 1998; Kelley \textit{et al.}, 1998; Scott \textit{et al.}, 1998; Topol \textit{et al.}, 1998).

Genetic linkage analysis is a powerful method not only for mapping new locations but also for refining intervals where deafness-causing loci have been previously mapped. This strategy has helped in gene identification studies for recessive loci. The candidate interval demited are usually too large for positional cloning. Only when a linkage has been obtained can other families be analyzed to check if they are linked to these.
locations, if they have recombination different from the family used to map the locus, they can reduce the candidate interval and facilitate the identification of position candidates for deafness causing genes.

In the background of the above, it is proposed to carry out linkage analysis in consanguineous families. Such families yield LOD score (Log off Odd Ratios) and hence are very suitable for identifying markers near the linked gene. It is anticipated that the present study will help to find out mutations responsible for deafness in population of Balochistan and also will lead to the discovery of new loci genes.
REVIEW OF LITERATURE

Deafness is defined as partial or complete hearing loss which leads to an impaired ability to develop speech, language and effective communication skills, as a result of this the affected children require intensive specialized education in order to optimize their potential. Approximately one in 1000 infants is affected by severe or profound deafness at birth or during early childhood, i.e. the prelingual period (Kalatzis and Petit, 1998; Friedman and Griffith, 2003).

The etiology of profound childhood deafness is markedly diverse and involves numerous environmental and genetic factors or combination of both. An accurate diagnosis of an underlying cause is essential for optimal management and prognostication and genetic counseling. The main contributing environmental factors are meningitis, mumps, prenatal complications, postnatal trauma, hypoxia or hypoglycemia of the fetus, maternal diabetes, neonatal erythroblastosis fetalis, and congenital viral infections like rubella. Other factors like iodine deficiency, toxic drugs, and very high fever also contribute to hearing loss (Chen et al., 1988). Regardless of the cause, deafness may be classified into three categories: Conductive hearing loss, Neurosensory hearing loss and Mixed.

The gene involved, GJB2, encodes the connexin 26 molecule. Connexin 26 is a component of gap junctions, the links that allow small molecules to pass from one cell to the next, and this protein is found in several places in the body, including the epithelial supporting cells surrounding the sensory ear cells of the cochlea and the fibrocytes lining the cochlear duct (Kikuchi et al., 1995). The sensory ear cells of the cochlea allow potassium ions to pass through their upper surface during normal reception of sound, and these potassium ions must be recycled through the base of the ear cells and the supporting cells and fibrocytes back into the high-potassium endolymph that bathes the tops of the ear cells.

The vascularis on the lateral wall plays a critical part in pumping, but the gap junctions between the supporting cells and fibrocytes may provide the route of recycling. GJB2 was the gene to be associated with non-syndromic deafness phenotype DFNB1 (Kelsell, et al.,...
1997) and it has turned out to be remarkably common as a cause of deafness, accounting for up to 50 percent of childhood deafness in some populations (Estivill, et al., 1998). It is a small gene, with the whole protein-coding sequence located in one exon, which makes it relatively easy to screen for mutations. One mutation has been found to be particularly common, the 30delG mutation (also known as 35delG), a deletion of one base in a sequence of six guanine residues that starts at position 30. This 30delG mutation appears to have arisen independently in many populations, suggesting that it is a hypermutable region. Previous studies reported that as many as 1 in 31 people may be carriers of a mutation in GJB2, with most of them carrying the 30delG mutation (Denoyelle et al., 1997). Inherited hearing impairment affects 1 in 2000 newborns. Up to 50% of all patients with autosomal recessive non-syndromic pre-lingual deafness in different populations have mutations in the gene encoding the gap-junction protein connexin 26 (GJB2) at locus DFNB1 on chromosome 13q12 (Ignacio et al., 2002).

Hereditary hearing loss is either syndromic with additional clinical features or on syndromic if there is no other recognizable phenotypes. Worldwide prevalence of hereditary hearing loss is 1 in 2000 children. The affected children lack speech, language and effective communication skills and require specialized education. To date 142 non-syndromic hearing loci have been localized. And 57 of these loci are autosomal dominant, 77 autosomal recessive and 8 are X-linked and only 22 genes have been identified for recessive loci. Pendred syndrome and Usher syndrome are the two common syndromes associated with recessive deafness. Some of the non-syndromic loci are allelic variants of the genes causing syndrome like DFNB4/Pendred, DFNB2/Usher IB, DFNB12/Usher ID, DFNB18/Usher IC, and DFNB23/Usher IF. Hereditary hearing impairment is highly heterogeneous and it is estimated that up to 100 genes may be responsible for this disorder (Bitner et al., 2002).
OBJECTIVES

1. To determine the prevalence of recessive deafness in some families from Balochistan.
2. To draw the pedigree, collect blood samples from affected and normal siblings, purify DNA and estimate the DNA with gel electrophoresis.
3. To perform genotyping of DFNB1 specific STR markers by PAGE and construction of haplotype to know the linkage of a family to DFNB1 locus.
4. To sequence GJB2 gene to find out causative mutation in the gene.
5. To screen out carrier individual in our population thus to develop genetic counseling strategies to prevent deafness in our population, provide facilities of parental diagnosis and genetic counseling strategies.

JUSTIFICATION AND BENEFITS

➢ The proposed study will help to determine the prevalence of DFNB1 in families having recessive deafness in Balochistan population.
➢ This study will help to screen out carrier individuals in our population that will help to develop genetic counseling strategies to prevent deafness in Balochistan population.
➢ These studies will help to provide facilities of prenatal diagnosis and genetic counseling strategies.
PLAN OF WORK AND METHODOLOGY

Enrolment of affected families

Families will be located through the files of students from the school of hearing impairment. Family history and pedigree will be collected personally by visiting the families. Families with two or more hearing impaired individuals will be selected other relatives affected with deafness will also be included in the study depending on their willingness and availability. Informed consent will be obtained for participating in the study. Detailed history will be taken from each family to minimize the presence of other abnormalities and environmental causes for deafness. Families will be questioned about skin pigmentation differences in the eye color, hair pigmentation, problem relating to balance vision night blindness, like meningitis and typhoid etc. Audiometric testing will be performed for all deaf individuals where possible.

Collection of blood samples

10ml blood samples will be collected from all the affected individuals their normal siblings parents and grand parents to trace mode of inheritance. EDTA will be used as an anticoagulant to prevent the clothing of blood, which will be stored at 4c for not more than a week before DNA extraction.

DNA Extraction

The blood samples will be treated according to a standardized in-organic protocol already in practice at BUITEMS, in order to extract the DNA from the white blood cells.

Pedigree analysis

Pedigrees will be drawn on Cyrillic soft ware with the help of data taken from affected families. At least four generations family data as sibs, cousin marriage, monozygotic twins, dizygotic twins and gender will be shown by biological symbols.

Exclusion analysis for known loci

An initial search will be done to screen the families to link to known loci. The micro satellite markers will be amplified by polymerase chain reaction (PCR), using genomic DNA as a template. These markers are listed in Genome data base (GDB) and only
unlabelled makers will be used. The PCR product of each marker along with formamide will be loaded into poly Acrylamide gel electrophoresis (PAGE). Gel will be stained by using ethidium bromide solution.

**Construction of haplotype**

By reading alleles haplotype will be constructed to check weather a family is linked or unlinked to already known loci.

**Mutational Analysis**

GJB2 gene will be sequenced to find out the causative mutation responsible for deafness in all the linkage families to DFNB1 phenotype.

**Place of work and facilities available**

The BUITEMS laboratory is well equipped to carry out work program to achieve the objectives. Blood samples will be collected and processed for DNA isolated in BUITEMS. LOD Score will be calculated using the computer program and software available at the university. All the facilities for linkage analysis PCR are available at University.
REFERENCES


