Original Article

CANDIDATE GENES PREDICTION IN PAKISTANI FAMILIES WITH HEARING IMPAIRMENT BY USING BIOINFORMATIC APPROACH

PREDIÇÃO DE GENES CANDIDATOS EM FAMÍLIAS PAQUISTANESAS COM DEFICIÊNCIA AUDITIVA, UTILIZANDO UMA ABORDAGEM DE BIOINFORMÁTICA

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ABSTRACT: Multiple factors such as genetic and environmental, are involved in causing hearing impairment (HI). Severe or profound hearing loss affects approximately one in 1000 children worldwide and half of these cases are due to genetic factors. In case of hereditary nonsyndromic HI, approximately 75–80% of cases are involved in autosomal recessive inheritance and 15% of cases involve autosomal dominant inheritance. HI represents extreme genetic heterogeneity. In nonsyndromic deafness, 135 loci have been mapped till now including 77 autosomal recessive genes of which only 29 corresponding nuclear genes have been cloned. This study was designed to apply bioinformatic approach for reducing large number of candidate genes responsible for deafness to a handy number for their mutation analysis. Databases of expressed mouse inner ear genes and the expressed human cochlear genes were used to cross-reference all genes present in particular locus predicting candidate genes for phenotypes of nonsyndromic hereditary HI. These candidate genes are a source of starting point for mutation analysis along with genetic linkage to refine the loci. After characterization, it was observed that KIAA119 and EDN3 are candidate genes for deafness. In present study, there were total 14 loci and two genes KIAA119 and EDN3 were identified as candidate genes in locus 48 and locus 65 respectively. If mutation analysis of the two characterized genes is done, it will not be a comparatively time taking and labor-intensive process as these genes are only two in number.

KEYWORDS: Bioinformatic approach. Hearing impairment. Genes. Loci. Mutation analysis.

INTRODUCTION

Hearing loss is a genetically heterogeneous trait and in human, it is one of the most common neurosensory disorders (FRIEDMAN; GRIFFITH, 2003; MORTON; NANCE, 2006). In developed countries, genetics is responsible in over 60% of deafness cases. Although, etiology of hearing impairment (HI) is multifactoral, heredity plays a major role (YINGSHI et al., 2004). About 0.16% of newborns are affected by congenital HI (MEHL; THOMSON, 1998; MEHL; THOMSON, 2002). Approximately half of these cases are affected genetically, and the remaining cases are affected by some environmental cause (ROIZEN, 2003). Identifying and understanding the genetic factors in patients and families with HI provides an excellent method to study the genes involved in the auditory system (YINGSHI et al., 2004).

Hereditary hearing impairment (HHI) can be classified as nonsyndromic and syndromic hearing loss. Almost 70% of HHI are nonsyndromic and 30% are syndromic. There are more than 70 genes involved in nonsyndromic forms and more than 400 syndromes are linked with hearing loss (GRUNDFAST et al., 1999; BAYAZIT; YILMAZ, 2006). Nonsyndromic deafness is responsible for 60–70% of hereditary deafness cases involving more than 100 different genes with the patterns of autosomal dominant, autosomal recessive, X-linked, and mitochondrial inheritance (BITNER-GLINZICZ, 2002; CORDEIRO-SILVA et al.; 2011), being the autosomal recessive form the most common one (CORDEIRO-SILVA et al.; 2011). According to the pattern of inheritance, HHI can also be classified as autosomal recessive, autosomal dominant, X-linked and mitochondrial (BAYAZIT et al.; 2003; VAN-CAMP; SMITH, 1999).

The autosomal recessive form accounts for 75-80% of all nonsyndromic hearing cases. Hearing loss is usually prelingual and severe except for DFNB8 in which hearing loss is postlingual and

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rapidly progressive. Although the genes causing a child to be deaf are present from the time of conception, not all forms of genetic deafness or hereditary hearing impairment are necessarily expressed at birth. Each gene has two alleles: one comes from mother and other from father. In a recessive inheritance, both alleles have to be mutated to cause hearing impairment. If only one of the alleles has disease-causing mutation and other allele is normal, subject is a 'carrier' (BAYAZIT; YILMAZ, 2006).

Autosomal recessive deafness is represented by DFNB. Each type is numbered in the same order in which it was explained. For example, DFNB1, DFNB2, DFNB3 and DFNB4 are particular types of autosomal recessive deafness. There are 95 loci of autosomal recessive deafness, which are identified worldwide up to date.

Autosomal recessive deafness is usually caused by alterations of structures in inner ear. The inner ear has three parts. First part is a snail-shaped structure called cochlea which helps to process sound, second part is nerves which send information from cochlea to the brain, and third part represents the structures which are involved in balance (BROWN et al.; 2008).

All nonsyndromic deafness is inherited 55% to 80% in an autosomal recessive pattern. In many populations, mutations in GJB2 gene cause most cases of nonsyndromic deafness. Certain mutations in this gene are most common in particular populations, such as people of Ashkenazi, Jewish, Asian, or Caucasian ancestry. In many world populations, 50% of persons with autosomal recessive nonsyndromic hearing loss have mutations in GJB2 (ZELANTE et al.; 1997; ESTIVILL et al.; 1998; KELLEY et al.; 1998). Other 50% of cases are attributed to mutations in numerous other genes, many of which have been found to cause deafness in only one or two families (HILGERT et al.; 2009). In many populations, main cause of autosomal recessive nonsyndromic deafness is a change in the Cx26 protein, a gap junction protein encoded by GJB2 gene (13q11-12, OMIM 121011) (ESTIVILL et al.; 1998; WILCOX et al.; 2000; CORDEIRO-SILVA et al.; 2011). A total of three connexin genes have been characterized as genetic causes of deafness, namely, GJB2 (Connexin 26/Cx26), GJB6 (Connexin 30/Cx30), and GJB3 (Connexin 31/Cx31) (BRUZZONE et al.; 1996; KUMAR; GILULA,1996; BITNER-GLINZICZ, 2002).

Hearing impairment is relatively more common in those geographical areas where strong caste system acts as strong social bonds and produced solidarity in such society with high consanguinity. In countries like Pakistan, recessively inherited diseases are more prevalent where cousin marriages are common (JABER at al.; 1998).

To determine the prevalence of specific mutations related to inherited deafness in a population can contribute to the development of more efficient and affordable molecular diagnostic protocols and help in the genetic counseling of patients and their families (YINGSHI et al., 2004).

The map locations of a large number of nonsyndromic autosomal recessive deafness phenotypes are known, but specific genes responsible for all these phenotypes have not been identified. The cloning of genes involved in such phenotypes requires refinement of the suspected genomic interval to as short a region as possible by linkage analysis. However, it is not always possible to map a gene within an interval that is amenable for mutation analysis. It is a labor-intensive approach to do mutation analysis of all genes which are in large genomic intervals.

This study is designed to apply bioinformatic approach for reducing large number of candidate genes responsible for deafness to a handy number for their mutation analysis. Databases of expressed mouse inner ear genes and the expressed human cochlear genes is used to cross-reference all genes present in particular locus predicting candidate genes for phenotypes of nonsyndromic hereditary hearing impairment.

MATERIAL AND METHODS

List of most current information and all loci, which were identified so far in Pakistan for the various nonsyndromic hearing loss, was obtained from Hereditary Hearing Loss Homepage (www.hereditaryhearingloss.org) and survey of latest literature. The list of deafness loci (Table 1) with unknown genes for autosomal recessive nonsyndromic hearing loss which were identified in Pakistan were also compiled from the same web based source. The example of list of genes located at DFNB26 (4q31) is given in Table 2. NCBI (National Centre of Biotechnology and Information, www.ncbi.nlm.nih.gov) was used to make a list of all cloned and identified genes which were present in genomic intervals. NCBI had all the information regarding genes and transcripts located in a genomic interval. All the genes and transcripts for each specific locus which were gathered by using NCBI were compared against two databases of ear gene-expression. First database has all genes which are

expressed in the developing ear (HOLME et al.; 1999). This database has numerous genes which are, during inner ear development, expressed at different stages in two animal species. Second database taken was Morton Hearing Research Group (SKVORAK; MORTON, 2009).

Table 1. List of autosomal recessive nonsyndromic loci obtained from Hereditary Hearing Loss Homepage

Locus Name	Location	Locus Name	Location
DFNB26	4q31	DFNB48	15q23-q25.1
DFNB38	6q26-q27	DFNB51	11p13-p12
DFNB42	3q13.31-q22.3	DFNB55	4q12-q13.2
DFNB44	7p14.1-q11.22	DFNB62	12p13.2-p11.23
DFNB45	1q43-q44	DFNB63	11q13.2-q13.4
DFNB46	18p11.32-p11.31	DFNB65	20q13.2-q13.32
DFNB47	2p25.1-p24.3	DFNB72	19p13.3

Table 2. List of genes location at DFNB26 (4q 31)

No.	Name of gene	No.	Name of gene	No.	Name of gene	No.	Name of gene
1	PCDH18	13	LOC729407	25	LOC729870	37	LOC729350
2	LOC100128578	14	IL15	26	ARFIP1	38	LOC644914
3	LOC644879	15	LOC100130178	27	ANXA2P1	39	LOC100129858
4	LOC100129986	16	INPP4B	28	TLR2	40	LOC152586
5	LOC729350	17	GYPE	29	RNF175	41	ELMOD2
6	RAB33B	18	LOC345041	30	SFRP2	42	ARHGAP10
7	SETD7	19	OTUD4	31	LOC729558	43	ASSP8
8	LOC644914	20	LOC100131639	32	SH3D19	44	LOC285423
9	LOC100131429	21	LOC729497	33	LOC729830	45	FBXW7
10	LOC152594	22	LOC100129572	34	PET112L		
11	FBXW7	23	DKFZP434I0714	35	TIGD4		
12	TRIM2	24	TIGD4	36	LOC391706		-

A standard was made for gene to be considered as candidate gene. This standard was that if genes are present in one of the above two mentioned databases then these genes will be considered as candidate genes. Genes which were not present in both databases were not considered. Through this standard a list of possible candidate genes for the various loci was compiled (Table 3). After it, gene characterization was done using OMIM (Online Mendalian Inheritance in Man, www.ncbi.nlm.nih.gov/omim).

It was found that there were few genes which are present in OMIM but show no disorder (Table 4). List of characterized genes and details of characterized given in Table 5 were obtained from OMIM. Bioinformatic approach which is used in the present study is given as Figure 1.

Table 3. List of genes, which are present in human cochlear database and mouse inner ear expression databases

Locus	Genes from Cochlea				Genes from Mouse
DFNB26	PCDH18	FBXW7	ARFIP1	FBXW7	
	TRIM2				
DFNB 38	SLC22A3	MAP3K4	AGPAT4	PSMB1	
	QKI	THBS2	PHF10		
DFNB42	NCK1	AMOTL2	PPP2R3A		RBP2
DFNB44	ZNF117				
DFNB45	RYR2	FMN2			
DFNB 46	COLEC12	MRCL3	MRLC2	EPB41L3	
DFNB 47					MYCNSDC1
DFNB 48	ANP32A	KIF23	NEO1	KIAA1199	CHRNA5

	IREB2 MORF4L1	CTSH	PSMA4	ARNT2	
DFNB51	ELP4	TRAF6	LRP4		
DFNB55	SGCB	USP46			EPHA5
DFNB63	SHANK2	FCHSD2	KIAA0280		
DFNB 65	PFDN4	CTCFL	TMEPAI	ATP5E	
DFNB 72	EDN3 KHSRP	RAB22A ZNF20	TUBB1		

Table 4. List of genes, which are present in OMIM but shown no disorder

Locus	*Location*	*Genes from Cochlea*			*Genes from Mouse*
DFNB26	4q 31	PCDH18	FBXW7	FBXW7	Nil
DFNB 38	6q26-q27	SLC22A3	PSMB1		Nil
DFNB44	7p14.1-q11.22	ZNF117			Nil
DFNB45	1q43-44	FMN2			Nil
DFNB 46	18p11.32-p11.31	COLEC12	EPB41L3		Nil
DFNB47	*2p25.1-p24.3*	KIDINS220	CPSF3	NAG	*MYCN*
DFNB 48	15q23-q25.1	KIF23 CTSH	IREB2 ARNT2	PSMA4	Nil
DFNB51	11p13-p12	ELP4	TRAF6	LRP4	Nil
DFNB55	4q12-q13.2	IGFBP7	EPHA5	UGT2B17	EPHA5
DFNB63	11q13.2-q13.4	SHANK2	PFDN4		Nil
DFNB 65	20q13.2-q13.32	TMEPAI	ATP5E		Nil
DFNB 72	19p13.3	KHSRP	ZNF20		Nil

Table 5. Details of genes, which have been characterized

Gene symbol	Locus	Location	Description	OMIM number	Disease
THBS2*	DFNB 38	6q27	Thrombospondin 2	188061	Lumbar disc herniation, susceptibility to
KIAA1199*	DFNB 48	15q24	KIAA1199 gene	608366	Deafness, nonsyndromic
EDN3*	DFNB 65	20q13.2 - q13.3	Endothelin-3	131242	Shah-Waardenburg syndrome, congenital, Hirschsprung disease
SGCB*, LGMD2E*	DFNB 55	4q12	Sarcoglycan, beta (43kD dystrophin-associated glycoprotein)	600900	Muscular dystrophy, limb-girdle, type 2E,
CHRNA5**, LNCR2**	DFNB 48	15q25.1	Cholinergic receptor, neuronal nicotinic,	118505	Lung cancer susceptibility 2

* Genes from cochlea of human, ** Genes from mouse

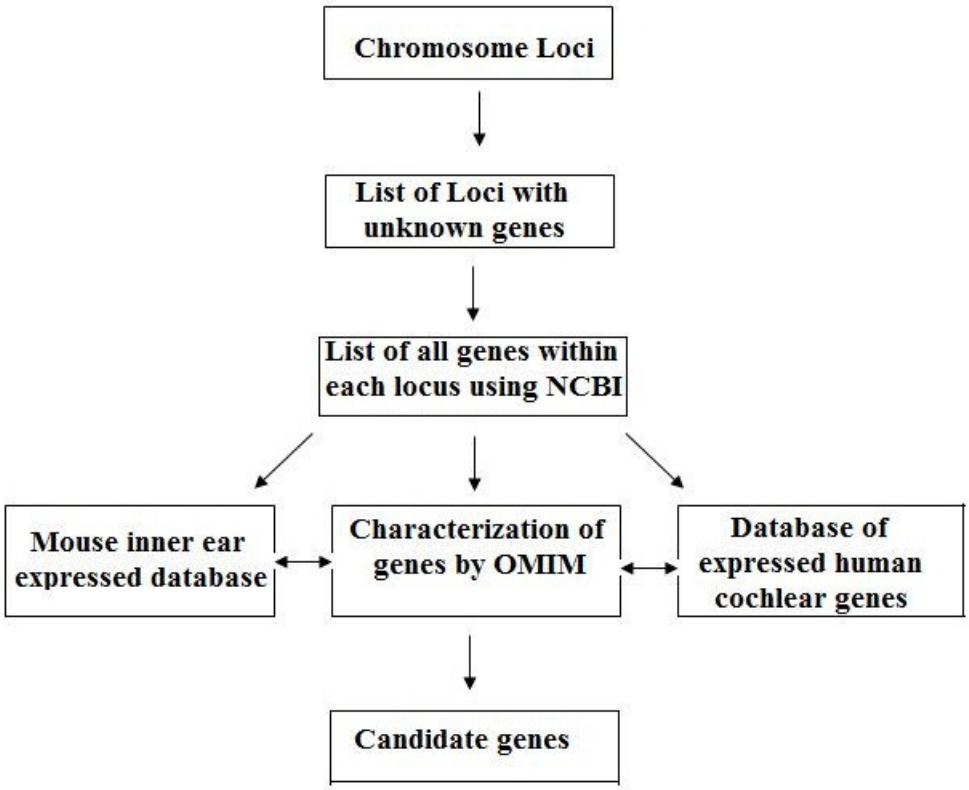

Figure 1. Scheme of predicting candidate genes. The rectangles show tasks that were processed in the sequence as indicated by arrows.

RESULTS AND DISCUSSION

To generate a set of candidate genes for the autosomal recessive nonsyndromic deafness, which are found in Pakistan, bioinformatic approach was employed.

The list of deafness loci with unknown genes for autosomal recessive forms identified in Pakistan were compiled from Hereditary Hearing Loss Homepage (Table 1).

NCBI is used to make a list of all cloned and identified genes which are present in each of listed genomic intervals. The example of list of genes located at DFNB26 (4q31) is given in Table 2. Similarly all genes present in Autosomal Recessive Nonsyndromic Loci were found.

Fetal cochlear cDNA library [22] and EST database (updated as of 2002) of the Morton Hearing Research Group [23] and database of mouse inner ear expression was used to make a list of genes, which are present in Human Cochlear database and mouse inner ear expression database (Table 3).

Genes which were not present in both databases were not considered. Through this standard, list of possible candidate genes for the various loci was compiled. After it, gene characterization was done using OMIM.

After characterization, it has been concluded that KIAA119 and EDN3 are candidate genes for deafness.

In countries like Pakistan, recessively inherited diseases are more prevalent where cousin marriages are common (JABER et al.; 1998). Nonsyndromic hearing loss (NSHL) is the most genetically heterogeneous trait known. It is estimated that 85% of nonsyndromic hearing loss is caused by autosomal recessive defects (COHEN; GORLIN, 1995; VAN-CAMP et al.; 1997). For autosomal recessive nonsyndromic HI, usually HHI has prelingual onset and it has all the frequencies and is severe to profound.

Autosomal recessive nonsyndromic childhood / prelingual hearing loss accounts for approximately 80% of genetic hearing loss cases (MORTON , 1991). Because of the improved molecular genetic techniques, 95 loci for autosomal recessive nonsyndromic HI are identified and to date, 40 of the corresponding genes have been identified. Many of these deafness loci have been mapped either in endogamous populations or in children of families having consanguineous marriages (FRIEDMAN; GRIFFITH, 2003).

However, of the autosomal recessive nonsyndromic HI genes, only a few GJB2, CDH23 and WFS1 have been well characterized in terms of prevalence and sequence variants found in different populations (SANTOS et al.; 2005). Among hereditary hearing deficiencies, approximately half is caused by mutations in the Gap Junction Protein Beta-2 (GJB2) gene, which encodes the protein Connexin 26 (Cx26). There are four mutations in this gene that present high prevalence in specific ethnical groups, namely, 35delG, 167delT, 235delC, and W24X (YINGSHI et al., 2004). There is now good evidence that up to 50% of recessive nonsyndromic hearing loss may be accounted for mutations in GJB2 encoding Cx26 in Caucasian and European populations (DENOYELLE et al.; 1997; ZELANTE et al.; 1997). The 35delG mutation in the GJB2 gene is the major cause of genetic deafness in Caucasian populations, and may be present in homozygosis or in compound heterozygosis (associated with other mutations in the genes GJB2 or GBJ6) (GABRIEL et al.; 2001; PIATTO; MANIGLIA, 2001; CORDEIRO-SILVA et al.; 2011). The mutation del (D13S1830/GJB6) is the second most frequent mutation related to autosomal recessive deafness in European populations, Jewish populations and also in Brazil (GABRIEL et al.; 2001; BATISSOCO et al.; 2009; CORDEIRO-SILVA et al.; 2011).

In present study, bioinformatic approach is used to predict candidate genes which are associated with nonsyndromic HI. NCBIdatabase was used to make a list of all cloned and identified genes which are present in genomic intervals. After it all the genes and transcripts of each locus were compared against databases of expressed mouse inner ear genes and the expressed human cochlear genes. If gene-by-gene approach is used for mutation analysis, there are too many genes and mutation analysis of all these genes will be time taking and labor-intensive approach. So we used the human cochlea (SKVORAK; MORTON, 2009), and mouse inner ear expression databases (HOLME et al.; 1999), to eradicate certain genes from the candidate list that were not expressed in these organs.

Mouse inner ear expressed database contains numerous genes present in two animal species and database of the expressed human cochlear genes is obtained from fetal cochlear cDNA library and EST database (updated as of 2002) of the Morton Hearing Research Group (SKVORAK; MORTON, 2009). The data present in this set was adapted from Unigene (PONTIUS et al.; 2003). The database has 14,805 ESTs, and Unigene sort 12,624 ESTs into 4,519 independent clusters. Remaining ESTs are not classified by Unigene because of possible contaminating sequences, very small inserts, or excessive repetitive elements. Those genes were considered for candidacies which were present in either of the previously mentioned databases. Genes which were not present in both databases were not considered. So we compiled a list of possible candidate genes for various loci. To further narrow down and refine this list, characterization of candidate genes was done by OMIM. After characterization, it is concluded that KIAA119 and EDN3 are candidate genes.

ALSABER et al., has also used bioinformatics to predict genes for deafness but he used different filters along with filters of databases to predict genes e.g. interacting proteins were also find in his approach. In his approach, there are many candidate genes and their mutation analysis will be a time consuming and labor-intensive process.

In present study, there were total 14 loci and two genes KIAA119 and EDN3 were identified as candidate genes in locus 48 and locus 65 respectively. In remaining 12 loci, no gene was identified as candidate gene because of the reason that there are many genes, which are not characterized yet according to OMIM Database. With the passage of time OMIM database will be updated and data about the characterization of genes will be available, we will be able to find more genes in the remaining loci in the future.

KIAA1199 is one of inner-ear-specific genes and it is expressed in the cochlea and vestibule tissues. The KIAA1199 protein may be vital for auditory function and its mutated forms may cause nonsyndromic hearing loss (ABE, 2003). It was reported that up regulation of the KIAA1199 gene is associated with cellular mortality (MICHISHITA, 2005).

Endothelin 3, also known as EDN3, is a human gene (CALDERONE, 1994). The protein encoded by this gene is a member of the endothelin family. Endothelins are endothelium-derived vasoactive peptides, which are involved, in different biological functions. The active form of this protein is a 21 amino acid peptide processed from the precursor protein. The active peptide is a ligand for endothelin receptor type B (EDNRB). Hirschsprung disease (HSCR) and Waardenburg syndrome (WS) are congenital malformations regarded as neurocristopathies since both disorders involve neural crest-derived cells (HIRSCHSPRUNG, 1888; WAARDENBURG, 1951; BOLANDE, 1973). The WS-HSCR association (Shah-Waardenburg syndrome) (OMMEN; MCKUSICK, 1979) is a rare autosomal recessive condition that occasionally has

been ascribed to mutations of the endothelin-receptor B (EDNRB) gene (PUFFENBERGER et al.; 1994; ATTIE et al.; 1995).

The map locations of a large number of nonsyndromic autosomal recessive deafness phenotypes are known, but the specific genes responsible for all these phenotypes have not been identified. The cloning of genes involved in such phenotypes needs short genomic interval which can be done by linkage analysis. If the mutation analysis of all genes is done, it will be time taking and labor-intensive process. Bioinformatic approach that is presented here can reduce very large number of genes into a number which can be managed for mutation analysis.

CONCLUSION

After characterization, it has been concluded that KIAA119 and EDN3 are candidate genes for deafness. If mutation analysis of these two genes is done, it will not be a time taking and labor-intensive process as these genes are only two in number. After mutation analysis, gene therapy of these two will also be possible.

RESUMO: Diversos fatores, tais como genéticos e ambientais, estão envolvidos na causa da deficiência auditiva (HI). A perda auditiva severa ou profunda afeta aproximadamente uma em cada 1000 crianças em todo o mundo e metade destes casos são devidos a fatores genéticos. Em relação a HI não-sindrômica hereditária, cerca de 75-80% dos casos estão envolvidos na herança autossômica recessiva e 15% dos casos envolvem herança autossômica dominante. HI representa extrema heterogeneidade genética. Em casos de surdez, 135 loci foram mapeados até agora, incluindo 77 genes autossômicos recessivos das quais apenas 29 genes correspondentes nucleares foram clonados. Este estudo foi desenhado para aplicar abordagem de bioinformática a fim de reduzir o grande número de genes candidatos responsáveis pela surdez a um número útil para a análise de mutação. Bases de dados de genes expressos do ouvido interno em camundongos e de genes expressos na cóclea em humanos foram usados para cruzar todos os genes presentes no locus específico prevendo genes candidatos para os fenótipos de HI não sindrômica hereditária. Estes genes candidatos são uma fonte de ponto de partida para a análise de mutação, juntamente com a ligação gênica para refinar os locos. Após a caracterização, verificou-se que KIAA119 e EDN3 são genes candidatos para a surdez. No presente estudo, houve um total de 14 locos e dois genes KIAA119 e EDN3 foram identificados como genes candidatos no locus 48 e locus 65, respectivamente. Se a análise de mutação dos dois genes caracterizados for feita, não será um processo comparativamente longo e trabalhoso uma vez que são apenas dois genes.

PALAVRAS CHAVES: Bioinformática. Deficiência auditiva. Genes. Loci. Análise de mutação.

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