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In Silico Profiling of Regulatory Micrornatargets in *GJB3* Gene

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Abstract: MicroRNAs are novel class of small non-coding RNAs that target and silence various genes across diverse signalling pathways comprising key physiological networks. Despite an important gene regulator, computational and experimental approaches are still infancy and an overall understanding of the importance of these regulatory transcripts is still far of satisfactory. Currently computer based prediction remains the only source for rapid identification of putative microRNA (miRNA) target. An increasing number of experimentally validated miRNAs targets are now available; utilizing this additional information in search of further targets may help to improve the specificity of bioinformatics based methods for predicting gene target site. An effort has been made in this pilot approach to investigate regulatory miRNA sequence and their location in the candidate gene of hearing impairment (*GJB3* gene) using online bioinformatics tool called miRDB. Using this online database, we identified eleven specific miRNAs (i.e: hsa-miR-466, hsa-miR-3613-3p, hsa-miR-16-1-3p, hsa-miR-144-3p, hsa-miR-561-5p, hsa-miR-326, hsa-miR-2110, hsa-mir-4510, hsa-mir-4419a, hsa-miR-3605-5p and hsa-miR-4738-3p). These targets different regions in the *GJB3*gene.Multiple sequence alignment to investigate whether similarities exists among mature sequences of these selected miRNAs were also performed. This data set will provide concrete bases and will help in experimental validation of these miRNAs.

Key words: Bioinformatics Tools · *GJB3* · Microrna · Multiple Alignment · Mirdb

INTRODUCTION

MicroRNAs (miRNAs) are small, endogenous, noncoding RNAs, usually between 18 and 25 nucleotides in length, involved in the regulation of cellular and developmental processes through post-transcriptional gene repression [1-3]. These are expressed either as single transcription unit or as polycistronic transcripts from miRNA clusters, encoded within intronic or intergenic regions of the genome [4, 5]. Primary or pri-miRNAs are formed by polymerase II that drives transcription of miRNAs as inverted repeats embedded in long primary transcripts, which spontaneously fold to form imperfect long hairpins [6-8]. It is then processed into shorter hairpin precursor miRNAs, or pre-miRNAs, in the nucleus byRNase III enzyme complex of DROSHA (RNASEN)/DGCR8 [9]. Pre miRNAs are transferred into cytoplasm by a trans-nuclear membrane protein called exportin 5 (XPO5) [7, 8]. In cytoplasm, the RNase III enzyme DICER1 cut the pre-miRNA to form a double-stranded miRNA duplex comprising a mature miRNA (guide strand) and a partially complementary passenger, or ‘‘star’’ (*), strand [10,11]. The fully matured miRNA associates with argonaute proteins in the RNA-induced silencing complex (RISC) to direct translational repression by binding to the regions of complementarily in 3'untranslated regions of target mRNAs [12,13].

RISC loading has been shown to be largely asymmetric, with only a single strand of miRNA duplex being incorporated to direct gene silencing [14]. However, some miRNA duplexes encode mature miRNAs on both strands and recent evidence suggests that strand based in miRNA expression may be influenced by tissue-specific processing factors [15]. The altered expression may be due to a variety of mechanisms including transcriptional regulation, amplification, deletion, mutation and epigenetic silencing [16]. Although miRNA signatures were established in tumor cells [17,18], recent studies revealed that the potential capabilities of miRNAs as blood-based biomarkers for cancer and other diseases[19].

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Approximately 98% of RNAs in mammalian cells do not code for proteins. The epigenetic factors are associated with hearing loss [20], elevating the possibility that noncoding RNAs, such as miRNAs, might also involve in inner ear development and hearing loss.

In Silico miRNA target identification: Both computational and experimental approaches show that thousands of human genes are regulated by miRNAs [21, 22]. The functional characterization of miRNAs has become one of the most interesting research domains because of their critical roles in gene expression regulation. However, accurate target prediction is one of the major issue faced bymiRNAsbasedresearch is the lack of computational tools.Various types of bioinformatics tools are developed to give insight into the molecular functionalities of microRNA gene regulatory network. One strategy for target prediction is to use machine learning approach but has not been applied to miRNA target prediction to a greater extant. An increasing number of experimentally validated microRNAs targets are now available that utilize this additional information in search of further targets. Which may help to improve the specificity of *in silico* based methods for target site prediction.

Structure and function of *GJB3:* The *GJB3* gene encoding the gap junction protein connexin 31 (Cx31) was initially mapped to chromosome 1p35- p33 and heterozygous mutations were shown to cause ADNSHL (Autosomal Dominant Non-Syndromic Hearing Loss) [23,24]. *GJB3* mutations have been reported to cause ARNSHLand a skin disorder callederythro keratodermiavariabilis (MIM 133200). Biallelic *GJB3* mutations causing ARNSHL have been reported once in two families in which patients were compound heterozygote for two different *GJB3* mutations [25]. Digenic inheritance of non-syndromic deafness caused by mutations in GJB2 and *GJB3* has recently been reported. Two different missense mutations (p.N166S and p.A194T) of *GJB3* were found in compound heterozygosity with the c.235delC and c.299delAT mutations of GJB2 in three simplex families from China [26].

One gap junction consists of a cluster of closely packed pairs of trans-membrane channels, the connexins, through which materials of low molecular weight diffuse from one cell to a neighbouring cell. Involvement in disease, *GJB3*is a cause of erythrokeratodermiavariabilis (EKV) [MIM:133200]. EKV is a genodermatosis characterized by the appearance of two independent skin lesions: transient figurate erythematous patches and hyperkeratosis that is usually localized but occasionally occurs in its generalized form. Clinical presentation varies significantly within a family and from one family to another. Palmoplantarkeratoderma is present in around 50% of all cases. Defects in *GJB3* are the cause of deafness autosomal dominant type 2B (DFNA2B) [MIM:612644]. DFNA2 is a form of sensorineural hearing loss. Sensorineural deafness results from damage to the neural receptors of the inner ear, the nerve pathways to the brain, or the area of the brain that receives sound information.

MATERIALS AND METHODS

List of genes associated with hearing loss identified in human were collected from literature and publicly available databases. All relevant publications were identified after searching PubMed (http://www.ncbi. nlm. nih. gov/pubmed) with key phrases, such as gene, genetics, hearing, candidate's genes for hearing, physiology of hearing impairments etc. Among the list of candidate genes, *GJB3* were selected for this study.

We used miRDB for potentialmiRNA target identification in *GJB3*. To briefly explain, miRDB is an online computational tool for miRNA target prediction and functional annotations [5]. All the targets were predicted by a bioinformatics tool MirTarget2, which was developed by analysing thousands of genes impacted by miRNAs with an SVM learning machine. Common features associated with miRNA target binding have been identified and used to predict miRNA targets. miRDB hosts predicted miRNA targets in five species: human, mouse, rat, dog and chicken.

RESULTS AND DISCUSSION

A large number of microRNAs have been identified across a variety of different species. Being as gene regulators, identification of microRNA targets has become an essential step toward understanding these regulatory mechanisms. *In silico* analysis of prediction presently remains the only source for rapid identification of a putative microRNA target because it helps in efficiently allocating experimental resources. *In silico* microRNA target prediction programs are based on specific parameters that can give slightly different results for the same target input. Such limitations can be partially compensated by predicting targets using more than one program. These approaches have been quite successful for a few top ranked results in different diseases' models. In the present study, an attempt was made to predict

target sites in the sequences of selected genes through advance bioinformatics approaches such as miRDB (online tool) were used.

Using this database, 11 potential miRNAs in the sequence of *GJB3* gene were identified, given in Fig. 2. This computer based approach for identification of target site in selected genes will provide base for experimental validation of these novel microRNAs in *GJB3*. If validated experimentally, these miRNAs may be used as novel biomarker for hearing impairment in human.

Salih *et al*. [27] carried out study on mutation in GJB3 and GJB4 genes involved in Deafness in two Sudanese families using next generation sequencing technique. Similarly a study was carried out in Kenyan and Sudanese children suffering from NSARD (non-syndromic autosomal recessive deafness) due to variants of GJB2 gene. As compare to other areas and other ethnic groups, deafness-associated variants of the coding region of GJB3 were rare in Kenya and Sudan, which clearly demonstrate a causative role of other genetic or epigenetic factors [28]. As compare to these studies our results also suggest that GJB3 may be possibly responsible for hearing impairment.

GJB2 deafness-associated mutations are ethnicity-specific [30] and occur witha frequency of 30 - 50% in most Caucasian populations and 17%in Ghanaians, but they are remarkably rare among Kenyan and Sudanese populations [31].Similarly the study of Lopez-Bigas *et al*. [29] pointed out the strong association of E204A with deafness. From these studies [27-29] it can be hypothesized that E204A mutation has pathogenic effect on Connexins' activity which lead to hearing loss.

Trotta *et al*. [32] analysed the genotypic composition of GJB2 gene and did not witnessed any significant difference between deaf and control group suggesting that GJB2 gene or its genotypic combination with any other gene is not attributing to deafness in Northern Cameroon. Their results were different than those studies conducted at Kenya and Sudan. This also indicate that there are many factors contributing to deafness which vary within different populations such as presence of infections, malnutrition, poverty and poor access to health care. These factors may play a more predominant role than hereditary factors [33].

Details names of the candidate miRNAs, their sequence order, size, seed location, target score, target gene symbol and protein size of the target gene is given for each microRNA (Fig. 3 to Fig. 13).

Multiple alignment of selected miRNA sequences targeting *GJB3:* After identification of list of selected miRNAs (i.e: hsa-miR-466, hsa-miR-3613-3p, hsa-miR-16-1-3p, hsa-miR-144-3p, hsa-miR-561-5p, hsa-miR-326, hsa-miR-2110, hsa-mir-4510, hsa-mir-4419a, hsa-miR-3605-5p and hsa-miR-4738-3p), it was investigated, if there is any similarities among the sequences of these selected miRNAs. Multiple alignment of the mature sequences of

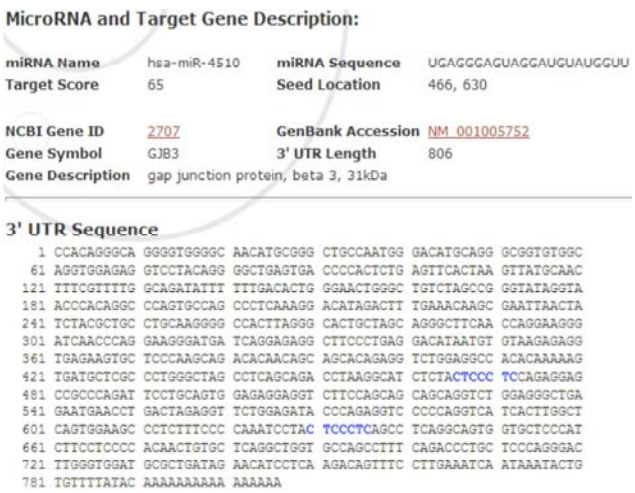
```
hsa-miR-466          auacacau-acacgca-------acacacau---
hsa-miR-3613-3p      -------acaaaaaaaaaagc--ccaacccuuc-
hsa-miR-16-1-3p      --ccaguauuaac--ugu--gcugcuga------
hsa-miR-144-3p       -uacaguauagaugaugu--acu-----------
hsa-miR-561-5p       ----------aucaaggauc-uuaaacuuugcc
hsa-miR-326          -----ccucugggcccuu--ccuccag-------
hsa-miR-2110         --------uuggggaa-acggccgcugagug---
hsa-mir-4510         ---------ugagggaguaggauguaugguu---
hsa-mir-4419a        ---------ugagggaggagacugca--------
hsa-miR-3605-5p      ---------ugaggauggau--agcaaggaagcc
hsa-miR-4738-3p      ---------ugaaacuggag--cgccuggagga-
```

Fig. 1: Represents multiple sequence alignment of 11 selected miRNA genes.

Gene 2707 is predicted to be targeted by 11 miRNAs in miRDB

Target Detail	Target Rank	Target Score	miRNA Name	Gene Symbol	Gene Description
Details	1	65	hsa-miR-4510	GJB3	gap junction protein, beta 3, 31kDa
Details	2	58	hsa-miR-4419a	GJB3	gap junction protein, beta 3, 31kDa
Details	3	57	hsa-miR-3605-5p	GJB3	gap junction protein, beta 3, 31kDa
Details	4	56	hsa-miR-16-1-3p	GJB3	gap junction protein, beta 3, 31kDa
Details	5	55	hsa-miR-144-3p	GJB3	gap junction protein, beta 3, 31kDa
Details	6	55	hsa-miR-3613-3p	GJB3	gap junction protein, beta 3, 31kDa
Details	7	52	hsa-miR-2110	GJB3	gap junction protein, beta 3, 31kDa
Details	8	51	hsa-miR-561-5p	GJB3	gap junction protein, beta 3, 31kDa
Details	9	51	hsa-miR-4738-3p	GJB3	gap junction protein, beta 3, 31kDa
Details	10	51	hsa-miR-466	GJB3	gap junction protein, beta 3, 31kDa
Details	11	51	hsa-miR-326	GJB3	gap junction protein, beta 3, 31kDa

Fig. 2: List of 11 miRNA predicated by online tool miRDB in the sequence of *GJB3* gene.



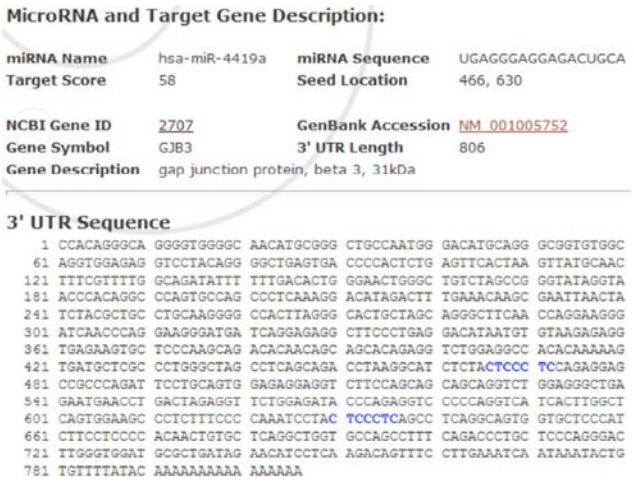
MicroRNA and Target Gene Description:

miRNA Name	hsa-miR-4510	**miRNA Sequence**	UGAGGGAGUAGGAUGUAUGGUU
Target Score	65	**Seed Location**	466, 630
NCBI Gene ID	2707	**GenBank Accession**	NM_001005752
Gene Symbol	GJB3	**3' UTR Length**	806
Gene Description	gap junction protein, beta 3, 31kDa		

3' UTR Sequence

```
  1 CCACAGGGCA GGGGTGGGGC AACATGCGGG CTGCCAATGG GACATGCAGG GCGGTGTGGC
 61 AGGTGGAGAG GTCCTACAGG GGCTGAGTGA CCCCACTCTG AGTTCACTAA GTTATGCAAC
121 TTTCGTTTTG GCAGATATTT TTTGACACTG GGAACTGGGC TGTCTAGCCG GGTATAGGTA
181 ACCCACAGGC CCAGTGCCAG CCCTCAAAGG ACATAGACTT TGAAACAAGC GAATTAACTA
241 TCTACGCTGC CTGCAAGGGG CCACTTAGGG CACTGCTAGC AGGGCTTCAA CCAGGAAGGG
301 ATCAACCCAG GAAGGGATGA TCAGGAGAGG CTTCCCTGAG GACATAATGT GTAAGAGAGG
361 TGAGAAGTGC TCCCAAGCAG ACACAACAGC AGCACAGAGG TCTGGAGGCC ACACAAAAAG
421 TGATGCTCGC CCTGGGCTAG CCTCAGCAGA CCTAAGGCAT CTCTACTCCC TCCAGAGGAG
481 CCGCCCAGAT TCCTGCAGTG GAGAGGAGGT CTTCCAGCAG CAGCAGGTCT GGAGGGCTGA
541 GAATGAACCT GACTAGAGGT TCTGGAGATA CCCAGAGGTC CCCCAGGTCA TCACTTGGCT
601 CAGTGGAAGC CCTCTTTCCC CAAATCCTAC TCCCTCAGCC TCAGGCAGTG GTGCTCCCAT
661 CTTCCTCCCC ACAACTGTGC TCAGGCTGGT GCCAGCCTTT CAGACCCTGC TCCCAGGGAC
721 TTGGGTGGAT GCGCTGATAG AACATCCTCA AGACAGTTTC CTTGAAATCA ATAAATACTG
781 TGTTTTATAC AAAAAAAAAA AAAAAA
```

Fig. 3: Illustrate the miRNA sequence (has-miR-4510), its seed sequence and it location in the sequence of target gene *GJB3*predicated by online tool miRDB.



MicroRNA and Target Gene Description:

miRNA Name	hsa-miR-4419a	**miRNA Sequence**	UGAGGGAGGAGACUGCA
Target Score	58	**Seed Location**	466, 630
NCBI Gene ID	2707	**GenBank Accession**	NM_001005752
Gene Symbol	GJB3	**3' UTR Length**	806
Gene Description	gap junction protein, beta 3, 31kDa		

3' UTR Sequence

```
  1 CCACAGGGCA GGGGTGGGGC AACATGCGGG CTGCCAATGG GACATGCAGG GCGGTGTGGC
 61 AGGTGGAGAG GTCCTACAGG GGCTGAGTGA CCCCACTCTG AGTTCACTAA GTTATGCAAC
121 TTTCGTTTTG GCAGATATTT TTTGACACTG GGAACTGGGC TGTCTAGCCG GGTATAGGTA
181 ACCCACAGGC CCAGTGCCAG CCCTCAAAGG ACATAGACTT TGAAACAAGC GAATTAACTA
241 TCTACGCTGC CTGCAAGGGG CCACTTAGGG CACTGCTAGC AGGGCTTCAA CCAGGAAGGG
301 ATCAACCCAG GAAGGGATGA TCAGGAGAGG CTTCCCTGAG GACATAATGT GTAAGAGAGG
361 TGAGAAGTGC TCCCAAGCAG ACACAACAGC AGCACAGAGG TCTGGAGGCC ACACAAAAAG
421 TGATGCTCGC CCTGGGCTAG CCTCAGCAGA CCTAAGGCAT CTCTACTCCC TCCAGAGGAG
481 CCGCCCAGAT TCCTGCAGTG GAGAGGAGGT CTTCCAGCAG CAGCAGGTCT GGAGGGCTGA
541 GAATGAACCT GACTAGAGGT TCTGGAGATA CCCAGAGGTC CCCCAGGTCA TCACTTGGCT
601 CAGTGGAAGC CCTCTTTCCC CAAATCCTAC TCCCTCAGCC TCAGGCAGTG GTGCTCCCAT
661 CTTCCTCCCC ACAACTGTGC TCAGGCTGGT GCCAGCCTTT CAGACCCTGC TCCCAGGGAC
721 TTGGGTGGAT GCGCTGATAG AACATCCTCA AGACAGTTTC CTTGAAATCA ATAAATACTG
781 TGTTTTATAC AAAAAAAAAA AAAAAA
```

Fig. 4: Illustrate the miRNA sequence (has-miR-4419a), its seed sequence and it location in the sequence of target gene *GJB3* predicated by online tool miRDB.

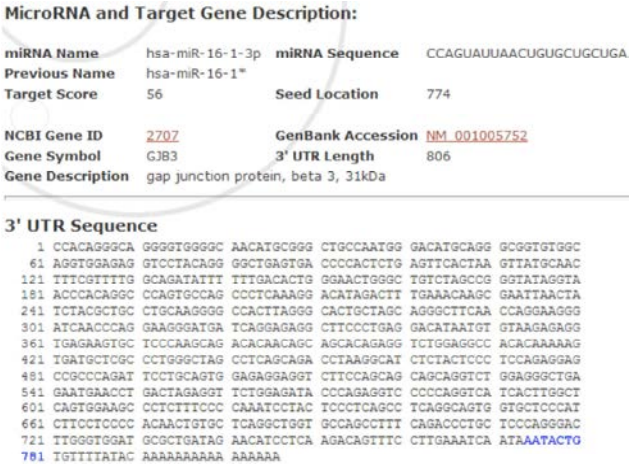
MicroRNA and Target Gene Description:

miRNA Name	hsa-miR-3605-5p	**miRNA Sequence**	UGAGGAUGGAUAGCAAGGAAGCC
Target Score	57	**Seed Location**	743
NCBI Gene ID	2707	**GenBank Accession**	NM_001005752
Gene Symbol	GJB3	**3' UTR Length**	806
Gene Description	gap junction protein, beta 3, 31kDa		

3' UTR Sequence

```
  1 CCACAGGGCA GGGGTGGGGC AACATGCGGG CTGCCAATGG GACATGCAGG GCGGTGTGGC
 61 AGGTGGAGAG GTCCTACAGG GGCTGAGTGA CCCCACTCTG AGTTCACTAA GTTATGCAAC
121 TTTCGTTTTG GCAGATATTT TTTGACACTG GGAACTGGGC TGTCTAGCCG GGTATAGGTA
181 ACCCACAGGC CCAGTGCCAG CCCTCAAAGG ACATAGACTT TGAAACAAGC GAATTAACTA
241 TCTACGCTGC CTGCAAGGGG CCACTTAGGG CACTGCTAGC AGGGCTTCAA CCAGGAAGGG
301 ATCAACCCAG GAAGGGATGA TCAGGAGAGG CTTCCCTGAG GACATAATGT GTAAGAGAGG
361 TGAGAAGTGC TCCCAAGCAG ACACAACAGC AGCACAGAGG TCTGGAGGCC ACACAAAAAG
421 TGATGCTCGC CCTGGGCTAG CCTCAGCAGA CCTAAGGCAT CTCTACTCCC TCCAGAGGAG
481 CCGCCCAGAT TCCTGCAGTG GAGAGGAGGT CTTCCAGCAG CAGCAGGTCT GGAGGGCTGA
541 GAATGAACCT GACTAGAGGT TCTGGAGATA CCCAGAGGTC CCCCAGGTCA TCACTTGGCT
601 CAGTGGAAGC CCTCTTTCCC CAAATCCTAC TCCCTCAGCC TCAGGCAGTG GTGCTCCCAT
661 CTTCCTCCCC ACAACTGTGC TCAGGCTGGT GCCAGCCTTT CAGACCCTGC TCCCAGGGAC
721 TTGGGTGGAT GCGCTGATAG AACATCCTCA AGACAGTTTC CTTGAAATCA ATAAATACTG
781 TGTTTTATAC AAAAAAAAAA AAAAAA
```

Fig. 5: Illustrate the miRNA sequence (has-miR-3605-5p), its seed sequence and it location in the sequence of target gene *GJB3* predicated by online tool miRDB.



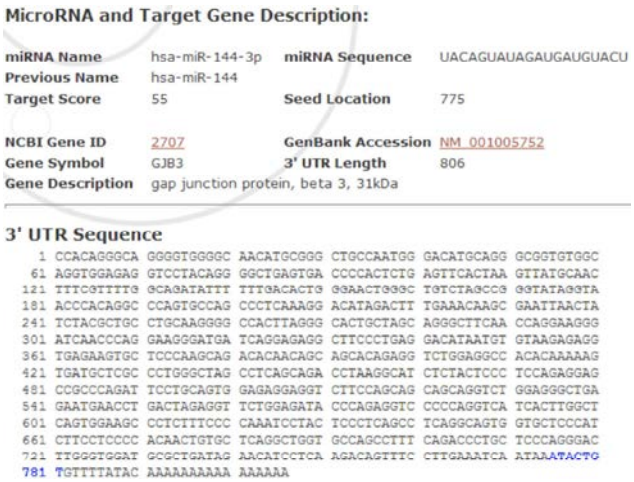
MicroRNA and Target Gene Description:

miRNA Name	hsa-miR-16-1-3p	**miRNA Sequence**	CCAGUAUUAACUGUGCUGCUGA
Previous Name	hsa-miR-16-1*		
Target Score	56	**Seed Location**	774
NCBI Gene ID	2707	**GenBank Accession**	NM_001005752
Gene Symbol	GJB3	**3' UTR Length**	806
Gene Description	gap junction protein, beta 3, 31kDa		

3' UTR Sequence

```
  1 CCACAGGGCA GGGGTGGGGC AACATGCGGG CTGCCAATGG GACATGCAGG GCGGTGTGGC
 61 AGGTGGAGAG GTCCTACAGG GGCTGAGTGA CCCCACTCTG AGTTCACTAA GTTATGCAAC
121 TTTCGTTTTG GCAGATATTT TTTGACACTG GGAACTGGGC TGTCTAGCCG GGTATAGGTA
181 ACCCCACAGGC CCAGTGCCAG CCCTCAAAGG ACATAGACTT TGAAACAAGC GAATTAACTA
241 TCTACGCTGC CTGCAAGGGG CCACTTAGGG CACTGCTAGC AGGGCTTCAA CCAGGAAGGG
301 ATCAACCCAG GAAGGGATGA TCAGGAGAGG CTTCCCTGAG GACATAATGT GTAAGAGAGG
361 TGAGAAGTGC TCCCAAGCAG ACACAACAGC AGCACAGAGG TCTGGAGGCC ACACAAAAAG
421 TGATGCTCGC CCTGGGCTAG CCTCAGCAGA CCTAAGGCAT CTCTACTCCC TCCAGAGGAG
481 CCGCCCAGAT TCCTGCAGTG GAGAGGAGGT CTTCCAGCAG CAGCAGGTCT GGAGGGCTGA
541 GAATGAACCT GACTAGAGGT CTGGAGATA CCCAGAGGTC CCCCAGGTCA TCACTTGGCT
601 CAGTGGAAGC CCTCTTTCCC CAAATCCTAC TCCCTCAGCC TCAGGCAGTG GTGCTCCCAT
661 CTTCCTCCCC ACAACTGTGC TCAGGCTGGT GCCAGCCTTT CAGACCCTGC TCCCAGGGAC
721 TTGGGTGGAT GCGCTGATAG AACATCCTCA AGACAGTTTC CTTGAAATCA ATAAATACTG
781 TGTTTTATAC AAAAAAAAAA AAAAAA
```

Fig. 6: Illustrate the miRNA sequence (has-miR-16-1-3p), its seed sequence and it location in the sequence of target gene *GJB3* predicated by online tool miRDB.



MicroRNA and Target Gene Description:

miRNA Name	hsa-miR-144-3p	**miRNA Sequence**	UACAGUAUAGAUGAUGUACU
Previous Name	hsa-miR-144		
Target Score	55	**Seed Location**	775
NCBI Gene ID	2707	**GenBank Accession**	NM_001005752
Gene Symbol	GJB3	**3' UTR Length**	806
Gene Description	gap junction protein, beta 3, 31kDa		

3' UTR Sequence

```
  1 CCACAGGGCA GGGGTGGGGC AACATGCGGG CTGCCAATGG GACATGCAGG GCGGTGTGGC
 61 AGGTGGAGAG GTCCTACAGG GGCTGAGTGA CCCCACTCTG AGTTCACTAA GTTATGCAAC
121 TTTCGTTTTG GCAGATATTT TTTGACACTG GGAACTGGGC TGTCTAGCCG GGTATAGGTA
181 ACCCCACAGGC CCAGTGCCAG CCCTCAAAGG ACATAGACTT TGAAACAAGC GAATTAACTA
241 TCTACGCTGC CTGCAAGGGG CCACTTAGGG CACTGCTAGC AGGGCTTCAA CCAGGAAGGG
301 ATCAACCCAG GAAGGGATGA TCAGGAGAGG CTTCCCTGAG GACATAATGT GTAAGAGAGG
361 TGAGAAGTGC TCCCAAGCAG ACACAACAGC AGCACAGAGG TCTGGAGGCC ACACAAAAAG
421 TGATGCTCGC CCTGGGCTAG CCTCAGCAGA CCTAAGGCAT CTCTACTCCC TCCAGAGGAG
481 CCGCCCAGAT TCCTGCAGTG GAGAGGAGGT CTTCCAGCAG CAGCAGGTCT GGAGGGCTGA
541 GAATGAACCT GACTAGAGGT CTGGAGATA CCCAGAGGTC CCCCAGGTCA TCACTTGGCT
601 CAGTGGAAGC CCTCTTTCCC CAAATCCTAC TCCCTCAGCC TCAGGCAGTG GTGCTCCCAT
661 CTTCCTCCCC ACAACTGTGC TCAGGCTGGT GCCAGCCTTT CAGACCCTGC TCCCAGGGAC
721 TTGGGTGGAT GCGCTGATAG AACATCCTCA AGACAGTTTC CTTGAAATCA ATAAATACTG
781 TGTTTTATAC AAAAAAAAAA AAAAAA
```

Fig. 7: Illustrate themiRNA sequence (has-miR-144-3p), its seed sequence and it location in the sequence of target gene *GJB3* predicated by online tool miRDB.

MicroRNA and Target Gene Description:

miRNA Name	hsa-miR-3613-3p	**miRNA Sequence**	ACAAAAAAAAAAGCCCAACCCUUC
Target Score	55	**Seed Location**	138
NCBI Gene ID	2707	**GenBank Accession**	NM_001005752
Gene Symbol	GJB3	**3' UTR Length**	806
Gene Description	gap junction protein, beta 3, 31kDa		

3' UTR Sequence

```
  1 CCACAGGGCA GGGGTGGGGC AACATGCGGG CTGCCAATGG GACATGCAGG GCGGTGTGGC
 61 AGGTGGAGAG GTCCTACAGG GGCTGAGTGA CCCCACTCTG AGTTCACTAA GTTATGCAAC
121 TTTCGTTTTG GCAGATATTT TTTGACACTG GGAACTGGGC TGTCTAGCCG GGTATAGGTA
181 ACCCCACAGGC CCAGTGCCAG CCCTCAAAGG ACATAGACTT TGAAACAAGC GAATTAACTA
241 TCTACGCTGC CTGCAAGGGG CCACTTAGGG CACTGCTAGC AGGGCTTCAA CCAGGAAGGG
301 ATCAACCCAG GAAGGGATGA TCAGGAGAGG CTTCCCTGAG GACATAATGT GTAAGAGAGG
361 TGAGAAGTGC TCCCAAGCAG ACACAACAGC AGCACAGAGG TCTGGAGGCC ACACAAAAAG
421 TGATGCTCGC CCTGGGCTAG CCTCAGCAGA CCTAAGGCAT CTCTACTCCC TCCAGAGGAG
481 CCGCCCAGAT TCCTGCAGTG GAGAGGAGGT CTTCCAGCAG CAGCAGGTCT GGAGGGCTGA
541 GAATGAACCT GACTAGAGGT CTGGAGATA CCCAGAGGTC CCCCAGGTCA TCACTTGGCT
601 CAGTGGAAGC CCTCTTTCCC CAAATCCTAC TCCCTCAGCC TCAGGCAGTG GTGCTCCCAT
661 CTTCCTCCCC ACAACTGTGC TCAGGCTGGT GCCAGCCTTT CAGACCCTGC TCCCAGGGAC
721 TTGGGTGGAT GCGCTGATAG AACATCCTCA AGACAGTTTC CTTGAAATCA ATAAATACTG
781 TGTTTTATAC AAAAAAAAAA AAAAAA
```

Fig. 8: Illustrate the miRNA sequence (has-miR-3613-3p), its seed sequence and it location in the sequence of target gene *GJB3* predicated by online tool miRDB.

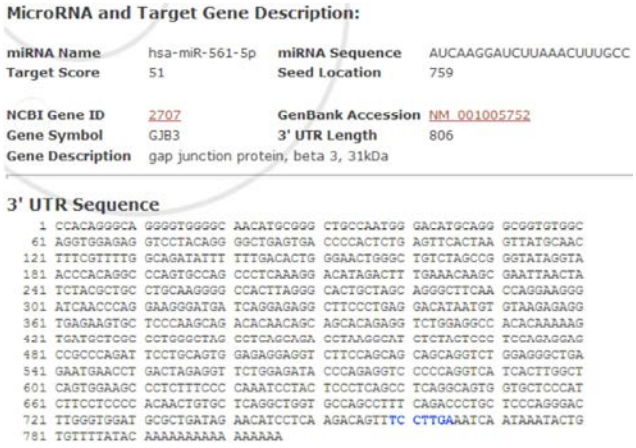
MicroRNA and Target Gene Description:

miRNA Name	hsa-miR-2110	**miRNA Sequence**	UUGGGGAAACGGCCGCUGAGUG
Target Score	52	**Seed Location**	616
NCBI Gene ID	2707	**GenBank Accession**	NM_001005752
Gene Symbol	GJB3	**3' UTR Length**	806
Gene Description	gap junction protein, beta 3, 31kDa		

3' UTR Sequence

```
  1 CCACAGGGCA GGGGTGGGGC AACATGCGGG CTGCCAATGG GACATGCAGG GCGGTGTGGC
 61 AGGTGGAGAG GTCCTACAGG GGCTGAGTGA CCCCACTCTG AGTTCACTAA GTTATGCAAC
121 TTTCGTTTTG GCAGATATTT TTTGACACTG GGAACTGGGC TGTCTAGCCG GGTATAGGTA
181 ACCCCACAGGC CCAGTGCCAG CCCTCAAAGG ACATAGACTT TGAAACAAGC GAATTAACTA
241 TCTACGCTGC CTGCAAGGGG CCACTTAGGG CACTGCTAGC AGGGCTTCAA CCAGGAAGGG
301 ATCAACCCAG GAAGGGATGA TCAGGAGAGG CTTCCCTGAG GACATAATGT GTAAGAGAGG
361 TGAGAAGTGC TCCCAAGCAG ACACAACAGC AGCACAGAGG TCTGGAGGCC ACACAAAAAG
421 TGATGCTCGC CCTGGGCTAG CCTCAGCAGA CCTAAGGCAT CTCTACTCCC TCCAGAGGAG
481 CCGCCCAGAT TCCTGCAGTG GAGAGGAGGT CTTCCAGCAG CAGCAGGTCT GGAGGGCTGA
541 GAATGAACCT GACTAGAGGT TCTGGAGATA CCCAGAGGTC CCCCAGGTCA TCACTTGGCT
601 CAGTGGAAGC CCTCTTTCCC CAAATCCTAC TCCCTCAGCC TCAGGCAGTG GTGCTCCCAT
661 CTTCCTCCCC ACAACTGTGC TCAGGCTGGT GCCAGCCTTT CAGACCCTGC TCCCAGGGAC
721 TTGGGTGGAT GCGCTGATAG AACATCCTCA AGACAGTTTC CTTGAAATCA ATAAATACTG
781 TGTTTTATAC AAAAAAAAAA AAAAAA
```

Fig. 9: Illustrate the miRNA sequence (has-miR-2110), its seed sequence and it location in the sequence of target gene *GJB3* predicated by online tool miRDB.



MicroRNA and Target Gene Description:

miRNA Name	hsa-miR-561-5p	**miRNA Sequence**	AUCAAGGAUCUUAAACUUUGCC
Target Score	51	**Seed Location**	759
NCBI Gene ID	2707	**GenBank Accession**	NM_001005752
Gene Symbol	GJB3	**3' UTR Length**	806
Gene Description	gap junction protein, beta 3, 31kDa		

3' UTR Sequence

```
  1 CCACAGGGCA GGGGTGGGGC AACATGCGGG CTGCCAATGG GACATGCAGG GCGGTGTGGC
 61 AGGTGGAGAG GTCCTACAGG GGCTGAGTGA CCCCACTCTG AGTTCACTAA GTTATGCAAC
121 TTTCGTTTTG GCAGATATTT TTTGACACTG GGAACTGGGC TGTCTAGCCG GGTATAGGTA
181 ACCCCACAGGC CCAGTGCCAG CCCTCAAAGG ACATAGACTT TGAAACAAGC GAATTAACTA
241 TCTACGCTGC CTGCAAGGGG CCACTTAGGG CACTGCTAGC AGGGCTTCAA CCAGGAAGGG
301 ATCAACCCAG GAAGGGATGA TCAGGAGAGG CTTCCCTGAG GACATAATGT GTAAGAGAGG
361 TGAGAAGTGC TCCCAAGCAG ACACAACAGC AGCACAGAGG TCTGGAGGCC ACACAAAAAG
421 TGATGCTCGC CCTGGGCTAG CCTCAGCAGA CCTAAGGCAT CTCTACTCCC TCCAGAGGAG
481 CCGCCCAGAT TCCTGCAGTG GAGAGGAGGT CTTCCAGCAG CAGCAGGTCT GGAGGGCTGA
541 GAATGAACCT GACTAGAGGT TCTGGAGATA CCCAGAGGTC CCCCAGGTCA TCACTTGGCT
601 CAGTGGAAGC CCTCTTTCCC CAAATCCTAC TCCCTCAGCC TCAGGCAGTG GTGCTCCCAT
661 CTTCCTCCCC ACAACTGTGC TCAGGCTGGT GCCAGCCTTT CAGACCCTGC TCCCAGGGAC
721 TTGGGTGGAT GCGCTGATAG AACATCCTCA AGACAGTTTC CTTGAAATCA ATAAATACTG
781 TGTTTTATAC AAAAAAAAAA AAAAAA
```

Fig. 10: Illustrate the miRNA sequence (has-miR-561-5p), its seed sequence and it location in the sequence of target gene *GJB3* predicated by online tool miRDB.

MicroRNA and Target Gene Description:

miRNA Name	hsa-miR-4738-3p	**miRNA Sequence**	UGAAACUGGAGCGCCUGGAGGA
Target Score	51	**Seed Location**	754
NCBI Gene ID	2707	**GenBank Accession**	NM_001005752
Gene Symbol	GJB3	**3' UTR Length**	806
Gene Description	gap junction protein, beta 3, 31kDa		

3' UTR Sequence

```
  1 CCACAGGGCA GGGGTGGGGC AACATGCGGG CTGCCAATGG GACATGCAGG GCGGTGTGGC
 61 AGGTGGAGAG GTCCTACAGG GGCTGAGTGA CCCCACTCTG AGTTCACTAA GTTATGCAAC
121 TTTCGTTTTG GCAGATATTT TTTGACACTG GGAACTGGGC TGTCTAGCCG GGTATAGGTA
181 ACCCCACAGGC CCAGTGCCAG CCCTCAAAGG ACATAGACTT TGAAACAAGC GAATTAACTA
241 TCTACGCTGC CTGCAAGGGG CCACTTAGGG CACTGCTAGC AGGGCTTCAA CCAGGAAGGG
301 ATCAACCCAG GAAGGGATGA TCAGGAGAGG CTTCCCTGAG GACATAATGT GTAAGAGAGG
361 TGAGAAGTGC TCCCAAGCAG ACACAACAGC AGCACAGAGG TCTGGAGGCC ACACAAAAAG
421 TGATGCTCGC CCTGGGCTAG CCTCAGCAGA CCTAAGGCAT CTCTACTCCC TCCAGAGGAG
481 CCGCCCAGAT TCCTGCAGTG GAGAGGAGGT CTTCCAGCAG CAGCAGGTCT GGAGGGCTGA
541 GAATGAACCT GACTAGAGGT TCTGGAGATA CCCAGAGGTC CCCCAGGTCA TCACTTGGCT
601 CAGTGGAAGC CCTCTTTCCC CAAATCCTAC TCCCTCAGCC TCAGGCAGTG GTGCTCCCAT
661 CTTCCTCCCC ACAACTGTGC TCAGGCTGGT GCCAGCCTTT CAGACCCTGC TCCCAGGGAC
721 TTGGGTGGAT GCGCTGATAG AACATCCTCA AGACAGTTTC CTTGAAATCA ATAAATACTG
781 TGTTTTATAC AAAAAAAAAA AAAAAA
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Fig. 11: Illustrate the miRNA sequence (has-miR-4738-3p), its seed sequence and it location in the sequence of target gene *GJB3* predicated by online tool miRDB.

Fig. 12: Illustrate themiRNA sequence (has-miR-466), its seed sequence and it location in the sequence of target gene *GJB3* predicated by online tool miRDB.

Fig. 13: Illustrate themiRNA sequence (has-miR-326), its seed sequence and it location in the sequence of target gene *GJB3* predicated by online tool miRDB.

these miRNAs were performed using Clustal Omega, which is maintained by EMBL-EBI (European Molecular Biology Laboratory- The European Bioinformatics Institute, i.e: part of the EMBL).

In our study the multiple alignment results showed that there is high level of sequences' variation among these selected miRNA except hsa-mir-4510, hsa-mir-4419a and hsa-miR-3605-5p where there is some sequence similarities, as shown in Fig. 1. As compare to the study of Mishra and Chandrasekharan [34], they used CLUSTAL-W alignment tool for alignment of all miRNA precursors in miRBase for selected species of hexapoda. They analysed conservation among *Apismellifera, Bombyxmori* and *Anopheles gambiae*. They concluded that about 82% mature sequences fall under conserved region while 13% are outside the conserved region by 1-2 nucleotides.

CONCLUSION

It was concluded that there are 11 target sites (seed sequences) for miRNA on*GJB3*gene. These are non-identical. Every gene containsmiRNA target sites that can be arranged using different bio informatics tools. This might help molecular biologists to diagnose genetic disorders such asunko gene expression rate, siRNA b ased drug designing and easy and short analysis of a genetically blemishedpedigree for a specific

loci. In future amiRNA based DNA micro array technology can be developed for mutation's identification.

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