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# IN-SILICO ANALYSIS OF P.LYS131ASN MISSENSE-DONOR LOSS VARIANT IN *MINAR2*

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## Abstract

In-silico analysis of underlying variants ensures the fact that the variant is pathogenic, and on top of it, this assists scientists and clinicians reach conclusion on the basis of guidelines provided by American College of Medical Genetics and Genomics. Discovery of deafness genes and elucidating their functions have substantially contributed to our understanding of hearing physiology and its pathologies. Here we report in-silico analysis of a variant in *MINAR2* (membrane integral NOTCH2-associated receptor) underlying autosomal recessive non-syndromic deafness. *MINAR2* is a recently annotated gene with limited functional understanding. We have reported three *MINAR2* variants, p.Trp48*, p.Arg138Valfs*10, and p.Lys131Asn previously, in 13 individuals with congenital or prelingual-onset severe to profound sensorineural hearing loss in recent past. The p.Lys131Asn variant is shown to disrupt a splice donor site. Here, we conclude that MINAR2 is essential for hearing in humans and its disruption leads to sensorineural hearing loss. In-silico analysis of MINAR2 variant p.Lys131Asn corroborates our previously reported findings and supports the fact that deleterious variants in MINAR2 underlie inherited non-syndromic hearing loss and MINAR2 plays an important role in normal hearing and development.

This in-silico work aims to determine whether mutation in MINAR2 protein affects the activity and stability of the protein, and also determines the pathogenicity of the p.Lys131Asn variant in MINAR2. Prediction of pathogenicity of variant will reveal if the mutation has a damaging effect on the native structure of protein or not. Prediction of protein stability will reveal whether the mutation has a stabilizing or destabilizing effect on the protein.

**Key words:** Autosomal recessive, deafness, hearing loss, MINAR2, NOTCH2, In-silico analysis of MINAR2 variant, p.Lys131Asn

## Introduction

Hearing loss (HL) is one of the most common sensory deficits, affecting ~1 in 500 newborns (1). Genetic factors are implicated in the majority of cases, with more than 80% of the inherited form exhibiting autosomal recessive transmission (2). No additional findings are present in over 70% of the cases, which are then classified as non-syndromic HL (Hereditary Hearing Loss Homepage, https://hereditaryhearingloss.org/) (3). Genetic testing for etiologic evaluation has become a standard of care in people with congenital or childhood-onset sensorineural HL, which is caused by pathologies of the inner ear and auditory nerve (4, 5).

Recent studies have shown that screening all recognized HL genes for variants reveals underlying cause in about half of the affected individuals, leaving a significant portion of people with HL with

an unknown etiology (6-9). In the era of emerging genetic therapies for HL, finding the etiology of HL in affected individuals has become a critical task. This is especially relevant for progressive HL, as genetic therapies may potentially stop progression while cochlear hair cells are still alive (10-12). MINAR2 has recently been identified, and based on its structural similarity to MINAR1, named as membrane integral NOTCH2-associated receptor 2 (13). Functional aspects of MINAR2 and consequences of its dysfunction in humans remain unknown.

**Methodology**

Any chemical or amino acid alterations in the protein will interrupt the interactions between pro-survival proteins and pro-apoptotic proteins. Understanding of these mutations will help us to understand if the mutation is involved in any disease. (14) This in-silico study helps us to define the role of variants of MINAR2, which may alter proteins native structure and its function. By examining the role of mutation on biological function, we can determine the correlation between the mutation and the disease. The p.Lys131Asn variant in MINAR2 was subjected for predicting the deleteriousness of the variants, the in silico pathogenicity prediction tools that were used were *PolyPhen-2* (Polymorphism Phenotyping v2), available as software and via a Web server, predicts the possible impact of amino acid substitutions on the stability and function of human proteins using structural and comparative evolutionary considerations It performs functional annotation of single-nucleotide polymorphisms (SNPs), maps coding SNPs to gene transcripts, extracts protein sequence annotations and structural attributes, and builds conservation profiles.(30), *Multiple sequence alignment* opens up exciting possibilities for understanding genomic evolution and functional elements across diverse organisms It involves comparing DNA sequences across multiple species, which can be more powerful for discovering functional elements than pairwise comparisons (31) *The Genome Aggregation Database* (GenomAD), is a coalition of investigators seeking to aggregate and harmonize exome and genome sequencing data from a variety of large-scale sequencing projects, and to make summary data available for the wider scientific community. (32) The Berkeley Drosophila Genome Project (BDGP) is a consortium of the Drosophila Genome Center developing informatics tools that support the experimental process, identify features of DNA sequence, and allow us to present up-to-date information about the annotated sequence to the research community. (33) *STRING* is a database of known and predicted protein-protein interactions. The interactions include direct (physical) and indirect (functional) associations; they stem from computational prediction, from knowledge transfer between organisms, and from interactions aggregated from other (primary) databases. (34) *FATHMM* (35), and *Mutation Assessor* (36), For predicting the effect of amino acid change on the native BCL-w protein, Protein stability analysis *I-mutant 2.0* is a web server that determines the change in stability due to point mutation or missense mutation. (37), *MUpro* web server is a program that predicts the protein stability due to alteration in the sequence. (38), and Integrated predictor *iStable* was used for predicting the stability of the protein, iStable may require both the sequence and the structure as an input. (39), *SAAFEC* is a web server used to compute the energy changes due to single mutation. (40), *SDM* (sitedirected mutator) is an online server that is also used for predicting the effect of point mutation on the protein stability. (41), *DUET* is a web tool for the estimation of consequence of single mutation on proteins stability and its function. (42), and *mCSM* a web tool used to estimate the impact of point mutation on protein stability, protein-protein binding, and protein-DNA binding. (43).

**Result**

In the exome data of two affected individuals from Family 3, filtering variants reveals only one variant for which both individuals are homozygous: *MINAR2*c.393G>T(p.Lys131Asn). SNP arrays show that this variant is located within the only shared homozygous run, >1 MB, in all seven affected individuals in Family 3. This homozygous run is flanked by markers rs13174854 and rs377767449, which is 2.96 MB on chr5:128,738,407-131,705,915(hg19).

In Family 4, two affected siblings share a 5.76 MB homozygous run on chr5:126,978,108-132,742,450 (hg19), flanked by markers rs11241936 and rs11242152. Exome sequencing showed that the proband in Family 4 is homozygous for the same *MINAR2* variant detected in Family 3.
None of the detected *MINAR2* variants is listed in dbSNP or gnom AD databases and all variants are predicted to be deleterious. Sanger sequencing confirmed all three *MINAR2*variants and showed that each variant co-segregates with autosomal recessive HL in all families.

| | Variant 1 | Variant 2 | Variant 3 |
|---|---|---|---|
| **Family ID** | Family 1 | Family 2 | Families 3 and 4 |
| **c. DNA position (NM_001257308.2)** | c.412_419delCGGTTTTG | c.144G>A | c.393G>T |
| **a.a. notation (NM_001257308.2)** | p.Arg138Valfs*10 | p.Trp48* | p.Lys131Asn |
| **Genomic location (hg19) (NC_000005.9)** | g.129100595_129100602delCGGTTTTG | g.129084027G>A | g.129096298G>T |
| **Variant type** | Frame-shift | Nonsense | Missense-Splice |
| **gnomAD global MAF** | absent | absent | absent |
| **dbSNP** | absent | absent | absent |
| **GERP RS score** | 3.608 (Mean) | 4.7199 | 4.7899 |
| **CADD Phred score** | NA | NA | 26.2 |
| **DANN score** | NA | 0.9952 | 0.9987 |
| **TraP Score** | NA | NA | 0.937 |

**gnomAD**: Genome aggregation database, **dbSNP**: The single nucleotide polymorphism database **GERP**: Genomic evolutionary rate profiling, **CADD:** Combined annotation dependent depletion, **DANN:** Deleterious annotation of genetic variants using neural networks, **TraP:** Transcript-inferred pathogenicity

***Figure 1.*** *Shows in Family 3 homozygous run is flanked by markers rs13174854 and rs377767449, which is 2.96 MB on chr5:128,738,407-131,705,915(hg19). In Family 4, 5.76 MB homozygous run on chr5:126,978,108-132,742,450 (hg19), flanked by markers rs11241936 and rs11242152.*

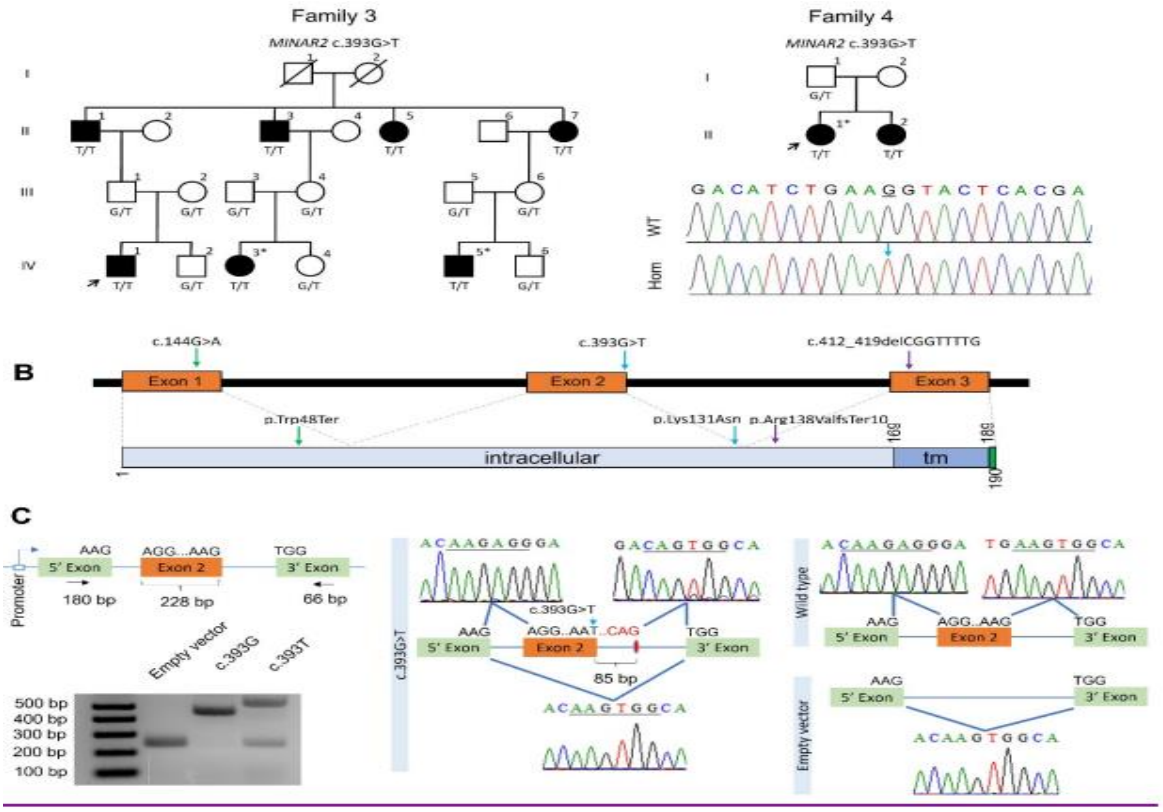



***Figure: 2*** *Illustrating exact location of c.393G>T, p.Lys131Asn variant in p.Lys131Asn mutated MINAR2 sequence.*

While it is a missense change, the c.393G>T(p.Lys131Asn) variant substitutes the last nucleotide of exon 2 and is predicted to abolish a splice donor site. Via exon trapping experiments, we show that this variant leads to an addition of 85 intronic nucleotides into exon 2, which alters the amino acid composition of the rest of the protein leading to a premature stop codon. The same variant also leads to skipping of exon 2 entirely. *MINAR2 c.393G>T (p.Lys131Asn) leads to aberrant splicing*.

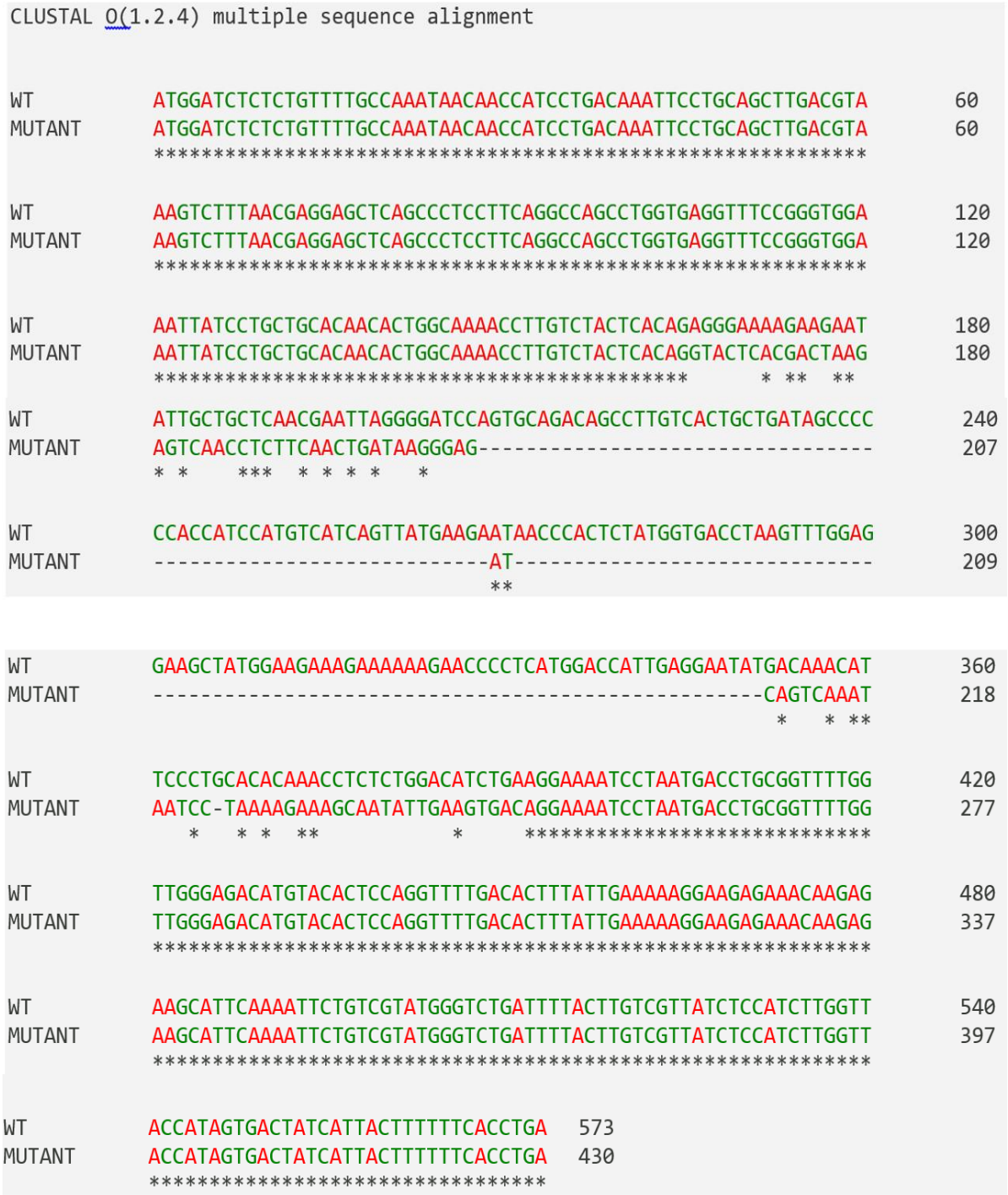



***Figure: 3*** *Shows sequence alignment of wild type MINAR2 cDNA sequence and p.Lys131Asn MINAR2 mutant sequence demonstrating premature stop codon occurring due to insertion of 85 bases in intron between exon 2 and exon 3 and skipping of entire exon 2 sequence in p.Lys131Asn mutant MINAR2 sequence.*

**In-silico prediction for MINAR 2**

**1) Multispecies alignment**

Multispecies alignment confirms that p.Lys131 in MINAR2 is a highly conserved amino acid residue across various different species including human. This suggests that p.Lys131 amino acid residue in MINAR2 plays an important role in normal growth and development of various different species and replacement of p.Lys131 amino acid in MINAR2 may result into deleterious effect on the transcript.

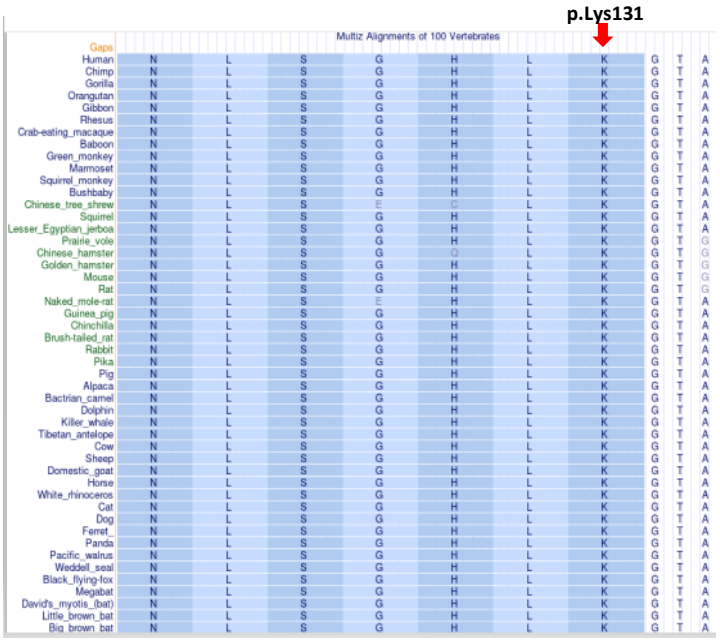



***Fig – 4*** *Shows Multispecies alignment of 100 vertebrates including human for p.Lys131 in MINAR2 is a highly conserved amino acid residue across various different species including human.*

2) HumDiv score for non-synonymous amino acid change p.Lys131Asn in MINAR2 is 0.999 whereas HumVar score for p.Lys131Asn variation in MINAR2 is 0.992. Thus PolyPhen-2 predicts that MINAR2 variant p.Lys131Asn is probably damaging.


| VARIANT DETAILS | SIFT PREDICTION | PolyPhen-2 PREDICTION |
|---|---|---|
| hg19 Chr5:129096298G>T; c.393G>T; p.Lys131Asn | DELETERIOUS (0) | PROBABLY DAMAGING (0.993) |

***Figure 5*** *shows non-synonymous amino acid change p.Lys131Asn in MINAR2 is 0.999 PolyPhen PREDICTION predicts that MINAR2 variant p.Lys131Asn is predicted to be deleterious as per SIFT prediction tool and probably damaging as per PolyPhen-2 prediction tool.*

**3) Variant Effect Predictor in Ensemble Genome Browser predicts** MINAR2 variant p.Lys131Asn to be deleterious as per SIFT mutation prediction tool with the score of 0 and PolyPhen predicts that p.Lys131Asn variant in MINAR2 is probably damaging with the score of 0.993. As variant Chr5:129096298G>T [hg19]; c.393G>T; p.Lys131Asn is not reported in GnomAd browser database. This indicates that this variant is extremely rare and is likely to be deleterious and pathogenic.

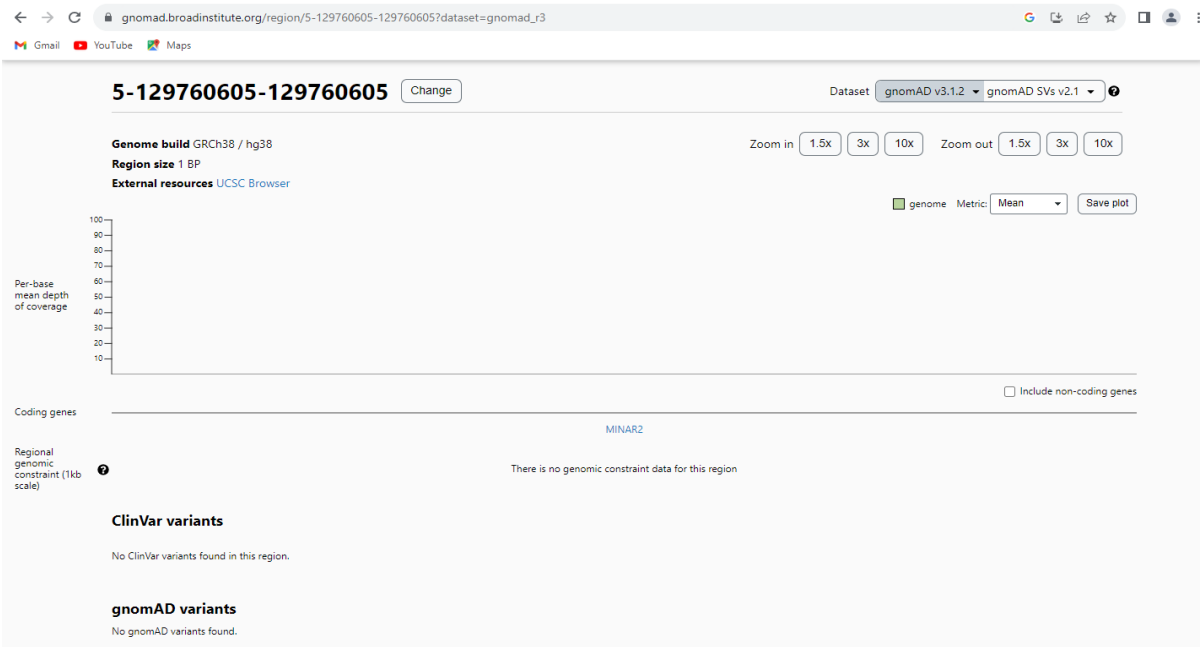
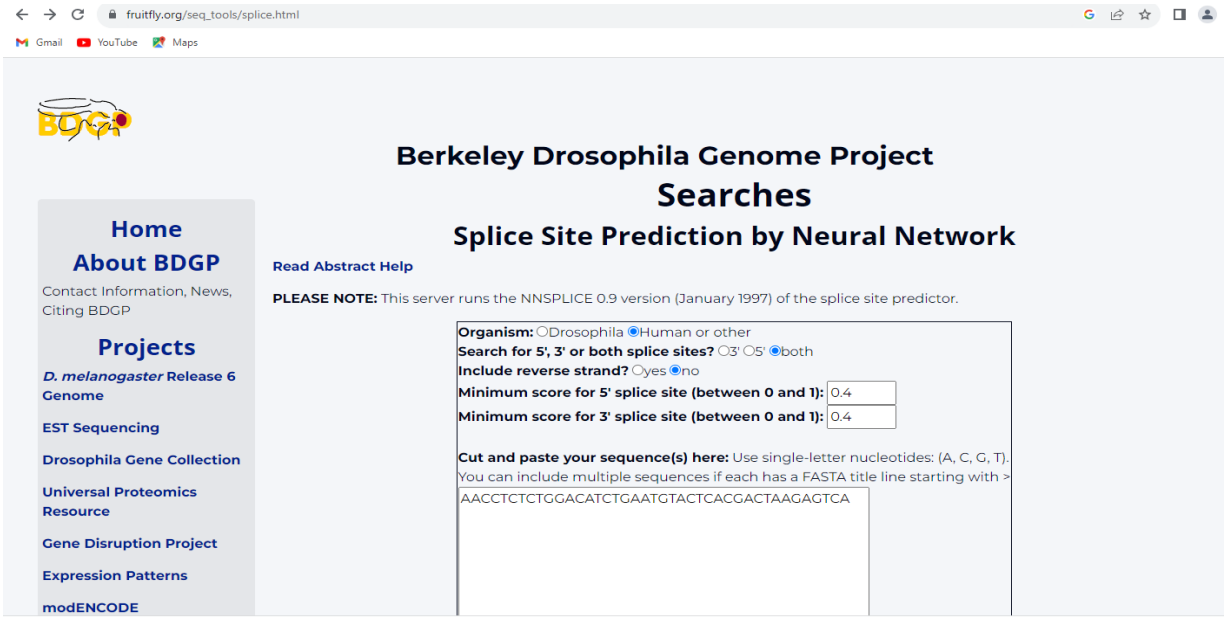



***Figure: 6*** *Illustrating that c.393G>T; p.Lys131Asn variant in MINAR2 is not reported in gnomAD browser database and hence indicating that it is a novel and extremely rare genomic variation.*

**4) Berkeley Drosophila Genome Project's** splice-site prediction tool predicts that the nucleotide change chr5:129096298G>T [hg19]; c.393G>T; p.Lys131Asn results into abolition of donor splice site in coding exon 2 of *MINAR2* gene. Splice AI Lookup predicts that donor splice site is lost due to Chr5:129096298G>T [hg19]; c.393G>T; p.Lys131Asn variant in MINAR2 gene with the score of 0.86.



**Splice site predictions for 1 sequence with donor score cutoff 0.40, acceptor score cutoff 0.40 (exon/intron boundary shown in larger font):**

**Donor site predictions for 10.42.0.175.825045.0 :**

```
Start   End    Score     Exon   Intron
```

**Acceptor site predictions for 10.42.0.175.825045.0 :**

```
Start   End    Score     Intron              Exon
```

| variant | gene | Δ type | Δ score | pre-mRNA position | REF score | ALT score |
|---|---|---|---|---|---|---|
| 5-129096298-G-T UCSC, gnomAD | MINAR2 (ENSG00000186367.7_8 / ENST00000564719.2_3 / NM_001257308.2) biotype: protein coding canonical transcript OMIM, GTEx, gnomAD, ClinGen, Ensembl, Decipher, GeneCards | Acceptor Loss | 0.00 | -227 bp | 0.91 | 0.81 |
| | | Donor Loss | 0.86 | 0 bp | 0.86 | 0.00 |
| | | Acceptor Gain | 0.00 | | 0.00 | 0.00 |
| | | Donor Gain | 0.16 | 85 bp | 0.18 | 0.34 |
| 5-129096298-G-T UCSC, gnomAD | ENSG00000251680 (ENSG00000251680.6_11 / ENST00000503616.5_4) biotype: lncRNA canonical transcript OMIM, GTEx, gnomAD, ClinGen, Ensembl, Decipher, GeneCards | Acceptor Loss | 0.00 | 57 bp | 0.08 | 0.03 |
| | | Donor Loss | 0.00 | 9 bp | 0.06 | 0.03 |
| | | Acceptor Gain | 0.00 | | 0.00 | 0.00 |
| | | Donor Gain | 0.00 | | 0.00 | 0.00 |
| 5-129096298-G-T UCSC, gnomAD | ENSG00000251680 (ENSG00000251680.6_11 / ENST00000653455.1_3) biotype: lncRNA OMIM, GTEx, gnomAD, ClinGen, Ensembl, Decipher, GeneCards | Acceptor Loss | 0.00 | 57 bp | 0.08 | 0.03 |
| | | Donor Loss | 0.00 | 9 bp | 0.06 | 0.03 |
| | | Acceptor Gain | 0.00 | | 0.00 | 0.00 |
| | | Donor Gain | 0.00 | | 0.00 | 0.00 |

***Figure: 7*** *shows Berkeley Drosophila Genome Project's splice-site prediction tool predicting that the nucleotide change chr5:129096298G>T [hg19]; c.393G>T; p.Lys131Asn results into loss of donor splice site.*

5) The STRING network analyses showed functional interactions between the MINAR2 gene and 9 non-HI genes (Figure 8). Of these, CTXN3 (schizophrenia), ISOC1 (cancer), ADAMTS19 (non-syndromic heart valve disease), PRRC1 (lymphoblastic leukemia), TEX43, C5ORF63, MARCHF3, GRAMD2B, ZNF608 (endometrial cancer).and one HI Genes OCM2 (non-syndromic hearing impairment), as shown in (Figure 4). The in silico functional interactions suggest a common biological pathway that represents systemic activities for hearing. Little is known about the functions of these associated non-HI genes in hearing MINAR2

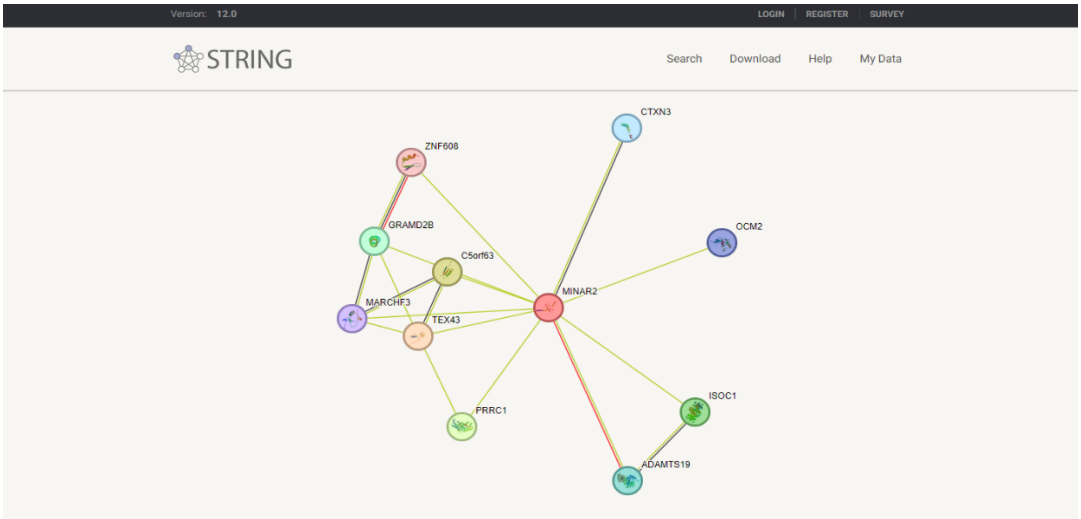



***Figure: 8*** *demonstrating all other proteins interacting with MINAR2.*

## Discussion

Multispecies alignment confirms that p.Lys131 in MINAR2 is a highly conserved amino acid residue across various different species including human. HumDiv score and HumVar score for non-synonymous amino acid change p.Lys131Asn in MINAR2 is 0.9. As per SIFT mutation prediction tool with the score of 0 and PolyPhen predicts variant in MINAR2 is probably damaging with the score of 0.993. Berkeley Drosophila Genome Project's splice-site prediction tool predicts that the nucleotide change results into abolition of donor splice site in coding exon 2 of *MINAR2* gene. The STRING network analyses showed functional interactions between the MINAR2 gene and other genes

The Notch pathway is a highly conserved intercellular signaling cascade that is activated by the interaction of transmembrane ligands (Delta and Jagged) with Notch receptors, which are usually expressed on the surface of neighboring cells. Binding of the Notch Ligand to receptor induces cleavage of the Notch receptors intracellular domain, which binds to multiple DNA-binding proteins in the nucleus .In the initial stages of angiogenesis, Notch activation is generally repressed to allow proliferation of endothelial cells in response to VEGF stimulation, and its expression is later upregulated when endothelial cells stop proliferating and the vessels begin to stabilize. While it is possible that the effects of MINAR2 on angiogenesis may play a role in hearing.

Most genetic and environmental causes of sensorineural HL lead to permanent loss of hair cells, reducing the chance of gene therapy or gene editing approaches. Progressive HL associated with a relatively normal appearance of hair cells makes *MINAR2* a promising target for genetic therapies. Further elucidation of its role in the maintenance of hair cell synapses and stereocilia bundles may open new avenues to treat more common forms of HL resulting from similar mechanisms.

## Conclusion

From all in silico prediction in MINAR2 suggests that *p.Lys131* amino acid residue in MINAR2 plays an important role in normal growth and development of various different species. In MINAR2 variant *p.Lys131Asn* lysine is replaced with asparagine is probably damaging with the score of 0.993.which is result into abolition of donor splice site in coding exon 2 of *MINAR2* gene. With different tools it is predicted p.Lys131Asn missense-donor loss variant in *MINAR2* is extremely rare and is likely to be deleterious and pathogenic. Further elucidation of *MINAR2* role in the hearing functions may open new avenues to treat more common forms of HL resulting from similar mechanisms.

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**Conflict of interest:** The authors declare that no conflict of interest exists.

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