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# MOLECULAR DIAGNOSIS OF A FAMILY WITH SYNDROMIC HEARING LOSS AND INTELLECTUAL DISABILITY USING WHOLE EXOME SEQUENCING IN KHYBERPAKHTUNKHWA, PAKISTAN

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**Keywords**
*Hearing Loss; COL11A2 Gene; Missense Variant; Whole Exome Sequencing; Intellectual Disability; Genetic Diagnosis.*

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**Abstract**

***Background:***
*Deafness or hearing loss is a condition in which a person is unable to learn to speak during their pre-or post-lingual years. Such patients either never speak or just occasionally produce meaningless sounds. Based on their causative agent, the severity varies from patient to patient and even from family to family. Primarily deafness is divided into two categories: syndromic and non-syndromic hearing loss. Along with other signs and symptoms, there are more than 400 conditions that result in hearing loss are referred to as syndromic hearing loss which are about 30% in different world populations. While 70% percent of prelingual hereditary HL is non syndromic (NSH).*

***Methodology:***
*In the present study, A family was recruited from the district Bannu, Khyber Pakhtunkhwa Province (KP), Pakistan, having medical problems including mental retardation and other physical disabilities. Molecular diagnosis of the family was performed using Whole Exome Sequencing to recognize the causal gene. First blood samples were collected from the targeted family in EDTA tubes and the samples were taken into the molecular lab at the Centre For Omic Sciences, Islamia College Peshawar. A standard phenol chloroform method was used to extract the DNA. Samples were quantified using Nano drop spectrophotometer and sample of one of the affected individuals was sent to 3billiom. Inc. for whole exome sequencing (WES). WES is NGS based method, which is the first line of molecular diagnostic tool for confirmation of the genome investigation.*

***Results:***
*The sequencing analysis results in a missense mutation in col11a2 at*

*cDNA: NM_080680.3:*
*c.3031C>A located on exon 41 of chromosome 6p21.32. Furthermore, the variant was selected for segregation analysis by sanger sequencing for validation. But the Sanger validations was inclusive as some samples were failed due to insufficient DNA and the concern family did not corporate as they refused to give us more blood samples.*

***Conclusion:***

*In this study, we identified a missense heterozygous variant in a Pashtun family suffering from deafness and intellectual disabilities in the district of Banuu, Pakistan. A very critical role is being played by next-generation sequencing (NGS) in the diagnosis of rare genetic diseases that were previously difficult to identify. It was time-consuming, expensive, and difficult to conduct genetic testing in the past due to a lack of resources. Genetic testing should be done on regular bases as Khyber Pakhtunkhwa is the hub for rare genetical disorders due to high consanguinity rate.*

## INTRODUCTION

The human ear is a specialized organ responsible for hearing and maintaining body balance. It consists of the outer, middle, and inner ear, which work together to receive sound and transmit auditory signals to the brain (Sundar et al., 2021). The outer ear includes the auricle, tragus, helix, and lobule (Ballenger and Snow, 2003; Alexander et al., 2011). The middle ear contains three ossicles—the malleus, incus, and stapes—which transmit sound vibrations towards the inner ear (Whyte et al., 2008). The inner ear contains the cochlea, labyrinth, and vestibule, which contribute to hearing and equilibrium (Rhode and Greenberg, 1992). Sound waves are converted into nerve signals in the inner ear and transmitted to the brain through the auditory nerve (Alberti, 2001; Stenfelt, 2016). The vestibular system also contributes to postural stability and coordination of head and eye movements (Khan and Chang, 2013).

Hearing loss, or deafness, is a common sensory disorder that can occur at any age and may result from genetic or environmental factors. Genetic causes include mutations inherited through autosomal dominant, autosomal recessive, X-linked, or mitochondrial patterns. Environmental factors, including maternal illness and complications before or after birth, may also contribute to hearing loss (Gatlin and Dhar, 2021; Sheffield and Smith, 2019). Medical and family histories, audiological assessments, physical examinations, and genetic testing help identify the causes and types of hearing loss (Oshima et al., 2010; Shearer and Smith, 2012). Approximately 70% of hereditary hearing-loss mutations are associated with non-syndromic hearing loss, while the remaining 30% are associated with syndromic forms (Petit et al., 2001). Syndromic hearing loss occurs alongside other clinical abnormalities, whereas non-syndromic hearing loss occurs without associated syndromic features. Both forms may follow different inheritance patterns (Morzaria et al., 2004).

Hearing loss is classified into sensorineural, conductive, and mixed types. Sensorineural hearing loss results from damage to the inner ear or auditory nerve and may be associated with genetic disorders, ageing, infections, or excessive noise exposure. Conductive hearing loss occurs when sound transmission through the outer or middle ear is impaired, while mixed hearing loss involves both sensorineural and conductive components. The identification of the underlying cause is essential for appropriate assessment and management. Non-syndromic hearing loss may be classified according to its inheritance pattern, including autosomal dominant, autosomal recessive, and X-linked forms (Smith and Jones,

2016). Genetic mutations, including those involving GJB2 and STRC, have been associated with different forms and severities of hereditary hearing loss.

Intellectual disability is a heterogeneous condition characterized by limitations in intellectual functioning and adaptive behaviour, including learning, reasoning, communication, decision-making, and independent daily living (Sheikh et al., 2018). Its causes include genetic and chromosomal abnormalities, prenatal infections, birth complications, brain injuries, and other neurological conditions. Recognized genetic conditions include Down syndrome, fragile X syndrome, and other chromosomal abnormalities (Ropers, 2008). Clinical assessment may involve blood and urine tests, genetic investigations, radiological imaging, electroencephalography, and other appropriate investigations to identify underlying causes and exclude alternative conditions (Patel et al., 2020).

Molecular diagnosis plays an important role in identifying genetic variants associated with inherited disorders. It supports disease detection, diagnosis, classification, prognosis, and monitoring of therapeutic responses. In patients with deafness and intellectual disability, molecular investigations can help identify disease-associated genetic variations and determine carrier status among potentially affected family members. These findings can also assist in estimating the risk of recurrence in future pregnancies and support appropriate genetic counselling.

## Methodology

### 2.1. Ethical approval

In compliance with the ethical guidelines of the university, the institutional bioethical committee of Islamia College University in Peshawar gave us its approval. All participants and legal guardians signed consent forms before the study started after being informed about it and given a copy of the Declaration of Helsinki's guiding principles. Anyone under the age of 18 or unable of understanding the consent form must have their legal parents or guardians sign it on their behalf. Clinical information and other important details were acquired from families through clinical examination and family member interviews.

### 2.2. Details about the research work

The Genomics Lab at the Center for Omic Sciences at Islamia College in Peshawar is where this work was carried out. For this investigation, a family in the Bannu district provided blood samples and clinical information. A molecular study was performed after their blood's DNA was extracted.

### 2.3. Sample collection

Blood samples from five family members were collected for analysis. First of all, patients were prepared for blood collection then we collected 3 to 8 ml of blood from a vein and placed it into an EDTA tube using sterile syringes. The obtain samples were than process and stored at -20C for long term and -4C for short term use. To avoid confusion, each sample was properly labeled.

### 2.4. DNA extraction

DNA extraction took place at the Center for Omic Sciences Islamia College Peshawar. Method, we use for extraction was phenol chloroform isoamyl alcohol most commonly used approach for liquid-liquid DNA separation. It separates the DNA from whole blood samples. Extraction of the DNA was done in several steps which are as under;

1. The EDTA tubes were stored at -20c in freezer. It was taken out from it and properly put it for melting
2. We added 200 to 250µl of blood in sterilized and labeled Eppendorf tubes with pipette
3. Then add other chemicals to the marked tube starting with 300µl of lysis buffer.
4. Add 5µl of Beta-mercaptoethanol (BME)
5. Add 6µl of proteinase-k (PK)
6. Add 200µl of dilution buffer
7. Now after adding all the components vortexing is essential for mixing lysis solution with blood
8. Then samples were shifted to water bath for 3 hours incubation with vortexing after each 20 minutes.

9.after 3hours of incubation add 400µl of phenol Chloroform Isoamyl Alcohol (PCI) to Eppendorf tubes

10. to get layers we centrifuge these tubes for 9 minutes at 1300rpm

11. After centrifugation three layers are formed in which the first layer has genomic DNA while the second layer consist of proteins and third one has lipids in it.

12. The genomic DNA was transfer to new marked Eppendorf tubes.

13. • A corresponding volume of cool isopropanol was added to the Eppendorf tube containing the supernatant, which was subsequently centrifuged at 13000 rpm for 8 minutes after being added and kept at -20°C for 40 minutes. Supernatant was eliminated in order to get genomic DNA free of impurities.

14. The pellet was then centrifuged for 5 minutes at 8000 rpm after being rinsed with 500 l of 70% ethanol. The resulting solution was then discarded.

15. Then we completely dried out the Eppendorf tubes and added 40µl of distilled water at room temperature for shorter period of time to dissolve DNA in it and then we can do molecular analysis on it.

The extracted DNA was run on Nano drop quantification to know its purity

This extracted DNA will be used for Sanger sequencing and whole exome sequencing.

**2.5. GEL ELECTROPHORESIS**

To evaluate the isolated genomic DNA's quantity and quality, it was run on an agarose gel, and the results were recorded on a gel documentation system after being seen under a UV light.

The procedures for gel electrophoresis are listed below.

1) The gel was poured
2) Samples were prepared
3) The gel was loaded
4) The gel was run on electric field (exposing it to an electric field) 5) The gel was stained.

**2.5.1. Procedure**

1.Take 250ml flask and add 100ml of TBE (Tris/Borate/EDTA) buffer and mixed it with 1 gram of agarose powder.

2.Heat the flask for 80 to 90 seconds to liquefy the agarose gel completely.

3.The agarose solution was cool, and ethidium bromide (EtBr), a fluorescent tag, was added. The final concentration was somewhere between 0.5 and 1 g/ml.

4.It took 20 minutes for the agarose solution to completely solidify after being added to the gel tray with a carefully placed comb.

5.The agarose gel was placed onto a gel tray and covered with 1xTBE buffer after setting up.

6.We took 5µl DNA was mixed with 2µl EtBr dye and then loaded it in the wells of the gel

7.• Electrophoresis was done for 40 minutes at 130 volts and 400 milliamps.

8.At the Center for Omic Sciences, the gel was visualized under UV light in the Gel documentation system.

**2.6. Identification of causative genes**

The following procedures were used to find the causative genes.

**2.7. WHOLE EXOME SEQUENCING (WES)**

WES was performed on afflicted family member based on phenotype and pedigree analyses. Then the collected DNA from one patient was sent for whole exome sequencing. Illumina platform HiSeq 2500 was used to sequence the chosen proband, with an average coverage of 150 produced by Macrogen (Geumcheon-gu, Seoul, South Korea). For each sample, FastQ files containing nucleotide sequence reads were created. There are around 24,000 coding genes in the human genome for identifying specific gene various procedures are involved in creating and analyzing WES data.

**2.8. Primer designing and Sanger sequencing;**

Sanger sequencing primers were made using information from Prime3

(https://bioinfo.ut.ee/primer3). The Ensembl Genome Browser (www.ensembel.org) was used to obtain the reference sequence for COL11a2.

**TABLE: 2.1 Primers for PCR amplification of *COL11A2* exon 41**

| Primers | Sequence | Length | Temp | Product |
|---|---|---|---|---|
| COL11A2_41F | 5'-CTGTCACAAGCACCAAGCAT-3' | 18bp | 64c | 561bp |
| COL11A2_41R | 5'-CCACACTCACTGGGACACC-3' | 18bp | 64c | |

**Table 2.2: Design primers attachment region**

| | |
|---|---|
| Col11a2 Exon 41 | CTGTCACAAGCACCAAGCAT CCTGAGTCCCCCCCCCGACC<br>CTGTCCCTGACCCCCTCCATGTCACT GGTGGACCTG<br>GAGCCTGTCTTCCCTCTCCAGCTCTC CCTGGCCC<br>AGTCTCATCTTCCCCAAATGTCTGGT GTGAGTCTGGGGTTCCGGAAGTGGTAGGA<br>GTTTGAAGGGGAATGAAGGTCCGTC AGGACCCGG<br>GAAAGGGGTGGGTGAGGAAGGGTGC GGCTCCCCTGGGGAACGAGG<br>CACCAGGGGCTGTGGGGCTGGGCAC<br>CCAGAATGCATCTGATTTTGGGAGCT<br>GAGGTTCTCTGGTATGGGCGGGCTG<br>TCTGACCATCTGCTTTCTCAG<br>TGCAGCAGGATCAGGGGGACCCATT<br>CCCA<br>GGTGTCCCAGTGAGTGTGG AGCA<br>GG |

**2.9. Sanger Validation**

Sanger sequencing was used to confirm only short-listed or chosen WES variations in all the affected family members. But the Sangers validations were inconclusive as some samples were failed due to insufficient DNA and the concerned family did not corporate as they refused to give us more blood samples. The Ensembl Genome Browser (https://asia.ensembl.org/Homo sapiens/Gene/) provided the Col11a2 gene wild type genomic DNA sequences. Primer selection was carried out using the online website Primers 3 Plus (www.bioinformatics.nl/primer3plus). Then the sample genomic DNA was amplified by PCR using the relevant primers. Each comprised a 25-l PCR reaction, 1-l of genomic DNA template (50 ng/l), and 1-l of reverse and forward primers (10 pmol/l) 12.5µl of GoTaq®

Green ready reaction mix (Promega, USA) and 9.5µl of distilled deionized water. Then for data generation of Sanger sequencing amplified PCR materials were cleaned from free nucleotides, unattached primers, and other ready reaction mix dyes. by using the Big-Dye

Terminator 3.1 cycle sequencing kit from Applied Biosystems Life Technologies, USA Creating single stranded sequences in different reactions with either forward or reverse primers as a template DNA the purified products of PCR. The genetic analyzer ABI 3500

Genetic Analyzer was used to run these samples (Applied Biosystems, USA). Analysis of

Sanger sequencing was done on the BioEdit editor version 7

(www.mbio.ncsu.edu/BioEdit/bioedit.html). The Sanger sequences were opened in BioEdit and aligned with the wild type sequences. Mismatches were documented in each participant, and results were cross-checked using Mutation Taster (http://www.mutationtaster.org/) an online website for checking the pathogenicity of variant.. The genomic sequences of each variant were copied from Ensemble Genome Browser. A primer for PCR amplification was chosen using the internet tool Primer3Plus.

### 3.1 Clinical history of Family

The family was recruited from district Bannu, KPK, Pakistan. It was an overall eight-member family that included two brothers, six sisters, and an affected brother (III-2, III-5 and III-8). Analyzing ancestry indicates autosomal inheritance (figure 1). Even though both of their parents had normal phenotypes, the pedigree research showed that this disease had skipped a generation, indicating that one of them was heterozygous. After carefully examining the medical histories, it was determined that the issue could not be caused by environmental factors or another reason. A complete medical and family history was documented. Mental retardation, physical disabilities, inconsistent urine, aggression, selfattack, laughter, and weeping were all visible in the affected individuals. In this study, we focused on five members of the same family.

**Table3.1: Symptoms of the disease patients and carrier. Yes = Present and No = Absent**

| Characteristics | III-2 | III-5 | III-8 | III-3 |
|---|---|---|---|---|
| Gender | Female | Female | Male | Female |
| Condition | Affected | Affected | Affected | Carrier |
| Genotype | G>T | G>T | G>T | |
| Deaf | Yes | Yes | Yes | No |
| Mute | Yes | Yes | Yes | No |
| Mental retardation | Yes | Yes | Yes | No |
| Physical disabilities | Yes | Yes | Yes | No |
| Urinary inconsistency | Yes | Yes | Yes | No |
| Laughing | Yes | Yes | Yes | No |
| Crying | Yes | Yes | Yes | No |

| | | | | |
|---|---|---|---|---|
| Self-attack | No | No | Yes | No |
| Aggression | Yes | No | Yes | No |

**Table 3.2: Members that were studied**

| Sample id | Age | Relation | Condition | Findings | Genotype |
|---|---|---|---|---|---|
| II-2 | ? | Mother | Normal | Heterozygous/carrier | C/A |
| III-1 | ? | Daughter | Normal | Homozygous for wild type allele | C/C |
| III-2 | 42 | Daughter | Affected | Homozygous for affected allele | A/A |
| III-5 | 30 | Daughter | Affected | Homozygous for affected allele | A/A |
| III-8 | 24 | Son | Affected | Homozygous for affected allele | A/A |

International Journal of Advanced Research

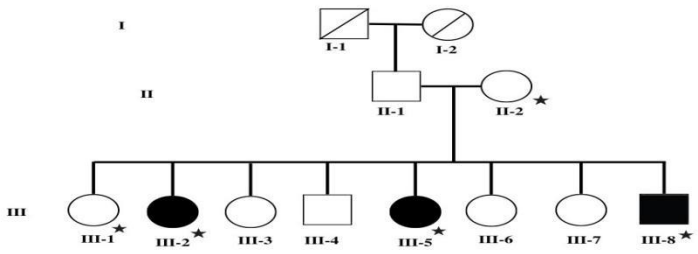



**Figure 3.1: The pedigree shown the autosomal recessive pattern of disease, the circle represents the female while the square represents male the box while the shaded represents the affected individual.**

**3.2Genomic DNA analysis by gel electrophoresis**

Gel electrophoresis was used to run the genomic DNA, and gel documentation was used to visualize it

**3.3Genetic analysis:**

The initial plane was to screen all coding exons of *COL11A2* gene through Sanger sequencing. However, we got the opportunity to perform WES of affected member of the family so we sent the affected member III-2 for the whole exome sequencing.

**3.3.1 Whole exome sequencing and prioritization of candidate gene**

Whole exome sequencing screens around 24,000 coding genes of human genome. Based on phenotype and pedigree analysis the extracted DNA was sent to Macrogen south Korea. Where Illumina HISeq4000 sequencer was used for sequencing the chosen probands.

Mutation at position c.3031C>A in Collagen Type XI Alpha 2 Chain (COL11A2). COL11A2 gene is 561 bp long and codes for collagen type proteins. Mutation at position c.3031 cytosine is converted in to thymine. Due to this missense mutation in protein proline1011 in to thr. It is heterozygous variant of uncertain significance (VUS) which means that there is a probability of pathogenesis. For further investigation sanger sequencing was done.

**3.3.1Potential candidates' prioritization**

On the basis of zygosity and the frequency of minor alleles, 250 variations in more than 100 potential genes for hearing loss have been connected to the disorder in various ways. One variant of col11a2 (c.3031C>A) was chosen for Sanger validation and three-dimensional protein modeling.

**3.5. Gene location:**

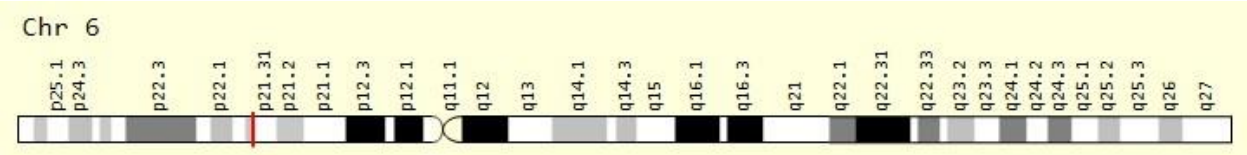



**Figure 3.2: Col11a2 is located on chromosome 6 (omim 609706).**

**PCR amplification and Sanger sequencing:**

The exon 41 of *COL11A2* was amplified by using *COL11A2*_F: 5'-CTGTCACAAGCACCAAGCAT -3' and *COL11A2*_R: 5'-CCACACTCACTGGGACACC -3' primers. The primers were optimized for annealing at a different temperature. ABI-3730 DNA analyzer was used for the sequencing at Macrogen Inc Republic of Korea.

**3.3.2Sanger sequence analysis**

The data we received from whole exome sequencing was analyzed by bio edit sequence alignment editor version 7.2.5 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The inherited variant in *COL11A2* which causes deafness and mental retardation in some populations was analyzed in family. The results we received from Sanger sequencing are as under.

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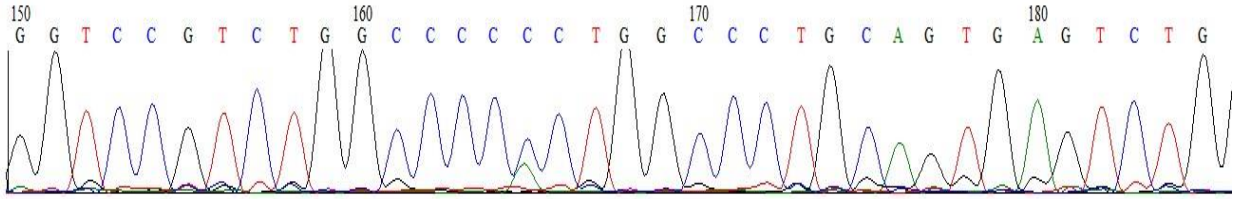



**Figure 3.3 Multiple sequence alignment shows carrier/wild type**

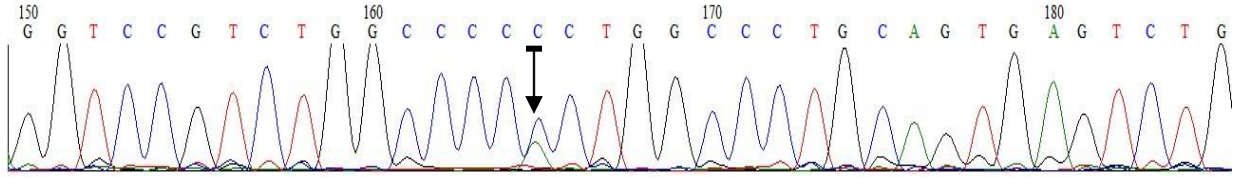



**Figure 3.4 Multiple sequence alignment shows carrier.**

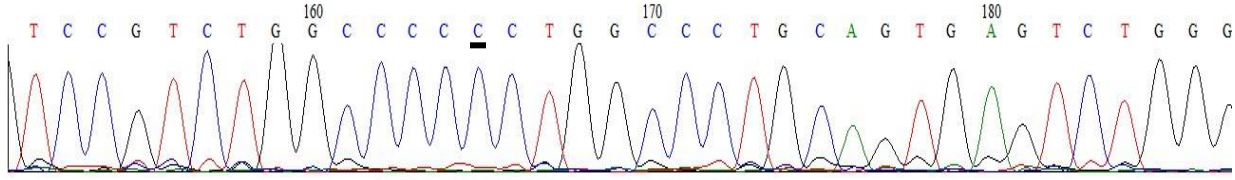



**Figure 3.5 Multiple sequence alignment shows normal.**

In figure 3.6 we used a Mutationtaster software for prediction of disease.

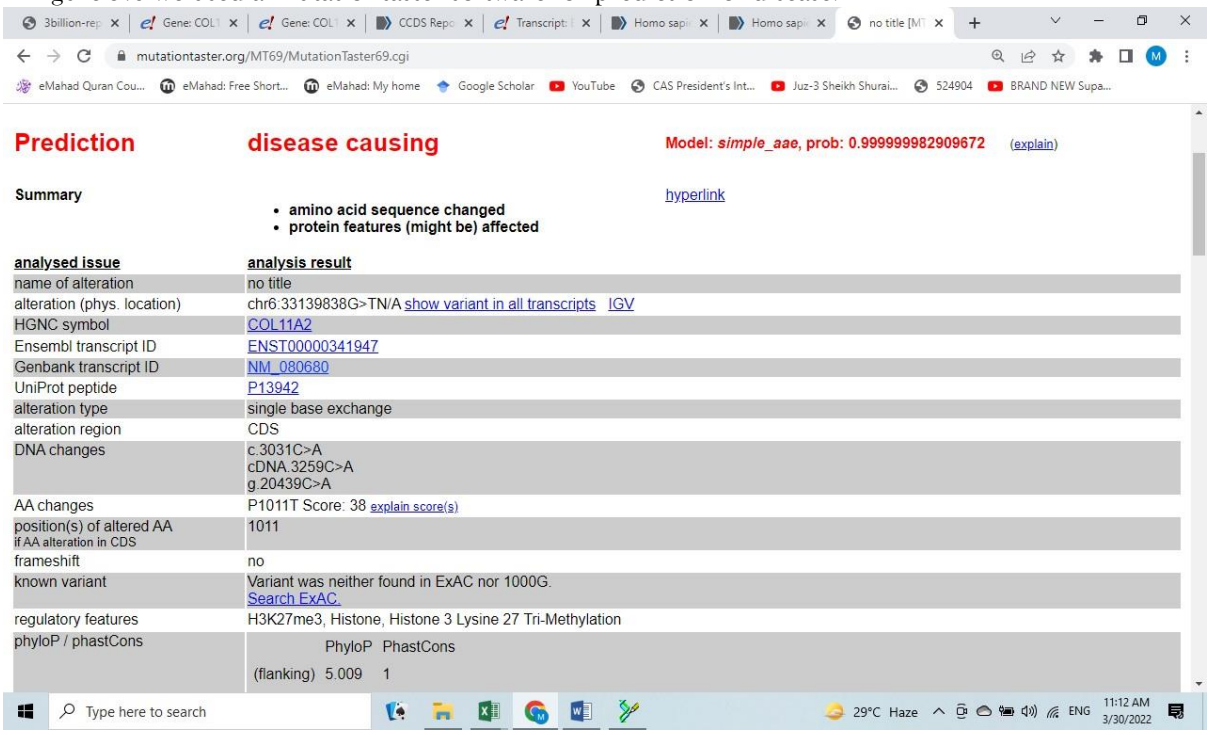



**Figure 3.6; Screenshot from Mutationtaster software**

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**Discussion**

In the current work, we employed next-generation high-throughput sequencing methods to identify the causative mutation of COL11A2 in a Pashtun family from the district of Bannu who had deafness, intellectual disabilities, and other disease signs and symptoms. The family had three probands and its pedigree showed autosomal recessive inheritance while their parents showed normal phenotype.

Hearing loss, often known as deafness, is one of the most prevalent neurosensory illnesses in people and one of the six main causes of disease in developed nations. An estimated 1 in 500 babies experience hearing loss at birth or in the first few years of life. Since the first deafness gene was discovered in 1997 More than sixty genes and a similar number of proteins have been linked to the pathogenesis of Non-Syndromic Hearing Loss, which is characterized by significant genetic variation. At least 30% of people will experience HL throughout their lives; it can be caused by variety of factors like hereditary or environmental. Deafness can be divided into a number of categories, including prelingual and post lingual, syndromic and non-syndromic hearing loss, degrees of HL (light, mildly intense, severe, profound), and types of HL (sensorineural, conductive, and mixed. There are four different inheritance patterns for deafness: autosomal dominant, autosomal recessive, X-linked, or mitochondrial.

An individual is said to have an intellectual disability if their capacity to understand at a typical level and function in daily life is limited. Mental disability in individuals is commonly overlooked by clinical professionals. This problem affects 2 to 3 percent of individuals, either as an isolated finding or as a part of a syndrome or more severe condition.

There are many different causes of mental retardation, including genetics and environmental factors. The diagnosis is significantly assisted by a thorough review of the patient's developmental history, personal and family medical history, and physical examination.

This *col11a2* gene encodes a minor febrile collagen, one of the two alpha chains of type XI collagen. It is situated on chromosome 6 extremely close to the retinoid X receptor beta gene, but apart from it. Despite being a heterotrimer, type XI collagen has an alpha 1 type II chain as the third alpha chain that has undergone post-translational alteration. This type XI chain undergoes proteolytic processing to become PARP, a proline/arginine-rich protein with an amino terminal domain. Mutations in this gene are linked to otospondylomegaepiphyseal dysplasia (OSMED syndrome), Weissenbacher-Zweymuller syndrome, type III Stickler syndrome, autosomal dominant non-syndromic sensorineural type 13 deafness (DFNA13), and autosomal recessive non-syndromic sensorineural type 53 deafness (DFNB53) and also to intellectual disability through this study.

**Table 4.1; mutations of col11a2 gene in bannu kpk along with those reported in other parts of the word**

| S. No | cDNA | Amino Acid | Codon Number | Phenotype |
|---|---|---|---|---|
| 1 | c.106G>T | p. Arg36Trp | 36 | Sensorineural hearing loss? |
| 2 | c.109G>T | p. Ala37Ser | 37 | Deafness |
| 3 | c.529C>T | p. Arg177* | 177 | Robin sequence |
| 4 | c.652T>T | p. Tyr218* | 218 | Dysmorphism |
| 5 | c.688G>T | p. Gly230Trp | 230 | Deafness |
| 6 | c.1333C>T | p. Pro445Ser | 445 | Hearing loss |
| 7 | c.1363C>T | p. Arg455Trp | 455 | Micrognathia |

| | | | | |
|---|---|---|---|---|
| 8 | c.1606C>C | p. Arg536Gln | 536 | Dysmorphism |
| 9 | c.1615C>T | p. Arg539Trp | 539 | Osteoarthritis, earlyonset |
| 10 | c.1636C>T | p. Arg546* | 546 | Otospondylomegaepiph yseal dysplasia |
| 11 | c.1861C>A | p. Pro621Thr | 621 | Non-syndromic hearing loss, autosomal recessive |
| 12 | c.1981C>A | p. Gly661Arg | 661 | Osteochondrodysplasia |
| 13 | c.2002C>T | p. Pro668Ser | 668 | Deafness |
| 14 | c.2206C>G | p. Gly736Val | 736 | Hearing loss |
| 15 | c.2335C>C | p. Pro779Leu | 779 | Sensorineural hearing loss, nonsyndromic |
| 16 | c.2422C>G | p. Gly808Glu | 808 | Deafness, autosomal dominant 13 |
| 17 | c.2491C>T | p. Ser831* | 831 | Otospondylomegaepiph yseal dysplasia |
| 18 | c.2491C>T | p. Ser831Leu | 831 | Otospondylomegaepiph yseal dysplasia? |
| 19 | c.2662C>A | p. Pro888Thr | 888 | Deafness |
| 20 | **c.3031C>A** | **p. Pro1011Thr** | **1011** | **Deafness, with intellectual disabilities** |
| 21 | C.3100C>T | p. Arg1034Cys | 1034 | Deafness, autosomal dominant 13 |
| 22 | c.3391C>C | p. Arg1131Gln | 1131 | Deafness |
| 23 | c.3742C>C | p. Pro1248Leu | 1248 | Hearing loss |
| 24 | c.3916G>G | p. Gly13306Asp | 1306 | Stickler syndrome, without eye involvement |
| 25 | c.3991C>T | p. Arg1331* | 1331 | Otospondylomegaepiph yseal dysplasia |
| 26 | c.4135C>T | p. Arg1379* | 1379 | Stickler syndrome, without eye involvement |
| 27 | c.4264C>C | p. Pro1422Leu | 1422 | Deafness? |
| 28 | c.2321G>G | p. Gly1441Glu | 1441 | Weissenbacher-Zweymuller syndrome |
| 29 | c.4465G>A | p. Gly1489Ser | 1489 | Stickler syndrome |
| 30 | c.4600C>T | p. Pro1534Ser | 1534 | Stickler syndrome |
| 31 | c.4750G>T | p. Gly1584* | 1584 | Otospondylomegaepiph yseal dysplasia |

## Conclusion

In the current study, we identified a heterozygous variant in a Pashtun family suffering from deafness and intellectual disabilities in the district of Banuu, Pakistan. Whole exome sequencing (WES), an NGS-based sequencing method was used for analysis. A critical role is being played by next-generation sequencing (NGS) in the diagnosis of rare genetic diseases that were previously difficult to identify. It was time-consuming, expensive, and difficult to conduct genetic testing in the past due to a lack of resources. Three members of the family were affected and the pedigree showed autosomal mode of inheritance. whereas their parents were normal. We revealed a heterozygous missense mutation through WES (NM_080680.3: c.3031C>A) in *COL11A2*. Genetic testing should be done on regular bases as Khyber Pakhtunkhwa is the hub for rare genetical disorders due to high consanguinity rate.

### 5.1: Significance of the study:

Syndromic Hearing loss is one of the major public health concerns which causes deafness worldwide. Instead of advances in its diagnostic approaches and management, huge number of its cases remains undetected till severe stages, empowering the need of more understanding of its genetic and molecular diagnosis. Genetic factors highly influenced its susceptibility, progression and therapeutic implications. There is a significant gap in the understanding of genetics of syndromic hearing loss in South Asian populations particularly in Khyber-Pakhtunkhwa, Pakistan due to lower rate of genetic studies on it. Pashtun population are at higher risk of developing it because of high consanguinity rate. Therefore, unraveling the genetic landscape of syndromic hearing loss in Khyber-Pakhtunkhwa is of highly importance. We found a missense mutation in *COL11A2* which is responsible for causing hearing loss and intellectual disability in Pashtun family which will support the identification of high-risk individuals by prenatal and career testing and development of personalized treatment and management strategies in this family. This study contributes a missense mutation in *COL11A2 gene* to the global understanding of syndromic hearing loss genetics which will significantly help in the knowledge of the molecular mechanism of syndromic hearing loss by analyzing the impact of this gene on protein function.

### 5.2: Limitations:

The Identified variant could not be validated because of lack of the blood samples. The small sample size in which only one family was subjected for WES limits the generalization of the findings. The genetic variants found may represent family specific mutation rather than generally relevant to other ethnic groups in Pakistan. Furthermore. WES can only sequence the exonic regions of the gene leaving the pathogenic variants in the intronic regions of the gene. Moreover, Functional biology of the identified variants was not performed to unravel the consequences of this variant on the mechanism of disease and protein function.

### 5.3: Future Directions:

It is recommended for future studies to revisit the same family and take a blood sample for sanger validation of the same variant and to include larger sample size by recruiting families from diverse ethnic regions of Pakistan to minimize the selection bias and overcome the limitation of generalizability of the findings. Furthermore, WES analysis of multiple affected and unaffected or Trio-based should be performed to strengthen the segregation analysis. Future studies should opt for whole genome sequencing to overcome the limitations of WES by detecting the variants in intronic regions of gene. Moreover, Functional studies or gene expression models should be performed of the identified variants in animal to unravel the underlying molecular mechanism and its functional consequences on protein function.

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