Molecular genetic study of Usher Syndrome in a cohort of consanguineous families from the local population

Hussan Bilal

Department of Zoology Faculty of Biological Sciences

Quaid-I-Azam University

Islamabad

2023

Molecular genetic study of Usher Syndrome in cohort of consanguineous families from local population

A dissertation submitted in partial fulfillment of the requirements for the degree of
Master of Philosophy
In
Molecular Biology
By
Hussan Bilal

Department of Zoology Faculty of Biological Sciences
Quaid-i-Azam University
Islamabad
2023

In the name of Allah Almighty, The Most Gracious, The Most Beneficent, The Most Merciful

CERTIFICATE

This dissertation "Molecular genetics study of Usher Syndrome in a cohort of consanguineous families from local population" submitted by **Mr. Hussan Bilal,** is accepted in its present form by the Department of Zoology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirement for the degree of Master of Philosophy in Molecular Biology.

Supervisor:

Dr. Sabika Firasat

External Examiner:

Dr. Ramla Shahid
Assistant Professor
Department of Biosciences
COMSATS University, Park Road,
Islamabad

Prof. Dr. Amina Zuberi
Chairperson

CHAIRPERSON
Department of Zoology
Quaid-i-Azam University
Islamabad.

Date: 19-05-2023

DEPARTMENT OF ZOOLOGY
ISLAMABAD
DATE 19-5-2023
QUAID-I-AZAM UNIVERSITY

CERTIFICATE

This dissertation "*Study of Clinical Profile and Molecular Basic of Niemann Pick Diseases (NPD)in Cases from Local Population"* submitted by ***Samra Aamir***, is accepted in its present form by the Department of Zoology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan as satisfying the thesis requirement for the degree of Master of Philosophy in Molecular and Cell Biology.

Supervisor _____

Dr. Sabika Firasat

Associate Professor

External Examiner _____

_____

Dr. Amina Zubari

Department of Zoology Quaid-i-Azam UniversityIslamabad

DECLERATION

I hereby declare that the work presented in this thesis is the result of my own efforts and research work, carried out in Molecular Biology Lab, Department of Zoology, Quaid-I-Azam University Islamabad.

This thesis is my own composition and no part of it has been presented for any degree previously, nor does it contain, without proper acknowledgement or reference, any material from the published resources to the best of my knowledge.

Hussan Bilal

DEDICATED

TO

MY BELOVED PARENTS WHO HAVE BEEN PILLARS OF SUPPORT, GUIDANCE AND LOVE IN MY LIFE

ACKNOWLEDGEMENT

All the praise and thanks to **Almighty Allah** for his countless blessings and for giving me strength to complete my research work successfully. Blessings of Allah be upon His **Holy Prophet (PBUH)** who brought us out of the darkness and enlightened the way to Heaven, who has guided His Ummah to seek knowledge from Cradle to Grave. All the glory and thanks to Almighty Allah for His countless blessings and for giving me the potency to finalize my research work successfully.

I owe deep sense of gratitude to my honorable research supervisor **Dr**. **Sabika Firasat,** Associate Professor, Department of Zoology, QAU, and **Dr.Hanif** an ophthalmologist at Al Hanif clinik Jhang Sadar, Pakistan, for their kind surveillance, incalculable guidance, constant inducement, and constructive propositions which remained exemplary throughout my research span. Their timely advice and scientific approach have helped me to a great extent to accomplish my research work. They have been a true provocation and the best mentors I have ever had.

I would like to express my gratitude to our Department Head, **Dr. Amina Zubari**, who equipped us with exceptional research and a productive environment.

I'd like to acknowledge my father **Muhammad Aslam** and my mother for their unwavering support; without them, I wouldn't be capable of finishing my studies. I am thankful to my sisters', brother for emotional support, which has been invaluable to me during this educational journey.

I am appreciative to my senior mentor **Raeesa Tehreem** for her undying support that aided me in compiling my whole research writing and for his insightful guidance. I am also pleased with my seniors **Asfandyar Ahmad Khan** immensely thankful for their serious and practical suggestions, which enabled me to finish my research task. I'd like to extend my heartfelt gratitude to my friends and fellows **Azmat Ullah, Rehan Khan, Muhammad Shafqat, Muhammad Shoaib and Samra Amir** for their delightful company throughout my studies.

Hussan Bilal

Table of Contents

Contents Page

LISTS OF FIGURES

LISTS OF TABLES

LIST OF ABBREVIATIONS

Abbreviation	Full Forms
RP	Retinitis pigmentosa
USH	Usher
USH1	Usher type 1
USH2	Usher type 2
USH3	Usher type 3
AA	Amino acid
EC	Extracellular
PBD	Protein binding domain
SAM	Stereocilia alpha motifs
CIB2	Protein binding domain
LE	Laminin-EGF-like domain
ASO	Antisense Oligonucleotides
ZFN	Zinc-Finger nucleases
AAV	Adeno-associated Virus
MyTH4	Myosin tail homology 4
ADGRV1	Adhesion G-protein coupled receptors 1
HC	Hair cells
HARS	Histidyl-tRNA synthetase
MET	Mechanotransduction
RPE	Retinitis pigment epithelium
AAV	Adeno-associated virus
ZFN	Zinc-fingers DNA binding domain
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
iPSC	Induced pluripotent stem cells
EDTA	Ethylenediaminetetraacetic Acid
PCR	Polymerase Chain Reaction
Rmp	Revolutions Per Minute
PK	Proteinase K
SDS	Sodium Dodecyl Sulphate
TE	Tris EDTA
TBE	Tris Borate EDTA

ABSTRACT

Retinitis pigmentosa is most prevalent form of inherited retinal degenerations (IRDs) that affect 1/4000 individuals worldwide. Most of the RP cases are non-syndromic while 20 to 30% of cases are associated with syndromic RP. Usher syndrome is most common form of RP that affects 1 to 4 in 25000 individuals. Congenital severe-to-profound sensorineural hearing loss with RP and, in rare cases, vestibular dysfunction defines USH. USH is divided into three clinical types; USH1, USH2 and USH3 depending upon the severity, age of onset and progression of disease.

Each type is genetically heterogenous with many genes that are cause of each specific type.75% cases in USH1 have USH1B subtype that occur by mutation in *MYO7A* gene that code for myosin VIIA. This USH1 subtype USH1B is most common in Pakistani population with 42.9% affected people followed by USH1D with 28.6% and *USH1C* and USH1F that affect 12.2%. Mutation in many other genes like *ADGRV1*, *CDH23* and *PCDH15* cause Usher syndrome.

In Pakistani population, autosomal recessive diseases are common due to consanguineous marriages specially in backward areas. In this study, we selected 6 families with Usher symptoms with recessive pattern of inheritance to screen genetic mutation and polymorphisms. Blood samples were collected from hospitals and DNA extracted from white blood cells. Electrophoresis was done to check integrity of DNA followed by PCR to amplify exon 6,7 and 8 of *MYO7A* gene.

Sanger's sequencing of purified PCR products was done to analyze genetic variant. Novel polymorphism were detected at position g.29179T>G, g.29186C>G and g.29181G>A in *MYO7A* gene (table 3.2). In silico analysis show that these polymorphisms affect the splice site that further change protein features including folding, integrity and structure of protein. Genetic counselling of affected families was done to avoid cousin marriages. However no homozygous disease-causing variant was found in this study.

Introduction

1.1 Usher Syndrome

Usher syndrome is a hereditary condition that causes progressive retinitis pigmentosa, hearing loss, and vestibular dysfunction (Keats & Savas, 2004). It accounts for more than half of all people who are both deaf and blind(Rosenberg, Haim, Hauch, & Parving, 1997). Usher syndrome is expected to affect 3.8-4.4 out of every 100,000 live births (Rosenberg *et al.,* 1997). Usher syndrome is classified into three types: USH1, USH2, and USH3. Significant hearing loss and impaired vestibular function are present in patients with Usher syndrome type 1 from birth. Patients with USH2 have milder hearing loss and normal vestibular function (Tsilou *et al*., 2002). While USH3 is characterized by progressive RP and hearing loss with vestibular dysfunctioning (Weston, Luijendijk, Humphrey, Möller, & Kimberling, 2004).

To carry out their regular activity, many Usher proteins interact with one another in a network (Maerker *et al*., 2008). Genes that cause usher syndrome form many protein classes and families ((Reiners, Nagel-Wolfrum, Jürgens, Märker, & Wolfrum, 2006) i.e. USH1B gene code for a motor protein called myosin VIIA, while *USH1C*, SANS (USH1G), and whirlin (USH2D) are scaffold proteins, cadherin 23 (USH1D), and protocadherin 15 (USH1F) are adhesion proteins, and *USH2A* and USH2C are transmembrane proteins.

The only known member of USH3 is Clarin 1 encoded by USH3A is a transmembrane protein. Mutations in the many Usher genes cause a wide range of ear and eye abnormalities (Adato *et al*., 2005); van Wijk *et al.,* 2006). In the Usher protein network, the USH1 and USH2 proteins interact with one another, with whirlin and harmonin playing important roles. (Adato *et al.,* 2005).

A number of Usher proteins have been discovered and described, including myosin VIIA, harmonin, cadherin 23, protocadherin 15, SANS, usherin, vlgr1, and clarin-1. Approximately 75% of people with type 1 Usher syndrome have USH1B, which is brought on by mutations in the *MYO7A* gene (Well *et al*., 1995).Cochlear hair cells and the retina express myosin VIIa (Well *et al*., 1995). USH1B is the most common

type of the disease in Pakistani citizens (42.9%), followed by USH1D (28.6%), and *USH1C* and USH1F (12.2%) (Z. Ahmed, Riazuddin, Riazuddin, & Wilcox, 2003).A PDZ-domain protein termed harmonin is encoded by the *USH1C* gene (Bitner-Glindzicz *et al.,* 2000; Verpy *et al.,* 2000). In protein-protein interactions, harmonin contains three PDZ domains that interact with other proteins (Sheng & Sala, 2001).

Mutations in *CDH23* cause USH1D (Bork *et al*., 2001). Waltzer mutant mice (Bolz *et al*., 2001), whose hair cell stereocilia are disorderly like those of shaker-1 animals, have mutations in the *CDH23* gene. Cadherins are a broad family of proteins that play a role in intercellular adhesion and have extracellular calcium binding domains. Myosin VIIA may deliver harmonin along the growing stereocilia's actin core, according to evidence from Boeda *et al*. (2002) and Siemens *et al*. (2002) that both myosin VIIA and cadherin 23 bind to harmonin through PDZ-domain interactions suggests harmonin is carried out by *MYO7A* along with actine core of developing stereocilia.

Patients with USH1F were found to have mutations in *PCDH15* (encoding protocadherin 15), another cadherin-like gene. The disordered hair bundles in the Ames waltzer mice, which were strikingly similar to those in shaker-1 and waltzer, were revealed to be deficient in *PCDH15*. Proline-rich regions in the cytoplasmic domain of a transmembrane protein, protocadherin 15 may mediate protein-protein interactions.

The USH1G locus is present on chromosome 17q, and the causal gene was identified as SANS, domain (Mustapha *et al.,* 2002). A novel scaffold-like protein is encoded by this gene that shows its association with harmonin in co-transfection experiment (Weil *et al.,* 2003).A protein Usherin is encoded by gene *USH2A* (Eudy *et al*., 1998). This protein shows its expression in retina and cochlea (Huang *et al.,* 2002). USH3 cases are mostly reported in Finnish population and caused by a mutation in the *USH3A* gene (Joensuu *et al.,* 2001). *USH3A* gene encodes a protein of 232 amino acids called Clarin-1 that contains transmembrane domain and ma b involved in synaptic transmission produced by hair cells.

It is proved by evidence that whirlin and hermonin bind to the cadherin 23, usherin, Protocadherin, VLGR1 and myosin VIIA that are components of USHER complex through PDZ domains (Brown *et al*., 2008).

Myosins are ATP hydrolyzing motor proteins that translocate with actin filaments and are responsible for cellular movements such as molecular transportation and localization of organelle. Several types of motor proteins have been discovered that are categorized on the basis of their primary motor domain. These proteins have conserved motor domain that contains binding sites for actin and certain nucleotides and regulatory domain that contain sites for binding of side chains related to calmodulin. The targeting or binding of molecules to cargo may be done by a c-terminal domain (Erickson *et al.,* 2003).In some cases, both syndromic and non-syndromic hair impairment is caused by mutation in single gene for example Usher syndrome (USH1G) and DFNA22/DFNA26 linked dominant deafness both caused by a mutation in SANS gene (Rosenberg *et al.,* 1997). Similarly, *MYO 7A* mutation can cause Usher syndrome type IB (USHIB), dominant (DFNA 11) and a recessive DFNB2 non-syndromic deafness (Lin *et al.,* 1997, Weil *et al*., 1995).

1.2 History:

Usher syndrome was first examined by a British ophthalmologist Charles Usher who examined Pathology and prevalence of this disease so called Usher syndrome. He reported 69 cases (Usher 1924). However, it was also examined by a pioneer of modern ophthalmology Albrecht Von Grafe (Von Grafe 1858). He examined two brothers having RP & deafness with same clinical symptoms.

One of his student Richard Liebreich studied disease pattern in a Berlin population (Liebreich 1861). He pointed out that Usher syndrome is recessive because it frequently affects siblings from blood-related marriages or families with patients who are in separate generations.

1.3 Prevalence:

Frequency 0f hearing loss is 1/2000 or 1/650 births (Morton & Nance 2006). According to an estimate,10 % deafness is caused by consanguineous marriages .RP affect 1 in 4000 individuals. A kind of hearing loss associated with RP is Usher syndrome that is highly heterogenous and associated with sensorineural deafness and Retinitis Pigmentosa (Keats & Savas 2004). Its frequency is 1 to 10 % in children and 3 to 5 in 10000 of common population. Between 4 to 17 out of every 100,000 people are affected worldwide by Usher Syndrome. Additionally, it is thought to account for

5% of cases of congenital deafness and 18% of cases of retinitis pigmentosa (Marazita *et al.,* 1993).

Usher syndrome affects 1 in 2300 individuals worldwide. It accounts for 3 to 4 in 10000 individuals in European population (Keat & Corey 1999). 1.86 in 1000 new born babies affect by this syndrome and half cases are due to genetic cause (Morton & Nance 2006).

Type 1 of the Usher syndrome is the most severe variant (Usher 1). Bilateral congenital sensorineural hearing impairment ranging from severe to profound is seen in Usher 1 patients, which is typically non-progressive and accompanied by vestibular areflexia. It accounts for 25–44% of all cases of Usher syndrome. Usher syndrome type 2 (Usher 2) accounts for more than half of all cases of the condition (Reiners *et al*., 2006). Usher syndrome type 3 (Usher 3) is uncommon in most groups, making up about 2–4% of cases overall, although it is especially common in Finland and among Ashkenazi Jews (Ness .,*et al*).

1.4 Subtypes of Usher Syndrome:

1/6th of RP patients are thought to affected by Usher syndrome (Williams 2008).In reduced severity of symptoms, Usher is divided into three categories I,II and III. People with Usher syndrome type are affected with hearing loss by birth and also loss their vision in adolescence age. They are also having balancing problems and problem in learning walking due to vestibular defaults. People with Usher type II have less severe symptoms than type I and they have normal vestibular function. They begin to loss their vision in adulthood. Milder deafness is recorded in Usher III patients but their hearing loss is progressive and half patients have vestibular disfunctioning. (Sadeghi, Cohn, Kimberling, Tranebjaerg, & Moller, 2005).

1.4.1 Usher Type I:

Usher syndrome type I causes congenital, bilateral, and significant hearing loss. Usher syndrome type I is predicted to affect 3 to 6 persons per 100,000 in the general population worldwide. Patients have communication problem. Another defining feature of this type is association of areflexia with deafness. Due to presence

of areflexia, children affected with this type learn to walk later as compared to normal childrens (Kimberling & Möller, 1995; Petit, 2001).

Old people seem uncoordinated and face injuries and accidental problems in balancing tasks such as cycling and riding. Children affected with this type thought to have non syndromic deafness and remain undiagnosed until symptoms of RP, night blindness become severe and noticed by parents and other members. RP is progressive loss of retinal cells called rod cells that adapt to dark condition. It start from peripheral portion of retina causes loss of peripheral vision that further lead to the night blindness.

One of the major heterogenous types of Usher syndrome is Type 1. There are seven gene loci have been reported until now. These are USH1B, *USH1C*, USH1D, USH1E, USH1F, USH1G, and USH1H) called USH1 loci and there are five USH1 genes have been identified (Z. M. Ahmed *et al.,* 2009). Genes that are mutated in Usher syndrome type I are *CDH23*,*MYO7A*,*PCDH15*,*USH1C* and *USH1G*.These genes are also responsible for non-syndromic hearing loss. Other genes responsible for non-syndromic hearing loss are *DFNB2*, *DFNB18*, *DFNB12* and *DFNB23* (Riazuddin *et al.,* 2008).

Proteins that are coded by these genes are involved in proper functioning and maintenance of structures of inner ear i.e Stereocilia. These are inner ear cells that are involved in sending signals to central nervous system that are produced in response to motion by sound waves. Mutation in these genes cause misfunctioning of proteins that lead to unbalancing (vestibular disfunctioning) and hearing loss. These genes also influence the form and functioning of photoreceptor cells called rod cells present in retinal epithelium. So, mutation in these genes also affect the vision and produce a clinical condition called RP.RP with hearing loss is called Usher syndrome.

1.4.2 USHER type II:

Ush type 2 is less severe than type 1 and characterized by mild to severe hearing loss with RP that is diagnosed at the age of puberty. Vestibular functioning is normal in this case. (Reisser, Kimberling, & Otterstedde, 2002).Most common tupe of Usher syndrome is Type 2 that account for more than 50% of all Usher cases (Eudy *et*

al., 1997). People affected with this type have difficulty in hearing high frequency sounds. For example, affected people have difficulty in hearing high frequency soft speech words like ‘’d’’ and ‘’t’’. Mutations in genes *USH2A*, *GPR98* and *DFNB31* cause Ush type 2. *USH2A* gene encode a protein called usherin that is supportive component of inner ear and retina.

Usherin is crucial for proper functioning and maintenance of inner ear that shows its role in vision and hearing. Other proteins are also associated with Usherin but functioning of that proteins are not yet understood.

1.4.3 Usher type III:

Usher type III was restricted to Finnish people before but now it’s been diagnosed in several other people of European ancestry. Therefor this type of syndrome is rare in other areas. In this case, hearing loss and RP associated with vestibular dysfunction occur later in life. These conditions become more severe with the passage of time. Hypermetropia and astigmatism are clinical conditions associated with Ush type III. Mutation in *CLRN1* gene cause Ush type III.*CLRN1* gene encode a protein called Clarin that is involved in maintenance and function of inner ear and retinal cells (Pakarinen, Karjalainen, Simola, Laippala, & Kaitalo, 1995).

1.5 FORM of Usher type 1 with Pathology and genetics:

1.5.1 USH1B:

The most common form of Usher syndrome type I is USH1B.It accounts for almost 75% of Ush1 patients. Mutation in *MYO7A* gene cause USH1B with encode a protein called myosin 7A.It was the first gene to isolated for Usher syndrome (Well *et al*., 1995). *MYO7A* contain 48 exons of which 17 made head,3 made neck and 28 made tail of protein molecule. Several alternatively spliced forms are also coded that are mainly different in motor head region. Its length is up to 100Kb.(Chen *et al*., 1996).Several mutant model organism like Zebra fish and mice that have defective vestibular hair cells and lateral line system carry mutations in *MYO7A* gene (Ernest *et al*., 2000).

Myosin is a large protein family that is divided into 18 families. These are motor proteins that move along the actin filaments by using energy produced by hydrolysis of ATP. N-terminus domain that is highly conserved head region in myosin proteins contain binding sites for both ATP and actin. Head is followed by neck region and tail region that varies from one myosin to myosin. Tail sequence of each myosin protein determines it function specificity because various protein-protein interacting domains are present in this region that bind to several cargo and regulatory molecules and other molecules that are involved in signal transduction pathways (Mermall, Post, & Mooseker, 1998). Other biological processes such as cytoplasmic extension, vesicular and mRNA transportation and signal transduction pathways are also done by the help of myosin.

1.5.1.1 Structure of Myosin VIIA:

The human *MYO7A* protein consist of 2215 amino acids ((AA) with mass 254kDa, in which motor head domain that contain ATP and acting binding domain consist of 729 AA, neck region consist of 126 AA that contain five IQ(isoleucine-glutamine) motifs that are thought to =bind with calmodulin and a tail with 1360 AA. Its recently demonstrated that head of *MYO7A* is involved in actin based motility (Udovichenko, Vansant, & Williams, 2000).

Tail of protein begins with a short coiled domain with 78 AA that is involved in formation of homodimers(Weil *et al.*, 1997).Two larger repeats of 460 AA follow the coiled domain with each containing MyTH4(Myosin tail homology 4) domain with unknown function and other is similar to a protein superfamily called FEMR(4.1, ecrin,radixin,meosin) which is a membrane binding domain (Chishti *et al.*, 1998).Myosin V and Myosin 15 also contain MyTH4 in vicinity of FEMR domains. Same vicinity is also present in plant kinesin that tells function importance of these two motifs. SH3 (src homology 3) is a poorly conserved region that separate these two motifs and this region is thought to bind with proline rich region (Nguyen *et al.,* 2000).

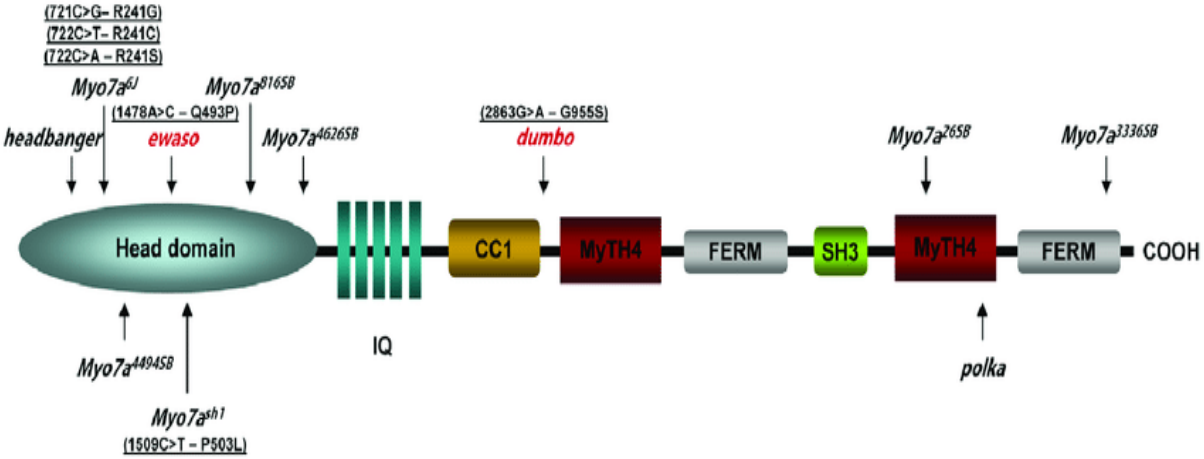

Figure 1.1: Schematic diagram of myosin VIIA (Miller *et al.,* 2012).

1.5.2 *USH1C*:

USH1C is another form of Usher type I caused by mutation in USHIC gene. It contains 28 exons with 51Kb length. *USH1C* encodes a protein called **Harmonin** that contain PDZ domain (Verpy *et al.,* 2000). From all the mutation that are reported in this until now, two very peculiar mutations are reported one of which is called expanded interonic VNTR that is supposed to affect transcriptional or post transcriptional process and exonic substitution that lead to the formation of new splice site with small in frame deletion and unstable transcript is formed (Bitner-Glindzicz *et al.,* 2000).Various transcripts are formed by alternative splicing of *USH1C* due to different use of eight exons. These varying transcripts have at least eight isoforms in murine inner ear that contain 420to 900AA and divided further into three categories on the basis of domains they have.

1.5.2.1 Structure of Harmonin:

These domains include PDZ domain that can be two or three in each transcript, coil-coiled domains could be one or two and proline-serine-threonine (PST) domains that could be present or absent. From these domains, coil-coiled domain is thought to involve in dimerization, PST domain is involved in binding of SH3 and Profilin (actin regulatory protein).

PDZ domain is 80 to 90 amino acid in length and very interacting domain (Sudol, 1998).Proteins that contain PDZ domains contain a vast variety of molecules some of

their representative are also present in bacteria. These molecules are involved in organization of large molecular complexes present along the surface of plasma membrane. In addition to binding with transmembrane proteins like ion channels and transporters, these molecules also bind with actin binding proteins (Kim, Niethammer, Rothschild, Nung Jan, & Sheng, 1995). These molecules accumulate and coordinate the activity of many transmembrane proteins and make their access to cortical cytoskeleton (Sheng & Pak, 2000).

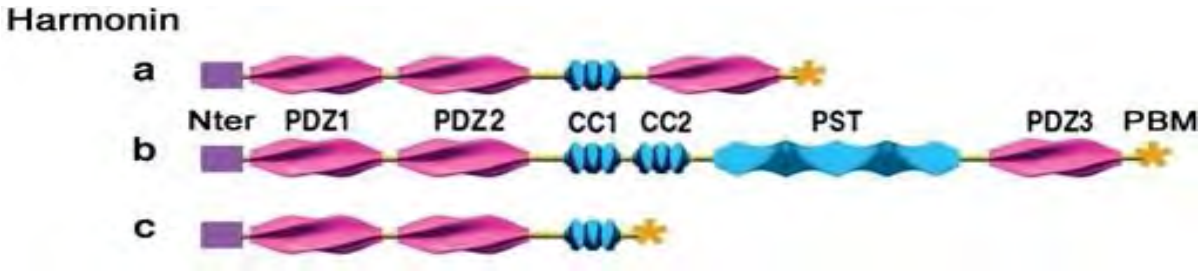

Fig.1.2: Schematic diagrams of Harmonin protein (Bahloul *et al.,* 2010)

1.5.3 USH1D:

This type of Usher syndrome I is caused by a mutation in *CDH23* gene. The second most common form of Usher type I is USH1D (L. M. Astuto *et al*., 2000). This gene consists of 69 exons and 300Kb in length. Chromosomal location of *CDH23* is 10q21-q22, also called DFNB12 locus. .It was found later that gene that cause USH1D overlap with DFNB12 locus. Later, Mollers groups identified these locus told about their mutations that cause USH1B and ARSNHL (DFNB12) (Bork *et al*., 2001).This gene encodes a protein called Cadherin related protein (Cadherin 23).

1.5.3.1 Catherin functions:

Cadherin proteins are the large transmembrane family that maintains calcium dependent intracellular and extracellular adhesion. They also function in establishing tissue integrity (Gumbiner, 2005).Other biological process that are done by Catherin to maintain tissue integrity are morphogenesis, mechanotransduction, tissue homeostasis and signal transduction (Schneider & Kolligs, 2015).Various types of diseases are associated with cadherins defect like cancer. **Cadherin-23** also known as Otocadherin and Cadherin-related 23 have remarkably long extracellular domains.

This is best known for its crucial function in mechanoelectrical transduction in ear cells (Xu, Oshima, & Heller, 2010).

Novel mutations that are reported in this gene are nonsense mutations, codon deletion, slice site mutation and truncated splice site mutations (von Brederlow *et al.,* 2002).Six isoform of *CDH23* exist due to alternative splicing. Three of these isoforms lack 68^{th} exon so 35 amino acids encoded by this exon is absent in these isoforms. Most prominent isoforms are A1 and A2. These are with 68^{th} exon (*CDH23*+$68^{th)}$ and without 68^{th} exon (*CDH23*-$68^{th)}$ respectively. Longest isoform that express in Organ of Corti is A1.This also express in rod photoreceptors of some primates (Xu *et al*., 2010).A2 isoforms primarily expressed in retina. Besides its expression in lungs, heart ,nose and brain, Cadherin 23 primarily expressed in neuroretina, vestibular and cochlear hair cell stereocilia (Lagziel *et al.,* 2005).

1.5.3.2 Structural pattern of Cadherin-23:

Cadherin-23 is a big (369 kDa) protein with 3354 amino acids and having 27 extracellular domains (EC) ,one transmembrane domain and one cytoplasmic domain (L. Astuto *et al.,* 2002).Mouse *CDH23* have two extracellular domains (EC) and their cytoplasmic PDZ domains. These domains are thought to interact with other proteins.

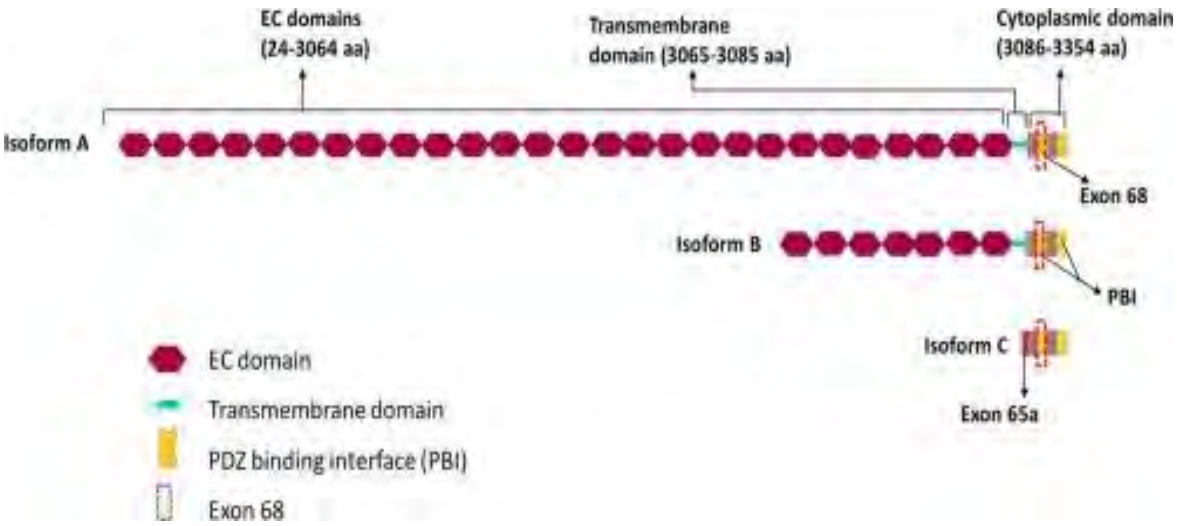

Fig. 1.3: Schematic diagram of Cadherin 23 showing isoforms with motifs

(Srisailapathy & Mohanram, 2018)

Its ectodomain that is extracellular possess 27 cadherin repeats most of which are bind to Ca+2. These calcium binding motifs helps in calcium binding interaction necessary for cellular adhesion. This domain is followed by 268 amino acids that are present in cytoplasmic region (Bork *et al.,* 2001).Cadherin is the part of tip links of mechanosensory hair cells present in inner ear.

Extracellular domain contains 110 amino acids forming Greek Key motifs in which two beta sheets formed beta sandwiched fold. These sheets are composed of seven beta strands. This feature is common to all cadhrins. Long N-terminal prodomain and alpha helix in different strands is unique to CDH-23. Classical cadherins have an angular bent in their structure but CDH-23 have linear EC1 and EC2 domains that represents their role in linear structure of tip links (Elledge *et al.,* 2010).

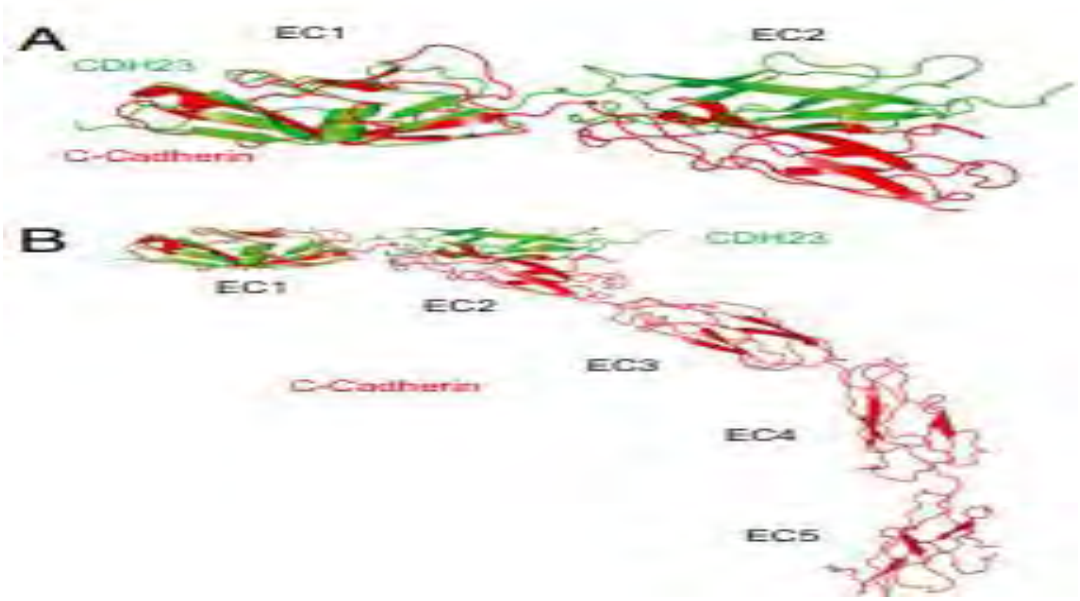

Fig.1.4: 3D structure of Cadherin (Protein Data Bank)

Similar to other cadhrins, calcium binding is property of cadherins that provide them mechanical strength and essential for keeping extracellular domains together. Crystal structure of CDH-23 showed that there are three conserved linker regions in EC1 and EC2 domains where calcium binds. This binding provides stability and maintenance to the CDH-23 structure. Cytoplasmic domain of CDH-23 contain two PDZ binding interfaces(PBI) that interact with PDZ domains of other proteins like Harmonin (Siemens *et al*., 2002).

1.5.4 USH1F:

PCDH 15 gene encodes a protein called Protocadherin 15 (PCDH 15). Mutation in this gene cause USH1F (Z. M. Ahmed *et al*., 2001). Protocadherin 15 is key component of mechanotransduction complex. This protein makes lower portion of tip links. These tip links mechanically gates transduction channels. This protein contain 11 extracellular motifs (EC), single transmembrane domain ,double proline rich regions and cytoplasmic domain contain C terminal PDM region through with this protein interact with PDZ2 domain of Harmonin protein (Adato *et al*., 2005). Here are three splice isoforms of PCDH 15 that differs in C-terminal region of cytoplasmic domain. These isoforms are C1, C2 and C3 (Z. M. Ahmed *et al*., 2008). But these isoforms have a common region (CR) that comes after TM. CD2 isoform is more prominent because this is necessary for proper mechanotransduction in mature hair cells (Webb *et al*., 2011).

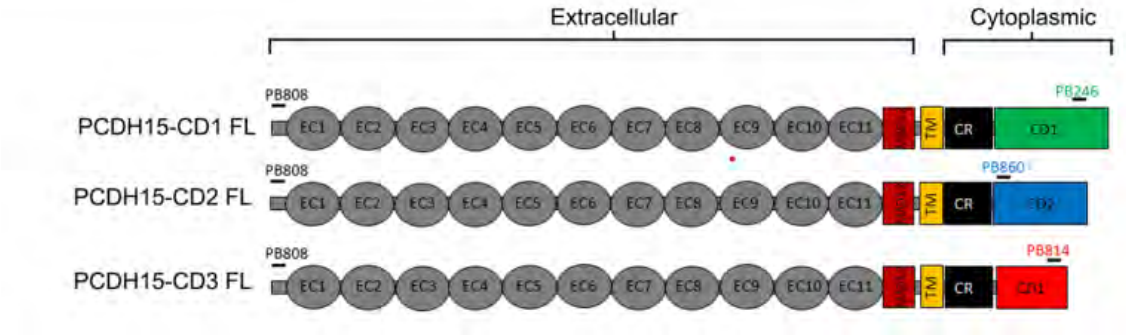

Fig.1.5: Schematic diagram showing Protocadherin domains

(Morishita & Yagi, 2007)

1.5.5 USH1G:

Mutation in a gene called USH1G cause this subtype of Usher syndrome. Product of this gene is a scaffold protein called SANS (Weil *et al* 2003). This protein contains several interactive domains that interact with other proteins, three ankyrin domains (ANK1-3) that are present at N-terminus, a sterile alpha motif (SAM) that is present after central region followed by class 1PBM that is a tripeptide and its C-terminus (Nourry *et al* 2003). SANS consist of 1380bp and encodes a protein that consists of 461 amino acids (Weil *et al*., 2003). Whirlin interacts with the class 1PBM of SANS through its distinct domains. These domains are PDZ1 and PDZ2 (van Wijk *et al*., 2006). Homodimerization of SANS occur by its central domain that interacts with *MYO7A*'s FERM domain. SAMS domain binds with PDZ1 and PDZ3 domain of Harmonin (Yan, Pan, Chen, Wu, & Zhang, 2010).

SANS and other Usher proteins are associated with primary cilia and these proteins also have essential role in ciliogenesis and ciliary maintenance. This suggested that Usher is a Ciliopathy (May-Simera, Nagel-Wolfrum, & Wolfrum, 2017) . Ciliopathies are group of disorders that occur by development, functioning and maintenance of cilia (Wheway, Parry, & Johnson, 2014).

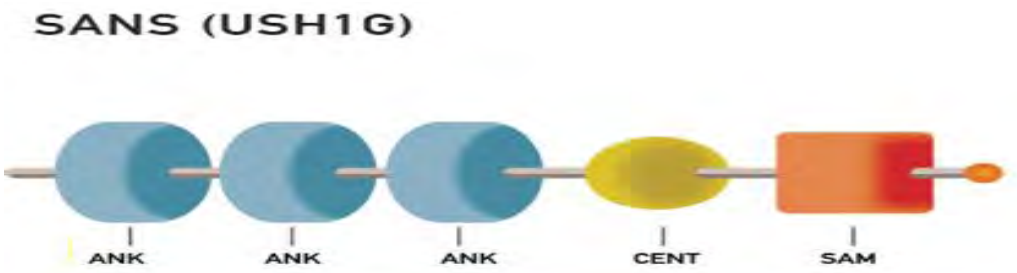

Fig.1.6: Schematic diagram of SANS domains

(Kremer, van Wijk, Märker, Wolfrum, & Roepman, 2006)

1.5.6 USH1J

Mutation in a gene called *CIB2* cause this subtype of Usher syndrome I. Locus of this gene is present on chromosome 15 (Seki *et al*., 1999). This gene code four different isoforms consist of 4 to 6 exons (Riazuddin *et al*., 2012). Some of the non-syndromic deafness type 48 (DFNB-48) and congenital muscular dystrophy type have been reported due to mutation in *CIB2* gene.

Calcium and intergrin binding protein 2 (*CIB2*) is encoded by this gene. Mass of this protein is 21.6 kDa, 187 amino acids residues and six exons. CIB1, CIB2, CIB3, CIB4 are members of this protein family. Common motifs to all of these members are EF-hands that change their conformation when bind with cations (Blazejczyk *et al*., 2009).*CIB2* contain three EF-hand motifs. Last two of these motifs are involved in binding of two cations specially Calcium and Magnesium (Michel *et al*., 2017). In opposite to prior research, it is proved that Ca2+ have low affinity for calcium and cannot work as calcium sensor under physiologic conditions. However, *CIB2* have more affinity for Mg+2 under same conditions, so it significantly functions as Mg+2 sensor (Vallone *et al.,* 2018).

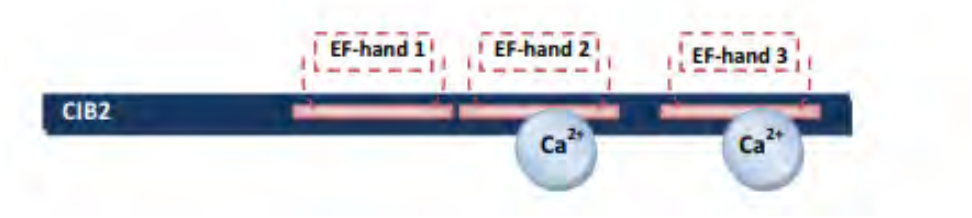

Fig.1.7: Schematic representation of *CIB2* protein motifs

(Jacoszek, Pollak, Płoski, & Ołdak, 2017)

1.6 Usher type 2:

USH2 patients have generally mild sensory neural hearing loss by birth and Retinitis pigmentosa which begins in late puberty or early adulthood (Mathur & Yang, 2015). Early it was observed that USH2 in non-progressive hearing loss but recent demonstrated that USH2 patients can also suffer with progressive hearing loss leading to severeness of symptoms (Hartel *et al*., 2016). Until now, three genes for USH2 have been reported including USH2B with their loci present on chromosomes 3 arm. After further molecular analysis, this gene is not present on USH loci (Emre Onat *et al*., 2013).Following are the further sub-types of USH2.

1.6.1 USH2A:

It was the first Usher locus to identify. Mutation in this gene cause USH type 2. *USH2A* accounts for half of the all USH cases (Emre Onat *et al*., 2013). 80% are found in *USH2A* gene that make it more prevalent type of USH2 out of two other types with specific gene mutations (Stabej *et al*., 2012). Locus of *USH2A* gene is present on chromosome 1q41. This gene encodes a protein called usherin which controls the development and maintenance of neurosensory cells present in cochlea and retina (Bhattacharya, Miller, Kimberling, Jablonski, & Cosgrove, 2002). Usherin is found to express within hair bundles of chochlear hair cells during development and it also express in apical inner segment of retina (Xiaoqing Liu *et al*., 2007). Usherin has a cytoplasmic tail that interact with one of the PDZ domain of Harmonin Whirlin causing *USH1C* and USH2D (Adato *et al*., 2005).A transcript have 24 additional region due to alternative splicing that express in cochlea and specified for it because this express highly in inner ear (Adato *et al*., 2005).This exon responsible for longer transcript was previously not included in *USH2A* transcript (van Wijk *et al*., 2004).

1.6.1.1 Structure:

It's a large protein that contain so many domains (Dona *et al*., 2018). Alternative splicing lead to isoforms A and B from which B is prominent with expression in retina. A is short isoform that contain 21 exons and code for a protein with 170kD mass and 1546 amino acids. This protein is an extra cellular matrix protein. This is long isoform that contain 72 exons and code a protein with 170kD mass and 5202

amino acids. This protein is crucial for cochlear hair cell development (van Wijk *et al.,* 2004). The thrombospondin extracellular protein family and this protein's thrombospondin-type LamG domain (TSNP-LG) are homologous (Nicosia and Tuszynski, 1994).

A protein of these family called thrombospondin-1 has functional diversity including activation of TGF-β1,activation and ligation of integrin and inhibition of MMP-2 AND MMP-9 and its presence in Usherin demonstrates its function in all of these activities (Murphy-Ullrich & Poczatek, 2000). Following this region is N-terminal lemining feature (LN module) that is crucial to polymerize leminins into special networks present in basement membranes (Bork, Downing, Kieffer, & Campbell, 1996).The domain is laminin-EGF-like (LE) that contain repeats of 60 amino acids with eight conserved cysteine residues (Engel, 1989). Between Usherin amino- and carboxy-terminal domains, this LE domain forms a rigid spacer with a rod-like tertiary structure and less flexibility (Beck, Hunter, & Engel, 1990). Four FN3 repeats are present at carboxylic terminus (Yan & Liu, 2010). These FN3 repeats form beta-plated sheets that are intended to interact with integrin molecules (Bowditch *et al*., 1994).

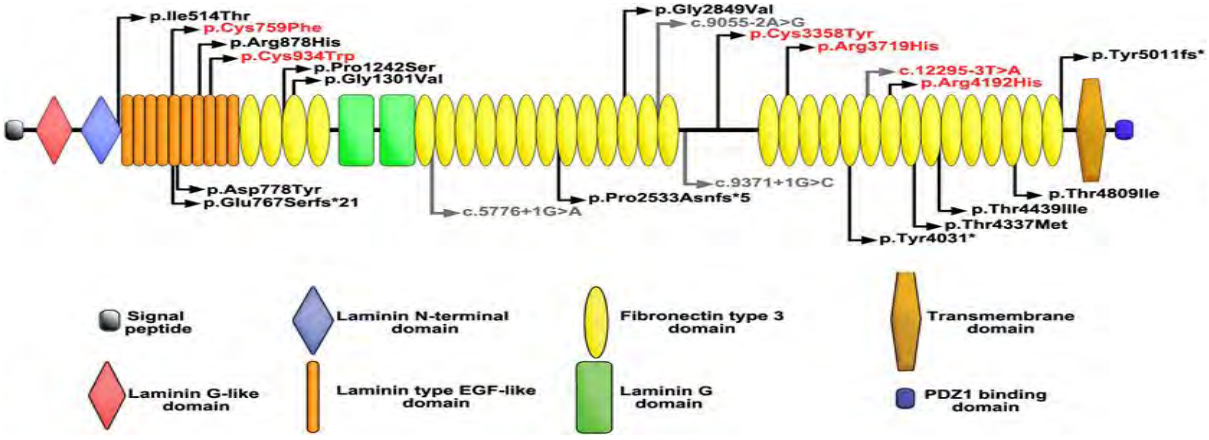

Fig.1.8 :Schematic diagram of Usherin (Lenassi *et al.,* 2015).

1.6.2 USH2C:

Causative gene of *USH1C* is ADGRV1 that is also known as VLGR1, MASS1 and GPR98. Location of this gene is 5q14.3-21.3 (Hilgert *et al*., 2009a).This gene encodes a protein called Adhesion G-protein coupled Receptor V1 (ADGRV1) a transmembrane receptor protein involved in development of cochlea and formation of

ankle links of stereocilia (Yang, Wang, Song, & Sokolov, 2012). ADGRV1 gene is included in one the most largest genes with 600kB genome and 90 exons (Zou *et al*., 2011). Due to large size, this protein contain many distinct domains and highly express in brain specially in ventricular region (McMillan & White, 2004). It is also involved in development of CNS, retina of fetus and other tissues (Hilgert *et al*., 2009b).

1.6.2.1 Structure of ADGRV1:

This protein called ADGRV1 belongs to sub-family that contain G-protein coupled proteolytic site for signaling of G-protein. This protein also relate with N-Terminal family with seven transmembrane receptor (Yang *et al*., 2012).Alternative splicing lead to the multiple variants with three isoforms that exclusively express in human ADGRV1a,ADGRV1b,ADGRV1c (Yang *et al*., 2012).The most predominant isoform is ADGRV1b that encode 6306 amino acids and expresses in inner ear and retina. ADGR1a is least abundant isoform because it starts after 64^{th} exon of isoform a and contain only 26 exons (Hilgert, 2009).

Isoform ADGRV1b isoform contain large extracellular region with Calx-β domain.EAR (epilepsy associated domain), transmembrane domain and some exonic fragments that are present between Calx-β domain (Myers *et al*., 2018). Same start codon is shared by ADGRV1c but it contains only 31 exons. 35 CalX-β domains that are present in tandem arrangement mediate interactions between two domains (Yang *et al*., 2012). 29 amino acids of ADGRV1b that are present at N-terminal are hydrophobic in nature and provide signal for cleavage of mature proteins (Hilgert *et al*., 2009a). The seven-bladed propeller structure of the EAR domain is made up of a series of repeating domains (Weston, Luijendijk, Humphrey, Möller, & Kimberling, 2004).

7 to 19% of USH2 cases are due to mutation in ADGRV1.More than 100 variants have been found until now 65% of being missens and 25% with small deletion mutations (García-García *et al*., 2013).

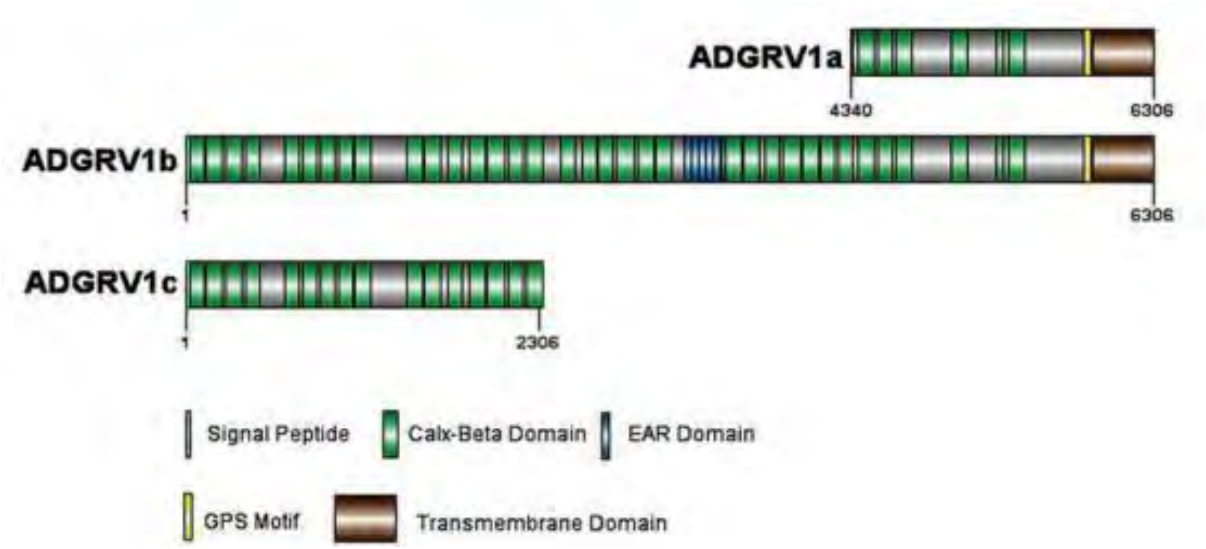

Fig.1.9: Isoforms of ADGRV1 protein (Whatley *et al*., 2020)

1.6.3 USH2D:

USH2D is caused by mutation in a gene called *DFNB31* present on chromosome 9q32-q34 with 12 exons. Protein encoded by this gene is a PDZ scaffold protein called Whirlin consist of 907 amino acids with five distinct domains (van Wijk *et al*., 2006). This gene is also responsible for non-syndromic hearing loss and USH2 type D (Aller *et al*., 2010). Whirlin is present in photoreceptor cells of retina and at the tips of mechanosensitive hair bundles called stereocilia (Ebermann *et al*., 2007). In the cochlea and retina, the protein isoforms of whirlin have distinct temporal and spatial functions.

It has been proven that only the C-terminal whirlin is present at the stereocilia tips of outer hair cells, however both full length and C-terminal whirlin are localized to the vestibular hair cells and inner hair cells and function in the elongation process of stereocilia in the inner ear. Only isoform that is present at the base pf al hair type of hair cells is full length whirlin that is crucial for interactions with USH2 protein network. In the cochlea and retina, the protein isoforms of whirlin have various spatial and temporal functions (Mathur and Yang, 2015).

1.6.3.1 Structure of Whirlin:

Gene responsible for usher has two splice sites that lead to the two isoforms that express in inner ear (Yang *et al*., 2010). All 12 exons are encoded by the full-length isoform, which also has two PDZ domains with N-termini, C-terminal PDZ domain and a proline-rich domain in its transcript (Yang *et al*., 2010). Other isoform do not have first five exons and encode 6-12 exons with no N-terminal PDZ domains but having C-terminal PDZ domain and proline rich domain (Yang *et al*., 2010). Long isoform only express in retina while both express in inner ear (Yang *et al*., 2010). Mutations that occur at different positions in whirlin cause non-syndromic deafness or USH2D (Mathur & Yang, 2015).

Mutations in DFNB3 gene is very rare. Most of the problems occur due to mutation in long isoform that lead to the non-syndromic deafness associated with vision problem because long isoform express in photoreceptor cells (Audo *et al*., 2011).

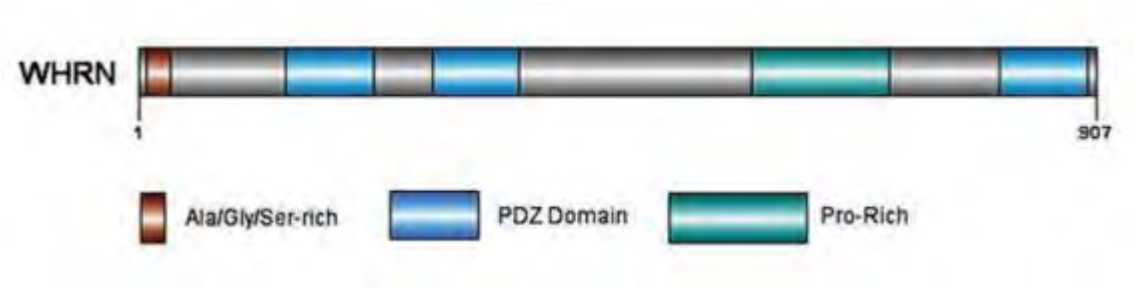

Fig.1.10: Whrilin protein motifs

(Whatley *et al*., 2020)

1.7 USHER type 3:

Symptoms in USH3 patients appear in mid-life that include progressive hearing loss RP that lead to the loss of night vision (nyctalopia), loss of peripheral vision and focus and progressive vestibular function (Fields *et al*., 2002). USH2 symptoms vary from patients to patients but vestibular dis function occur in 50% of the cases but loss of hearing and vision vary in patients. Due to the late onset and relatively slow course of the disease, USH3 has treatment possibilities (Geller *et al*., 2009).

1.7.1 USH3A:

USH3A occur due to mutation in a gene called *CLRN1* that is located on chromosomes 3 at position 25.1 (3q25.1) (Joensuu *et al*., 2001). This gene expresses in all tissues throughout the body. Its expression is identified in hair cells and ganglionic cells that penetrate in sensory epithelium of cochlea by mouse model. *CLRN1* specifically plays role in synaptic transmission of photoreceptor cells and HC (Adato *et al*., 2002). Its role is also similar to USH2 proteins that function in development and maintenance of cochlear hair cell bundles but have different role in vestibular organization (Mathur & Yang, 2015).

1.7.1.1 Structure of Clarin-1:

This gene is made up of 1619 bp that code for a protein called clarin-1 with 232 amino acids forming four transmembrane domains (Adato *et al*., 2002). Alternative splicing lead to the 11 variants with main variant consist of 3 exons (Västinsalo *et al*., 2011).

Mutation in *CLRN1* gene cause USH2D. Mutant protein have unstable structure and localization. This further disturb cellular trafficking.300T>C (p.Y176X) mutation is common in Finland people and c.143T>C (p.N48K) is common in Ashkenazi jews .In other population, this USH2D is very rare (Millán *et al*., 2011).

1.7.3 USH3B:

This type of Usher syndrome is caused by a gene called *HARS* (Histidyl-tRNA synthetase). *HARS* encodes a protein called *HARS*1 that is member of family aminoacyl-tRNA synthetases (Abbott *et al*., 2017). Histidyl-transfer RNA (tRNA) is produced by *HARS* that is necessary for conversion of histidine into protein. As the mutations are found in *HARS* gene of USH3B patients so this gene is responsible for USH3B (Puffenberger *et al*., 2012). Although presence of *HARS* in inner ear and its interaction with USH interactome is still unclear that need further research plans (Nolen *et al*., 2020).

1.8 Inner ear and expression of Usher proteins:

Human inner ear perform two main jobs, the first one is to transduce sound waves into neurochemical signal that is accomplished by cochlea and other one is to

maintain body posture and position that is done by vestibular system (Motallebzadeh, Soons, & Puria, 2018). The main functional unit in cochlea that make it enable to identify as separate variety of sound frequencies is erectile part of sensory epithelium that is also called as organ of Corti. It contain membranous labyrinth, supporting and hair cells and basil membranes with nerve endings (Appler & Goodrich, 2011). The ear contains two kinds of hair cells (HC), inner hair cells that are true sensory hair cells responsible for transmission of impulses through auditory nerves. These cells have sensory elements that translate sound induced motion of basilar membrane into electrochemical signals. These mechanosensory elements are stereocilia that are made up of actin filaments having ability to detect sub-nanometer deflection.

Additionally, the sensory and motor components found in outer hair cells contribute to hearing sensitivity, selectivity, and frequency by intensifying sound waves. So outer hair cells increase working capacity i.e. quality and quantity of sound (Simoni *et al*., 2017). These cells are called hair cells because tuft of stereocilia are present on each cell that protrude from apical surface of each cell (Simoni *et al*., 2017). These hair bundles form V-shape array. Every stereocilium is attached to the shaft of follow by tip links. Just like muscles, these cells have actin filaments that contract upon the vibration of basilar membrane and mechanical massage is proceeded through special signal transduction channels (Ashmore, 2008).

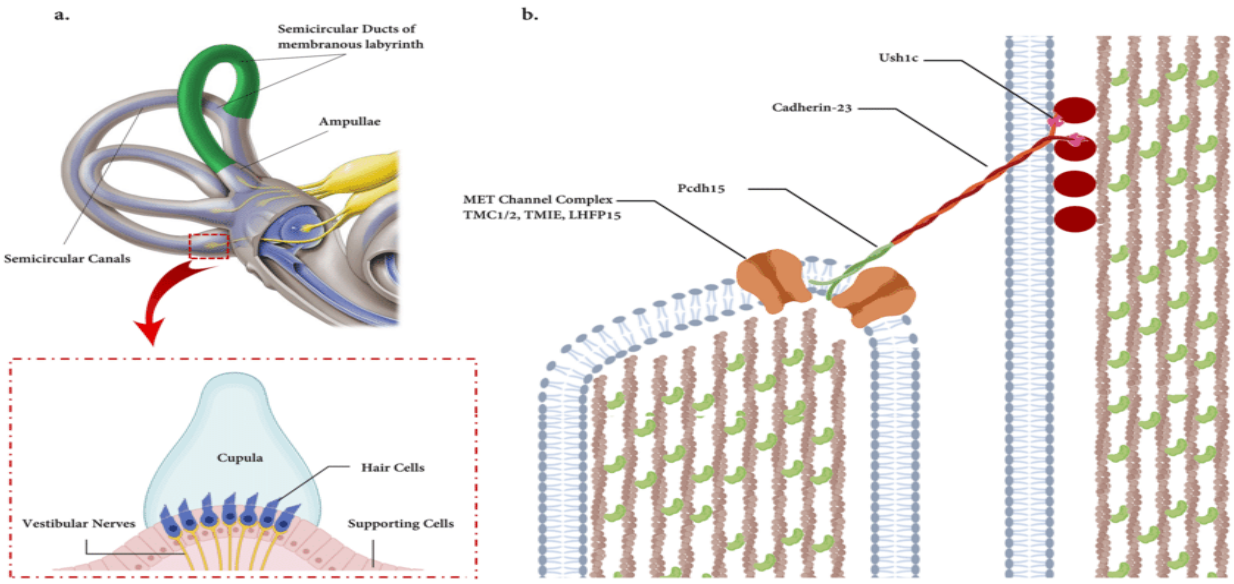

Fig.1.11: Stereocilia tip links with transduction channels and proteins

(Zardadi *et al*., 2020)

1.9 Protein interaction:

Molecules that have crucial role in development and maintenance of stereocilia are exclusively identified and studied. Mutation in genes encoding many of these molecules cause syndromic and non-syndromic deafness (Richardson, de Monvel, & Petit, 2011).These molecules are proteins that only express and play their roles in inner. These proteins are important part of mechanotransduction (MET) complexes and tip links. These MET channels have different sub-units whose proteins are transmembrane inner ear protein (TMIE), transmembrane proteins of hair cells stereocilia (TMHS), transmembrane channel like protein (TMC1). For machanotransduction process, these MET channel sub-units interact with the protocadherin 15 C-terminal domain that is present at tip links. (Kurima *et al*., 2015).

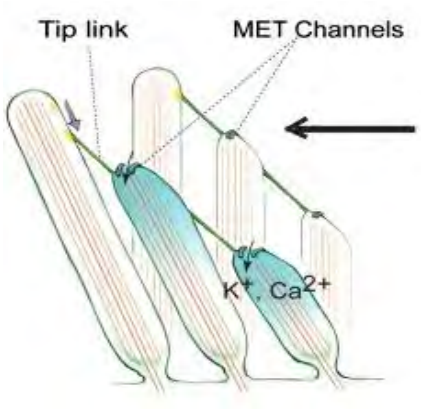

Fig.1.12: Machanotransduction in tip links

1.9.1 Cadherin and protocadherin:

Domains of the USH proteins interacts with several other USH partners, most important pair that maintain the integrity of stereocilia formation is cadherin 23 and protocadherin 15. These two proteins are localized in upper and lower parts of stereocilia respectively and form connection between stereocilia that is crucial for stability of stereocilia structure. Any mutation that affects the structure of any of these protein affect the ability of these protein to interact that lead to the defects in

stereocilia structure (Kazmierczak *et al*., 2007). These tip links interact with MET complex proteins and transmit force to these sub units (Maeda, Pacentine, Erickson, & Nicolson, 2017).

Components that form MET complex are Myosin VIIA, SANS and harmonin which are localized to taller stereocilium region where tip links anchor and interact with cadherin 23 for regulation and MET and tip links tension (Cunningham & Müller, 2019). Most of the time, Cadherin 23 form complexes with harmonin myosin-1c that is necessary for stability and shape of stereocilia hair bundles (S. Liu, Li, Zhu, Cheng, & Zheng, 2012). Through their binding to the PDZ domains, these proteins serve as a link between cadherin 23 and the stereocilium cytoskeletal actin core, linking cadherin 23 intracellularly to the actin filaments. (Adato *et al.,* 2005a).

1.9.2 Harmonin:

Harmonin is regarded as fundamental unit of USH protein interactome because of its functioning as a scaffold protein (Reiners, Marker, Jurgens, Reidel, & Wolfrum, 2005). In particular, the high affinity interaction between the SAM domain on SANS and the module formed by the PDZ1, PDZ3 domains and harmonin N-terminal domain led to the creation of a stable complex (Yan *et al.,* 2010). This whole complex binds with myosin VIIA tail to form upper tip link motor complex that further bind with CD of cadherin to anchor tip links with taller stereocilia (Yan *et al*., 2010).

Mutations in genes that code these proteins disrupt the structure and hence interaction of these proteins that lead to the impairment of normal hearing mechanism (Yan *et al.,* 2010). In vitro study shows the binding of cytoplasmic domain of cadherin protein to PDZ2 domain of harmonin (Senften *et al*., 2006). USG2 proteins and harmonin are co-localized in HC and retina that indicate that *USH2A* and ADGRV1 are involved in supramolecular USH-proteins network (Reiners, Van Wijk, *et al*., 2005). PDZ1 domain of harmonin binds with PDZ binding motifs present in C-terminal of Usherin and ADGRVI (Reiners *et al.,* 2005b). According to an in vitro study, all other harmonin isoforms' PDZ1 and PDZ2 domains can bind with the SCC domain of harmonin isoform b (Adato *et al.,* 2005b).

1.9.3 Myosin VIIA:

Myosin VIIA has central role because of ATP binding and hence controlling USH proteins trafficking and stereocilia connection (Woolner & Bement, 2009).Specifically, the USH modifier, PDZD7, and the harmonin and whirlin PDZ domains bind to MyTH4-FERM, C-terminal domain of myosin VIIA (Li *et al*., 2017). Myosin VIIA also interact with CD domain of cadherin suggesting their role in maintaining development and integrity of stereocilia (Senften *et al*., 2006). *CIB2* interacts with Myosin VIIA and harmonin among all USH proteins. Interaction of SANS is found to occur with both tip links protein, Cadherin and Protocadherin that demonstrate its function in formation of multiprotein complex (Caberlotto *et al*., 2011).

1.9.4 Usherin and whirlin:

A multiprotein complex is formed by interaction of USH2 proteins; Usherin, whirlin and ADGRV1 called ankle link complex (Mathur & Yang, 2015). Myosin VIIA is supposed to carry each of these proteins to the stereocilia base where they will temporarily connect to form an ankle link (Michalski *et al*., 2007). During development, thin fibers appear that join stereocilia are called ankle links (Michalski *et al*., 2007). So, any defect in this ankle link complex formation lead to the disruption of stereocilia bundles. So mutation in genes that code for USH2 proteins cause hearing loss due to disorganization of ankle link structures during developmental process (Michalski *et al*., 2007).

1.9.5 Clarin-1:

This protein is less studied than other proteins although it is thought to regulate actin cytoskeleton of stereocilia by interacting with myosin VIIA (Tian *et al*., 2009). Evidences are there for synergistic effect of both Clarin-1 and *MYO7A* products. So symptoms will be more severe by mutation in *MYO7A* with Clarin-1 (Adato *et al*., 1999). Additionally Protocadherin 15 interact with c-terminal domain of Clarin-1 and known to make efficient the assembly of Usher proteins by modulating vesicle recycling (Ogun & Zallocchi, 2014).

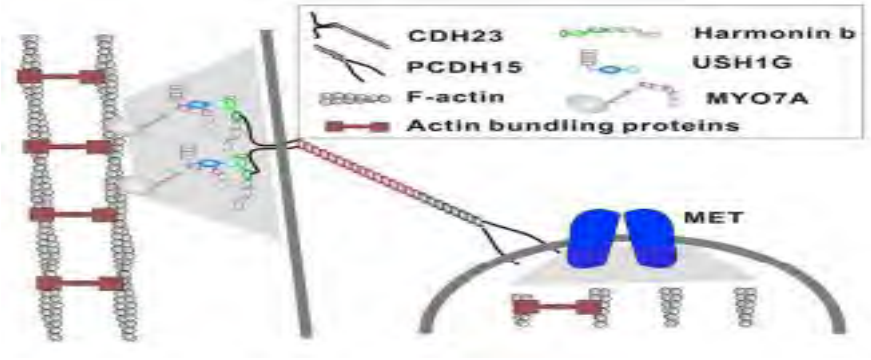

Fig. 1.13: Protein interaction in stereocilia (Li, He, Lu, & Zhang, 2016)

1.10 Usher proteins expression in Retina:

It has been demonstrated by evidence that by connecting cilia of photoreceptors, Myosin VIIA transports opsin from inner to outer segment through connecting cilium (Xinran Liu, Udovichenko, Brown, Steel, & Williams, 1999). Further studies shows that in ciliary and periciliary regions of rods and cons, domains of USH1 and USH2 proteins interact to each other. In the periciliary ridge complex, which serves as the docking location for post-golgi vesicles with cargo loaded, the proteins SANS, Usherin, and ADGRV1b are observed to be associates. Usherin and VLGR1b can form linkages through homomeric or heteromeric interactions in the extracellular space between the inner membrane segment and the connecting cilium. Usherin and VLRV1 intracellular domains bind to whirlin in the cytoplasm. Now whirlin interact with myosin VIIA and SANS which will interact with microtubules and actin filaments (Maerker *et al*., 2008). At periciliary membrane, this molecular motor is anchored by a multi protein complexed formed by cadherin-23, vezatin and some other partners (Roepman & Wolfrum, 2007).

So in periciliary ridge, usher proteins function in regulation of vesicle docking and cargo transfer, by connecting cilium with inner segment mechanically. Like hair cells in organ of Corti, Usher proteins are localized in photoreceptor where they function in trafficking of synaptic transmission (Kremer, van Wijk, Märker, Wolfrum, & Roepman, 2006).

The fact that the capacity of pigment epithelial cells to phagocytose in outer segment discs is significantly diminished when myosin VIIA is lacking suggests that myosin VIIA may play a function in the exocytosis and phagocytosis of the distal outer segment discs by the retinal pigment epithelium (RPE). Myosin VIIA has also been

linked to a function in the RPE cells that involves the transport of melanosomes intracellularly (Kremer *et al*., 2006).

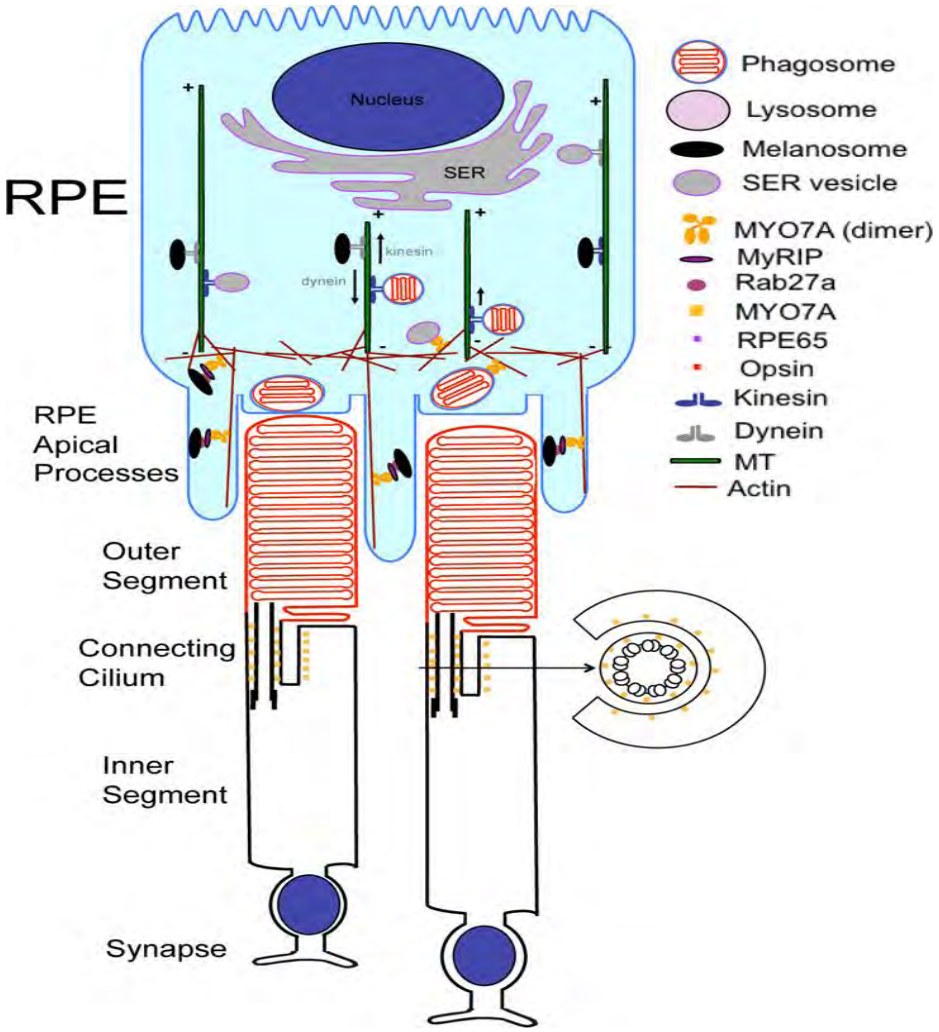

Fig.1.14: Usher proteins expression in retina (Sorusch *et al.*, 2019)

1.11 Therapeutic approaches:

Unfortunately, regardless of mutations in causative gene and subtype, there is still no cure for USH despite tremendous advancements in the field. Patients can choose from a variety of treatments to help them control USH symptoms, particularly HL symptoms. Treatment options for vision and balance problems are relatively restricted, with the majority of methods merely offering assistance to patients in carrying out activities of daily living. All forms of HL, including USH, are currently being treated with cochlear implants or hearing aids for auditory rehabilitation. These innovations can greatly improve communication, but they cannot yet match the clarity

of natural hearing, and more crucially, they do not address the HL's fundamental causes. A number of novel treatment approaches are now being explored, making use of cutting-edge technology like gene editing and cell-based therapeutics. Some of these approaches are even currently undergoing clinical trials.

1.11.1 Viral base gene therapy:

In order to create functional protein, the mutant gene can be replaced with a normal copy of the gene as a treatment strategy for hereditary illnesses like USH. Using viral vectors like adeno-associated virus (AAV) and lentivirus is a popular method for delivering normal genes. In SANS knockout mice, local delivery of rAAV2/8 harboring a normal Sans gene into the inner ear totally recovered hearing and vestibular function (Emptoz *et al*., 2017). Due to limited genetic capacity of AVV, it's not possible to fit many DNA sequences in vector (Akil, 2020). Scientists are trying to use multi or dual system AVV but protein expression is very small (Maddalena *et al*., 2018).

1.11.2 Genome editing based therapy:

This therapy is done by using ZFN (Zinc-Finger Nucleases) and CRISPR/Cas9.It is a promising technique to treat USH in vivo by site specific manners while preserving normal functioning of gene. ZFN is created by joining ZF domain (zinc-finger DNA-binding domain) to a DNA cleaving domain forming a chimeric protein. ZEN was applied to treat nonsense mutation (p.R31X) in cell line of *USH1C* that was lead to normal harmonin expression with no side effect (Overlack, Goldmann, Wolfrum, & Nagel-Wolfrum, 2012).

Another site-specific editing methodology is CRISPR. Because of its ease of use, dependability, and great efficiency, the Cas9 system is currently the most sophisticated and commonly used approach. *USH2A* (c.2299delG) mutation was successfully treated in patient derived fibroblast by using this technique (Fuster-García *et al*., 2017). This technique was also applied on patient-derived iPSC with muted *MYO7A* gene. These iPSC were treated with efficiently and specificity with no mutagenesis and retaining their pluripotency and genetic stability (Sanjurjo-Soriano *et al*., 2020).

1.11.3 Drug therapy:

This therapy is done by ASO (antisense oligonucleotides). These are single stranded synthetic oligodeoxynucleotides with a short length that is complement to the targeted mRNA (Rinaldi & Wood, 2018). Cryptic splicing mechanism was done by using ASO in *USH1C*.216A knock-in mice. In *USH1C* mice, correction lead to the elevated level of harmonin, improve structure of steroecilia and rescued vestibular and auditory response (Donaldson *et al*., 2018). A new drug QR-421a (ProQR Therapeutics) action in *USH2A* mutant Zebra fish model showed a successful exon skip that lead to restored electroretinogram and Usherin expression (van Diepen *et al*., 2019). Further clinical trials of this drug are in process.

1.11.4 Cell based therapy:

Pluripotent The ability of stem cells to develop into several cell types,so applied to regenerative medicine (van Diepen *et al*., 2019). The stem cells are mostly embryonic stem cells (ESC),adult stem cells (ASC) and induced pluripotent stem cells (iPSC). PSC can differentiate, assemble into three-dimensional aggregates, and change into various lineages, such as inner ear and retinal organoids. This differentiation of PSCs into organoids technology is more efficient for patient specific medications and disease models for USH so that genomic profile of these in vitro disease model must be similar with patients for therapeutic purposes.

Materials and Methods

2.1 Ethical Approval

Ethical approval was obtained to conduct the current research project from Institutional Review Board (IRB), Faculty of Biological Sciences, department of Zoology, Quaid-i-Azam University Islamabad, Pakistan and AL-Shifa Trust Eye-Hospital Rawalpindi, Pakistan.

2.2 Identifications of Families

Based on clinical diagnosis, several families possessing the patients of Retinitis Pigmentosa (RP) were selected. The demographical details of study subjects (RP patients and their first-degree relatives) were different.

2.3 Family Pedigree

A detailed interview was conducted from the normal elder individual of family to determine the genetic relationship among family members. Pedigree analysis was used to identify the mode of inheritance. Pedigree of each family was drawn using HaploPainter1.043 where male represented as square while female as circles. Furthermore, the normal individuals represented as hollow symbols while RP patients as filled symbols. The deceased individuals were represented through diagonal line on the symbols and the cousin marriage were represented through double lines between symbols. Roman numerals in pedigree represents the different generation while Arabic numerals were used to represents the individuals in each generation.

2.4 Blood collection

Sampling was done from different regions of Pakistan including Khalifa Gul Nawaz Hospital, Bannu, KPK and District Headquarter Hospital Bannu, KPK and Al-Shifa Trust Eye Hospital Rawalpindi, Punjab. Study subjects were confirmed as RP patients through clinical diagnosis as per criteria of WHO and voluntarily participated in the research study.

2.5 Exclusion Criteria

RP patients without any family history of RP were excluded.

Patients with other eye related co-morbidities were excluded.

Patients those were not willing to participate in the study were excluded.

2.6 Inclusion criteria

RP confirmed individuals those were willing to participate and possessing the family history of RP were selected to carry out the research project.

2.7 Sample Collection

After getting the written consent from RP patients, we collected 5 ml blood from study subjects in labelled EDTA vacutainer tubes. To ensure the sample differentiation, individual's and their parents name and a Unique Anonymous Identification (UAI) number were mentioned on the tubes. Tubes were gently shaken to mix the blood with EDTA to prevent blood clotting and later stored at -4ºC in the refrigerator.

2.8 Genomic DNA Extraction

An organic extraction method i.e., Phenol-Chloroform method was to extract genomic DNA, with the following protocol:

A. Day 1

i. Blood samples stored at -4 ºC were placed for 10-15 minutes at room temperature to allows the lysis of red blood cells.
ii. Afterword the blood was transferred to UAI labelled, 50 ml falcon tubes.
iii. About 1000 ml of washing buffer was prepared using 10 ml of 1M Tris-HCL, 4ml of 2mM EDTA and raising the volume with autoclaved water, and mixed.
iv. 40 ml washing buffer was added to 5 ml of blood in falcon tube.
v. Tubes were vortexed to mix it thoroughly and later kept at room temperature for about 10/15 minutes.
vi. Centrifugation was proceeded for 20-25 minutes at 3000 rpm and 20 ºC.
vii. We got the supernatant, that was discarded using glass pipettes and kept the pellet containing WBCs at the bottom of tubes.
viii. Vertexing or tapping was done to break the pellet.
ix. About 40 ml of washing buffer was added to blood and thoroughly mixed through tapping or vortexing.
x. Centrifugation was proceeded for about 20 minutes at 3000 rpm and 20 ºC.
xi. We got the supernatant, that was discarded using glass pipettes and kept the pellet containing WBCs at the bottom of tubes.
xii. The last four steps were repeated multiple times till we got the clear pallet of WBCs.

xiii. 5 µl of 20% SDS, 3 ml of TNE buffer and 25µl of Protein Kinase to dissolve the broken clear pellet. Afterword the tubes were incubated overnight at 37 ºC.

B. Day 2

i. Next day, the pellet digestion was confirmed at room temperature, and if undigested add some more protein kinase and vortex for complete digestion. Tubes were further incubated for 2-3 hours at 37 ºC.

ii. 500 µl of 6M NaCl was added and placed on ice for about 10/15 minutes after vigorous shaking.

iii. PCI mixture (1 ml) was added to each tube and mixed through gently inverting the tubes.

iv. Centrifugation was proceeded for about 20 minutes at 3000 rpm and 20 ºC.

v. We got the three layers, where the top layer contains the DNA, proteins in the middle one and PCI solution in the lower layer.

vi. The upper layer was carefully transferred to another falcon tubes and mixed with 5 ml of isopropanol till the DNA threads became apparent and left at room temperature for 5-10 minutes.

vii. Centrifugation was proceeded for about 20 minutes at 3000 rpm and 20 ºC. Supernatant was discarded and DNA pellet remained stick to wall of tube.

viii. 70% ethanol (5 ml) was added to pellet, followed by centrifugation for 20 minutes at 3000 rpm at 20 ºC to ensure maximum removal of all contaminations. Again, supernatant was discarded, and pellet was air dried.

C. Day 3

i. Parafilm strips were used to properly seal the caps of falcon tubes.

ii. Heat shock was provided by placing the tubes at 70 ºC in water bath for about 1 hour. It was done to inactivate the nucleases and prevent denaturation from DNA.

iii. Afterword, for about 5 minutes tubes were kept at room temperature.

iv. Tubes were provided with short spin to mix the DNA with buffer, then transferred to another pre-labelled tubes and stored at -20 ºC.

Table 2.1: Concentration and composition of solutions of DNA extraction

Sr.NO	Solutions	Chemical Composition
1.	**Tris EDTA buffer (pH 8.0)**	2mM EDTA (0.029g) 10mM Tri's hydroxyl (methylamino) methane
2.	**TNE buffer**	2mM EDTA 10mM Tris HCl (pH 8.0) 400mM NaCl
3.	**SDS**	20%
4.	**Ethanol**	**70%**
5.	**Proteinase K**	100 mg/ml
6.	**NaCl**	6M
7.	**Isopropanol (Chilled)**	100%
8.	**Chloroform: Isoamyl alcohol**	24:1
9.	**PCI (Phenol :Chloroform : Isoamyl alcohol)**	50:48:2

2.9 Agarose Gel Electrophoresis (1%)

Gel electrophoresis was proceeded to confirm the extracted DNA sample with the following protocol:

i. 0.5 gm of agarose powder was dissolved in 50 ml of 1X TBE buffer to prepare 1% agarose gel.

ii. 1000 ml of 1X TBE buffer was prepared by adding 100 ml of 10X TBE buffer in 900 ml of distilled water. Solution was heated in oven for a minute to get complete dissolution and clarity.

iii. Ethidium Bromide (5 µl) was added in solution of gel and mixed through gentle shaking. It is an intercalating agent and required for identification of DNA under UV light.

iv. Gel tray was set, and combs were placed followed by careful pouring of clear solution to avoid bubble formation.

v. It was left for about 30-40 minutes at room temperature. Once gel got solidified, we carefully removed the combs and caster was transferred to gel tank already filled with 1X TAE buffer.

vi. Loading sample including 3µl of extracted DNA with 3µl of 6X of loading dye (Bromophenol blue).

vii. The gel was run under at 120 volts for 25 minutes.

viii. UV based gel documentation system (Cleaver Scientific Limited) was used for gel visualization.

Table 2.2: List of Reagents for Gel Electrophoresis and their Chemical Composition

Sr.NO	Chemical	Composition
1.	**1%Agarose gel (**50 ml)	• 0.5g agarose gel • 1X TBE buffer (50ml) • Ethidium bromide (2µl)
	2%Agarose gel (50 ml)	• 1.0g agarose gel • 10X TBE buffer (5ml) • Ethidium bromide (5µl) • Distilled water (45ml)
2.	**10X TBE**	• Tris Base (0.89 M, 54g)

		• Boric Acid (0.025M, 27g) • EDTA (3.65g, pH 8.3) • Deionized water (500ml)
3.	**1X TBE**	• 10X TBE (10ml) • Distilled water (90ml)
4.	**Ethidium Bromide (50 ml)**	• EtBr (0.4g) • Distilled water (50ml)
5.	**Bromophenol blue (25 ml)**	• Bromophenol blue (0.0875%) • Sucrose (10g) • Distilled water (25 ml)

2.10 Nanodrop

Nanodrop (Thermo-Scientific 2000) was used for quantification (concentration) and to check quality (purity) of extracted DNA. T.E buffer was used as blank.

2.11 FASTA Sequence of Selected Exon and Mutation

2.11 Primer designing

Primers were designed using Primer-3 software https://primer3.ut.ee/ to amplify the *MYO7A* gene to analyze mutations and polymorphisms. The general conditions including annealing temperature, size of amplicon, concentration of salt and length of primer were selected of optimum level. Reference sequence critical for primer designing was obtained from Ensemble website http://asia.ensembl.org/Homo_sapiens/Info/Index. To confirm the specificity of selected primers, BLAT (Blast Like Alignment Tool) on UCSC genome browser was used and In-Silico PCR tool for verification of amplicon size. Further details of mutation locus, primer sequence, melting temperature (Tm) and size of product for primers are mentioned in table 2.3.

Table 2.3: Primers for Selected Mutation

Gene	Exon	Locus	Primer	Sequence (5'….>3')	Primer length (bp)	Product size (bp)
MYO7A	6/7	11q13.5	Forword primer	GAGGGTCCGTATTGTCAGCT	20	556
MYO7A	6/7	11q13.5	Reverse primer	AGCAATACGGGCAGCAATAC	20	556
MYO7A	8	11q13.5	Forword primer	CACCATGAAACCCACCGATG	20	395
MYO7A	8	11q13.5	Reverse primer	CCACTTCTCCTTGGTGTCCA	20	395

2.12 Primer Dilution

The commercial concentration of ordered primer was 50 picomole/µl that was diluted up to 10 picomole/µl (final concentration).

2.13 Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was done for amplification of selected exon in all study subjects. PCR tubes of 200 µl (Axygen USA) and the details of chemicals with their used concentration and volumes are given in the table 2.4.

Table 2.4: Chemicals used in PCR Mixture

Sr.No	**Chemical**	**Concentration**	**Volume (µl) for single PCR reaction**
1	Taq. Buffer	10X	2.5 µl
2	dNTPs	2.5mM	2.5 µl
3	MgCl2	25mM	2 µl
4	Forward Primer	10 pmol/ µl	0.5 µl
5	Reverse Primer	10 pmol/ µl	0.5 µl

6	DNA	>100 ng/ µl	2 µl
7	Taq Polymerase	5U/ µl	0.5 µl
8	PCR water		14.5 µl
	Total Volume		25 µl

Before placing into thermo cycle (Bio-Rad T100), PCR tubes were provided a brief spin to mix the content well at 3000 rpm for 1 minutes. The thermal details are mentioned in PCR reaction in table 2.5.

Table 2.5: Thermo Cycler Profile for Touch Down PCR at 65 to 55°C

Step	**Temperature**	**Time**	**Cycle**
Initial denaturation	96°C	5 min	1X
Denaturation	95°C	45 sec	40X
Annealing	65°C	45 sec	
Extension	72°C	60 sec	
Final extension	72°C	10 min	1X
Hold	25°C	∞	

2.14 PCR Product Confirmation:

A solution of 2% agarose gel was prepared by dissolving 1.6g of agarose in 80 ml of 1X TAE buffer and 2µl of EtBr for confirmation of PCR product. Later, the product (3-4µl) was mixed with loading dye (2 µl). Gel was run at 120V for 40 min along with 1kb ladder. UV based gel documentation system (Cleaver Scientific Limited) was used to confirm the amplified targeted sequences.

2.15 Purification of PCR Product:

The Purification kit (Thermo-scientific) was used to purify the PCR product with the following protocol:

i. An equal volume of binding buffer was mixed with PCR product and left for 1 minute at room temperature after gentle tapping.
ii. Centrifugation was proceeded for 2 minutes at 13000 rpm and then transferred to pre-labeled spin column tubes.
iii. Again, centrifugation was performed for 1 minute at 13000 rpm.
iv. Washing buffer (350 µl) was added to sample and again centrifuged at 13000 rpm for 1 minute.
v. Samples were left for 1 minute at room temperature.
vi. Elusion buffer (25 µl) stored at 70°C, was added to each sample and spin column tubes were placed in pre-labeled Eppendorf tube and left for 2 minutes at room temperature.
vii. Centrifugation was proceeded for 1 minute at 13000 rpm.
viii. 2% agarose gel was run to verify the purity of PCR product.

2.15 Sequencing

Sanger's sequencing was performed for identification of nucleotide sequence of each sample. Sequencing of samples was done from Hussein Ebrahim Jamal (HEJ) Research Institute, University of Karachi, under the grant of PCSIR to project. For sequencing purpose, purified PCR product (8µl) was mixed with 4 µl of forward primer and sent for sequencing after proper sealing. Sequencing reaction was performed through Big Dye Terminator (Genetic analyzer). Capillary electrophoresis method was used to separate labelled fragments of DNA and later detection was performed through spectrum analyzer. For documentation, specific dyes were used for labelling of each nucleotide including Adenine, Cytosine, Thymine, and Guanine.

2.16 Mutation analysis

Reference genome taken from Ensemble genome browser(https://www.ensembl.org/index.html) was used for alignment of sequenced

data using Bio-edit (v.7.2.0). For conflict determination, mutation tester was used. Further computational tools including PROVEAN (https://bio.tools/provean), I-Mutant (http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi) and gnomAD (https://gnomad.broadinstitute.org/) were used to determine reported and novel variants. The effect of variant at protein level was determined using Uniport (https://www.uniprot.org/). Analysis of chromatogram was performed through Chromas 2.66 and other tools was also used including SIFT (https://sift.bii.a-star.edu.sg/) and ITASER (https://zhanggroup.org/I-TASSER/) for confirmation. The effect of amino acid substitution on structure of protein and chemical nature was identified through using HOPE (Have Your Protein Explained) tool (https://www3.cmbi.umcn.nl/hope/).

Results

3.1 Clinical characteristics:

Blood samples of nineteen families were collected from Al-Shifa Trust Eye Hospital Rawalpindi and Al-Hanef Clinic Jhang Sadar. These all families were affected with Retinitis pigmentosa with positive family history. From these nineteen families, 6 families were affected with Usher syndrome. Affected members of these families were diagnosed with vestibular disfunctioning, hearing loss along with RP. From each family, proband was selected to analyze genetic change in exon 6,7 and 8 of *MYO7A* gene. These all probands were affected with vestibular disfunctioning, hearing loss and visual impairment. Proband of each family have typical fundus observation with bony spicules, attenuated arteries, pigmentation and waxy polar disc.

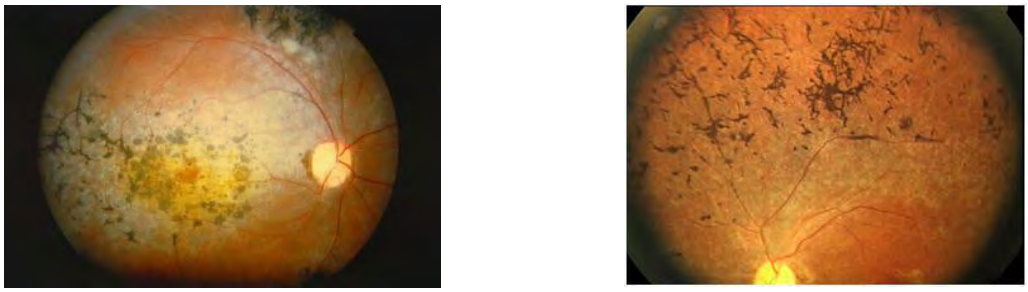

Fig.3.1: Representative fundus photographs of Usher patients.

3.2 DNA isolation:

DNA was extracted from white blood cells of each blood sample and mean concentration of extracted DNA was up to 50 ng/µl with purity value 1.8 of each DNA sample. The DNA content of each sample was recorded by spectrophotometry as summarized in table 3.1. However, to check integrity of DNA, 2 µl samples was loaded in agarose gel and selected results are shown in figure 3.2.

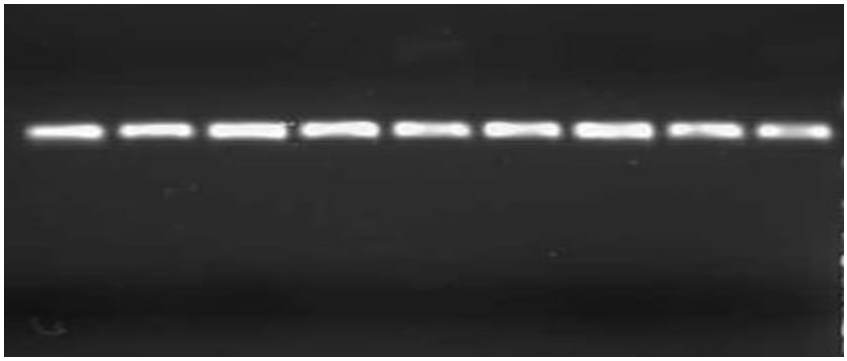

Fig.3.2: Agarose gel electrophoresis photograph

Table 3.1: Nano drop results of USH families

Family ID	Conc. (ng/µl)	A 260/280
RP 3	243.61	1.74
RP 13	196.93	1.79
RP 37	199.65	1.83
RP 47	185.54	1.76
RP 42	119.46	1.76
RP 30	213.31	1.81

3.3 Primer optimization:

For optimization of primer gradient PCR was used. Annealing temperature was 65°C at optimization. Primer pairs were design to analyze Usher mutations in three exons 6,7 and 8 of Usher gene *MYO7A.*

3.3.1 Polymerase chain reactions:

PCR was performed to amplify the DNA samples of all probands of collected families. Two primer pairs were optimized at 65 to 55 °C touch down PCR profile.

3.3.2 PCR product purification:

For purification of PCR product purification kit (thermo scientific) was used. All the PCR products of probands were purified and eluted in elusion buffer. The confirmation of amplicon was obtained by loading 2% agarose gel electrophoresis with 2 µl purified PCR product.

3.4 Sanger sequencing:

All the purified samples were sent to HEJ (Hussain Ebrahim Jamal) international center for chemical and biological sciences, University of Karachi for Sanger's sequencing under the grant of PCSIR (Pakistan council of scientific and industrial research, Islamabad, Pakistan. Big dye terminator chemistry (an automated ABI PRISM 3730 genetic analyzer) was applied to accomplish sequence reactions. The labelled fragments of DNA were separated by capillary electrophoresis and were detected by spectrum analyzer. Specific dye was used for labelling of each nucleotide (A,T,G,C) for documentation. Chromas 2.6.6 was used to visualize Sanger's sequencing results.

3.5 Genetic analysis:

Mutation in *MYO7A* gene is known to cause most of the Usher cases in many populations globally. Exon 6,7 and 8 of *MYO7A* gene (Transcript: ENST00000409709.3) was selected to screen polymorphisms and mutations in 6 USH families as shown in table 3.2. Sequence alignment was done by using Cluster W. All analyzed sequences are given below.

Family RP 3

Pedigree analysis

RP3 family belongs to Jhang Sadar, Punjab, Pakistan. Most members oof this family were affected with RP and hearing loss specially proband that is mention in figure. The descriptive data was taken from members of same family. Affected members of this family have RP by birth with progressive hearing loss. Affected members have syndromic RP with hearing loss due to continuous consanguineous marriages. Pedigree in Figure 3.3 shows the relationship of proband with other members.

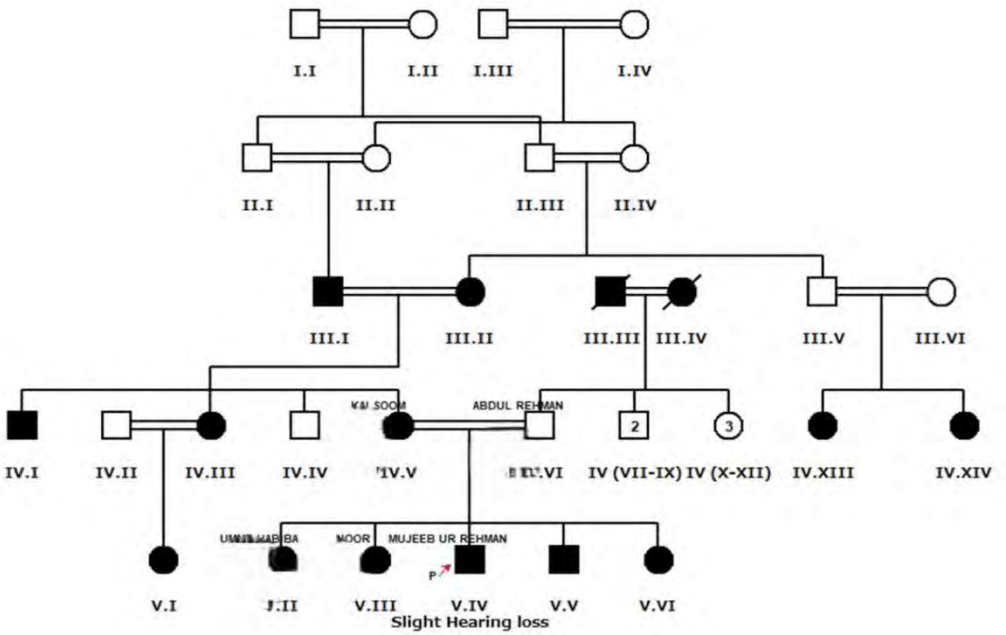

Fig.3.3: Pedigree of RP 3 family

Genetic analysis: Sanger's sequencing of proband shown in upper pedigree was done in which exon 6,7 and 8 were targeted to check mutation. However no sequence variant was detected in any of these three analyzed exons.

Family RP 13:

Pedigree analysis:

This family belongs to Jhang, Punjab, Pakistan. Affected members of this family have bilateral RP with hearing loss. Descriptive data was taken from other family members. Pedigree in figure 3.4 shows the consanguineous marriages and relation of proband with other members.

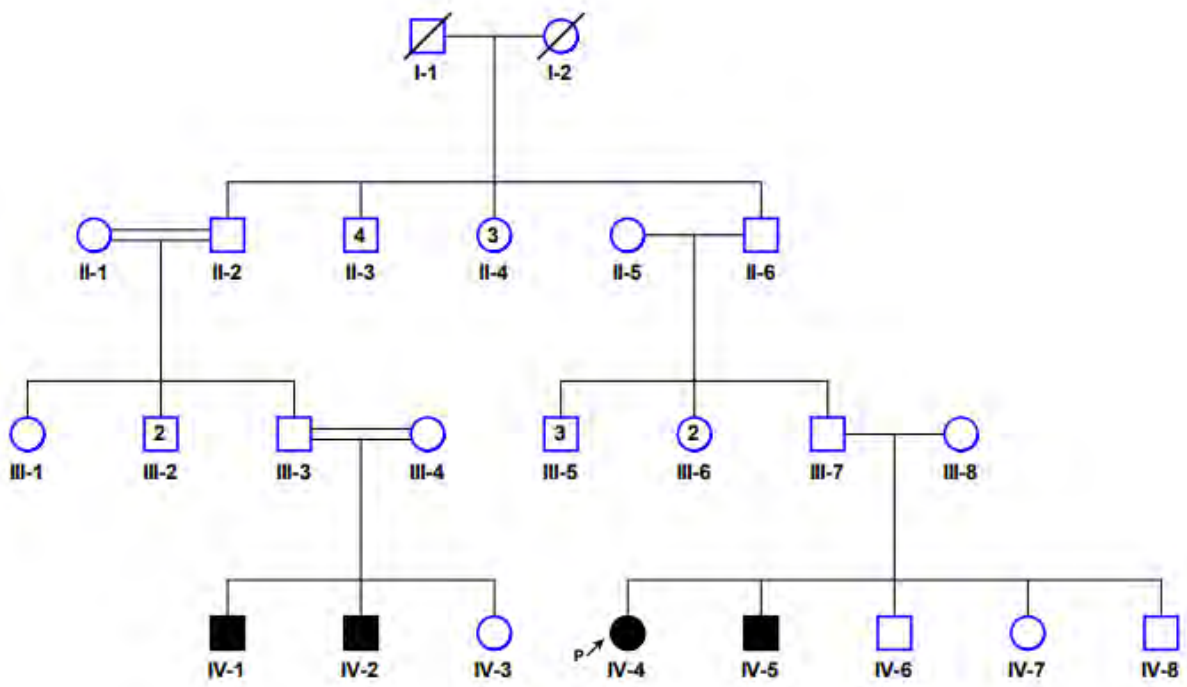

Fig.3.4: Pedigree of RP 13 family

Genetic analysis:

Sanger's sequencing of proband shown in pedigree was done in which exon 6,7 and 8 were targeted to check mutation. Two polymorphisms were found in interonic region of *MYO7A* exon 8 at position g.29179T>G and g.29186C>G.

In silico analysis shows that this polymorphism can change splice site that can further affect protein feature.

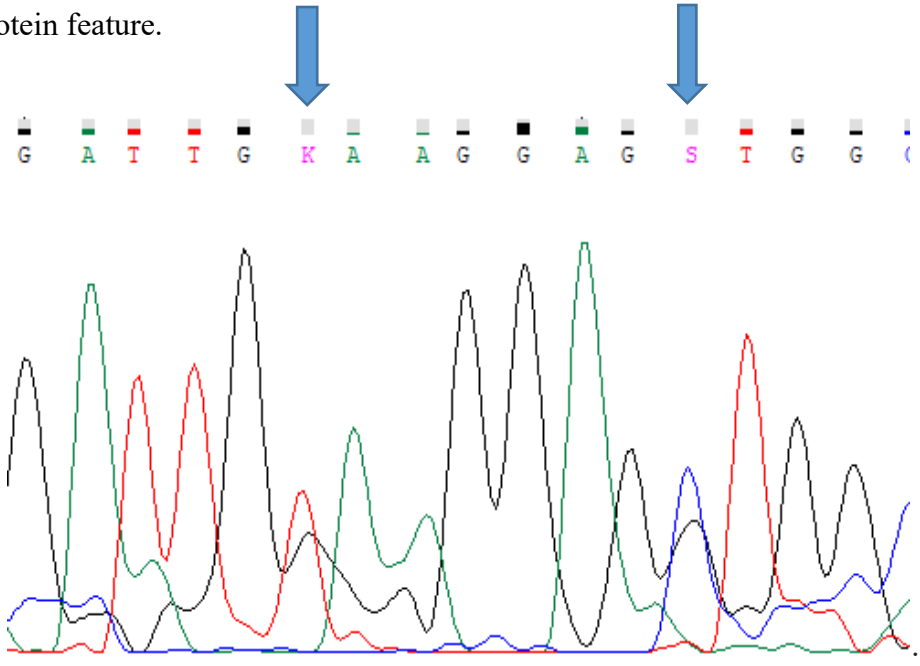

Fig.3.5: Sequence chromatogram showing polymorphism at position g.29179T>G and g.29186C>G

RP family 30:

Pedigree analysis: Blood sample and detail of this family was collected form Al Shifa Trust Eye hospital Rawalpindi. Symptoms in proband include RP and hearing loss with vestibular disfunctioning. Following pedigree shows the consanguineous marriages and relation of proband with other family members.

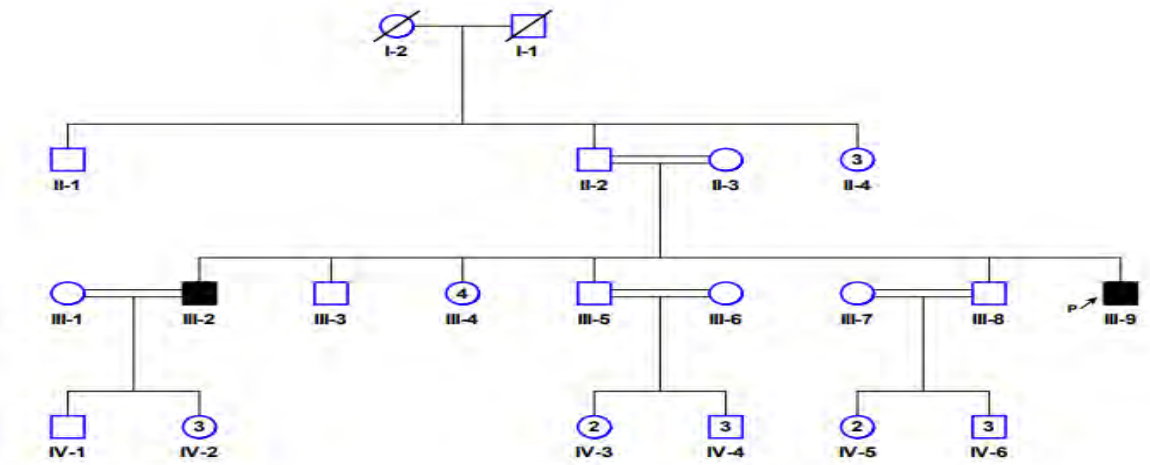

Fig.3.6: Pedigree of family RP 30

Genetic analysis:

Genetic analysis revealed a polymorphism in coding region at position c.783T>C in exon 8 of *MYO7A.*

No amino acid change was detected through in silico analysis by this polymorphism. Chromatogram is shown in Figure 3.7.

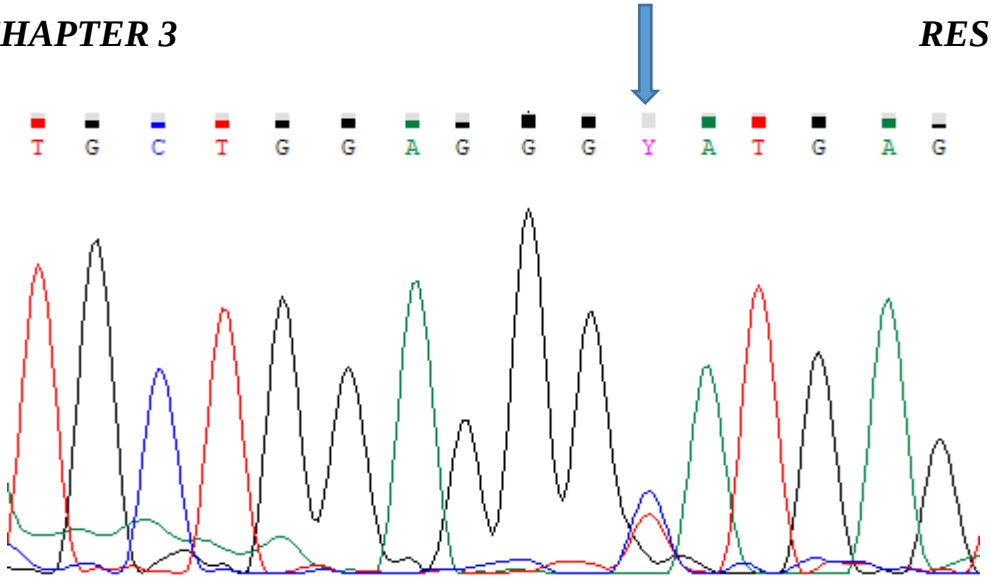

Fig.3.7: Chromatogram showing polymorphism at position c.783T>C

No sequence variant were detected in exon 6 and 7.

Family RP37:

Pedigree analysis:

Blood samples and descriptive data was obtained from Al Shifa Trust Eye hospital Rawalpindi. Following pedigree shows the details of proband and affected members.

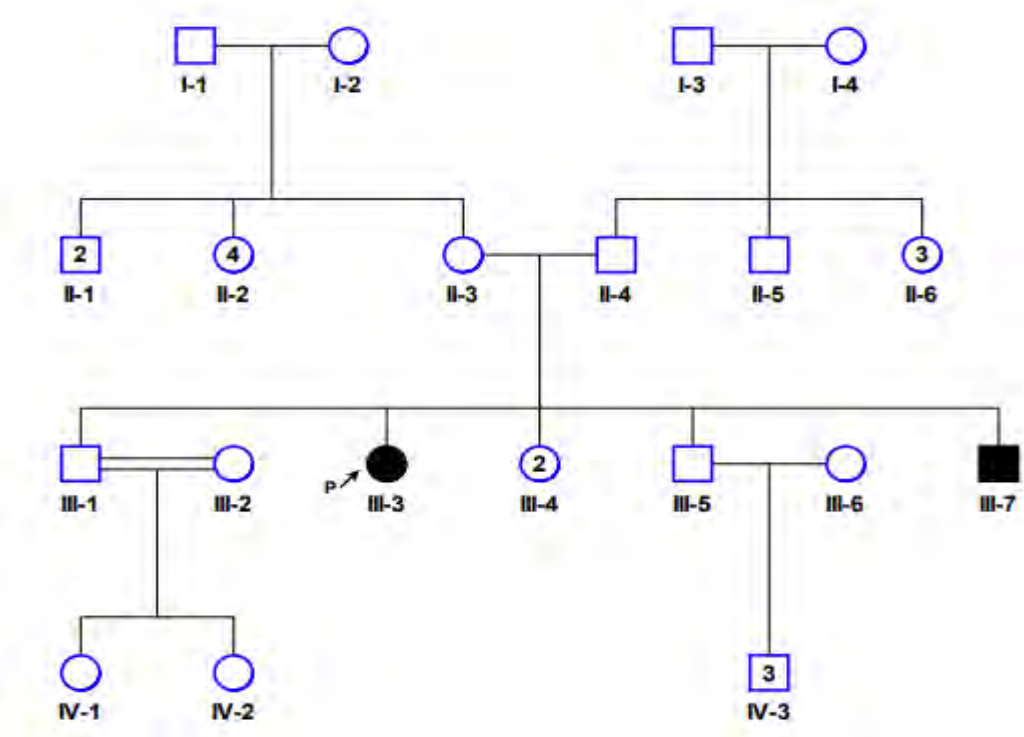

Fig.3.8: Pedigree of family RP 37

Genetic analysis:

A polymorphism was detected in interonic region of exon 8 at position g.29181G>A in which G was replaced by A as shown in figure 3.9.

In silico analysis shows that his polymorphism can change protein feature and splice site can also be affected.

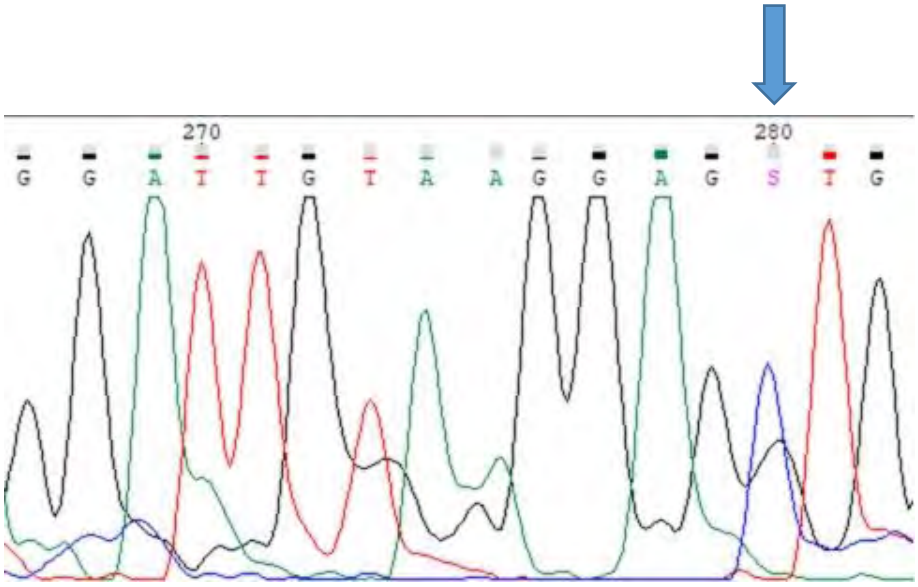

Fig.3.9: Chromatogram showing polymorphism at position g.29181G>A

No any variant was detected in exon 7 and 8.

Family RP 47:

Pedigree analysis:

Descriptive data and blood samples of this was collected form AL Shifa Trust-Eye Hospital. Following pedigree shows the positive family history of patients with RP and vestibular disfunctioning.

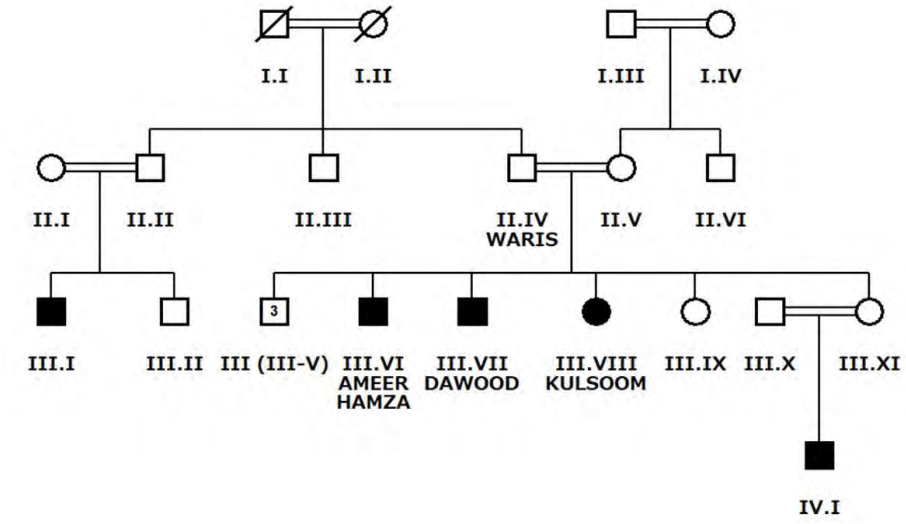

Fig.3.10 : Pedigree of family RP 47

Genetic analysis: A polymorphism was found in coding sequence of exon 8 at position c.783T>C in which T was replaced by C as shown in fig.

Result: No amino acid change was detected by this polymorphism.

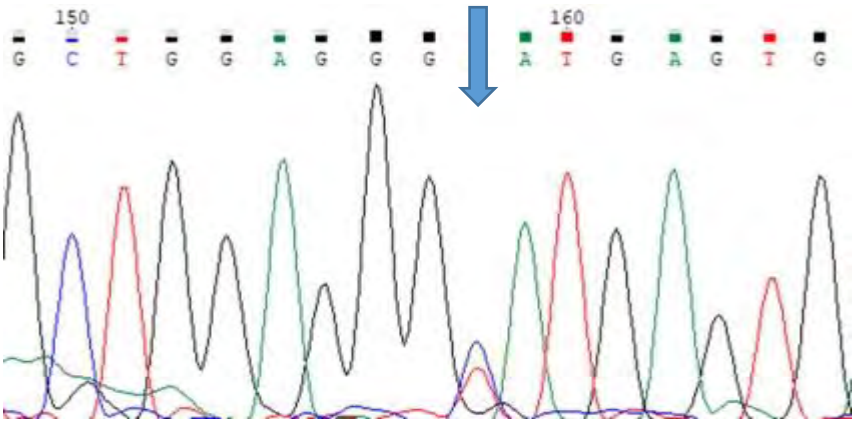

Fig.3.11: Chromatogram showing Polymorphism at position c.783T>C

Family RP 42:

Pedigree analysis:

Family history and blood samples of this family was collected from AL-Shifa Trust Eye hospital Rawalpindi. Family pedigree in figure 3.12 shows positive family history of patient and relationships of family members.

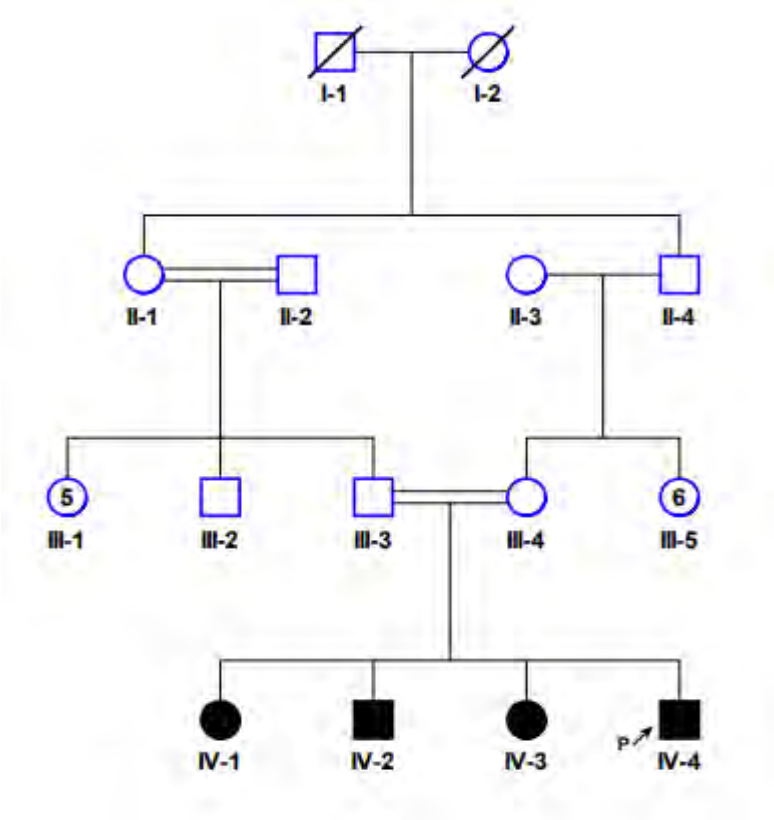

Fig. 3.12: Pedigree analysis of RP 42

Genetic analysis:

A homozygous polymorphism was detected in exon 8 as shown in figure 3.13 at Position: c.783T>C. However, this polymorphism does not affect protein features.

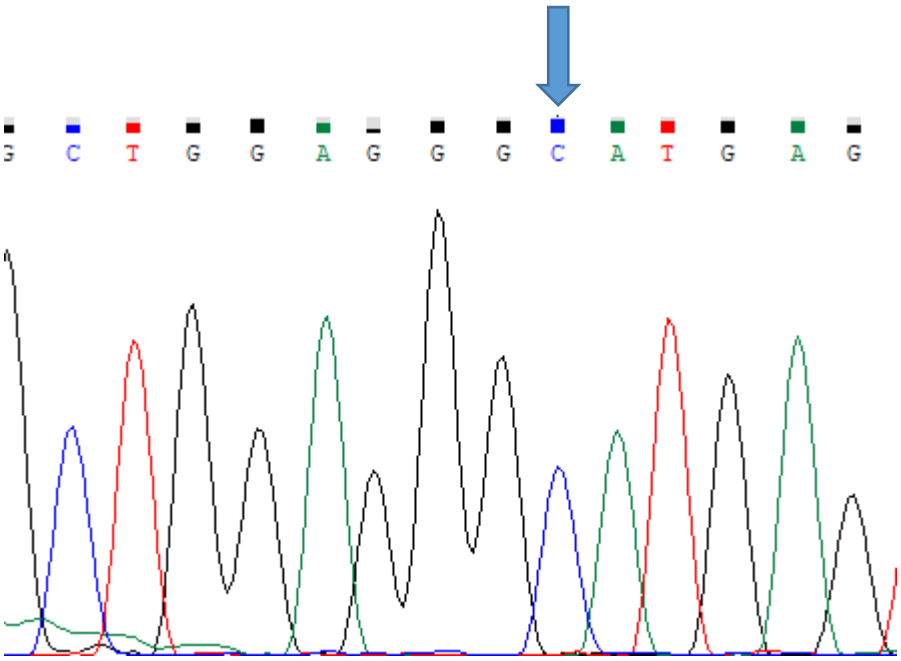

Fig.3.13: Chromatogram showing polymorphism at position c.783T>C

Table 3.2: Summary of sequence variations identified in exon 6,7 and 8 of *MYO7A* gene in this study

Family ID	**Genomic location**	**Chromosomal Location**	**Effect**	**rs.ID**	**Mutation taster**	**Status**
RP13	g.29179T>G g.29186C>G	chr11:76868488T	Protein features affected	n/a	Polymorphism	Novel
RP30	c.783T>C	chr11:76868372T>C	Splice site change	rs762667	Polymorphism	Reported
RP37	g.29181G>A	chr11:76868490G>A	Splice site affected	n/a	Polymorphism	Novel
RP47	c.783T>C	chr11:76868372T>C	Splice site affected	rs762667	Polymorphism	Reported
RP42	c.783T>C	chr11:76868372T>C	No	rs762667	Polymorphism	Reported

Discussion

The most prevalent genetic form of deaf-blindness is known as Usher syndrome (USH). It is defined by the triad of sensorineural hearing loss (SNHL), rod-cone dystrophy, and varied vestibular dysfunction and has an autosomal recessive inheritance pattern (Friedman, Schultz, Ahmed, Tsilou, & Brewer, 2011). An early prevalence estimate ranged from 3.6 to 6.2 per 100,000 people (2–6). Although the prevalence of USH may reach 16.7 per 100,000 people (Kauffman, Moron, Consalvo, Bello, & Kochen, 2008). There are currently three recognized clinical subtypes of USH, which can be characterized by the magnitude and chronology of hearing loss as well as the presence of vestibular dysfunction. All three kinds have a retinal degeneration known as rod-cone dystrophy or retinitis pigmentosa as their common ophthalmic manifestation (RP). Type 1 (USH1) manifests as juvenile onset of retinitis pigmentosa, significant congenital SNHL, and peripheral vestibular areflexia. Type 2 (USH2) causes retinitis pigmentosa and mild to severe congenital SNHL, but not vestibular dysfunction. Retinitis pigmentosa, increasing SNHL, and varied vestibular impairment are the effects of type 3 (USH3) (Smith *et al*., 1992).

Genetic heterogeneity is character of Usher syndrome. Usher syndrome 1 can be caused by any pathogenic variant in five of the following genes: *MYO7A* that code for myosin vii a, *USH1C* that code for harmonin,*CDH23* that code for cadherin 23, *PCDH15* that code for protocadherin and USH1G that code for SANS protein (Bolz *et al*., 2001). Usher syndrome type can be caused by pathogenic variant in any of the following genes: *USH2A* that codes for Usherin, ADGRV1 that code for G protein coupled receptor or Whirlin and mutation in gene *CLRN1* that code for Clarin protein cause Usher syndrome type 3 (Joensuu *et al*., 2001).

USH genes code variety of proteins that have different functions like myosin VIIA is a motor protein, harmonin, Whirlin and SANS are scaffold proteins. Usherin and clarin 1 are transmembrane proteins, cadherin 23 and protocadherin 15 are adhesion proteins. These all SH proteins interact to form Usher interactome (Cosgrove & Zallocchi, 2014). This interactome formation is important for visual and hearing because of expression of this interactome in inner ear and retina.

Some of the genes are ultra-rare genes. One of these genes is PDZ7 gene that contain a PDZ domain with 23.3 kb size, 16 exon and locus is present on chromosome 10

encodes a scaffold protein. This gene is highly similar with *USH1C* and WRLN, so it was declared as USH gene but now it has been proved by experiments that PDZ7 interact with *USH2A*, ADGRV1 and WHRN to form USH2 complex in vivo and invitro (Morgan *et al*., 2016). A pathogenic variant of PDZ7 gene, c.166dupC was found in USH patient. A novel deletion of PDZ7 c.2194_2203delTGCACACCCC was identified by Ebemann *et al*. (Ebermann *et al*., 2010).

Other ultra-rare gene is *HARS* that encodes a product that helps in charging tRNA molecules with histidine amino acid for translational process. A homozygous pathogenic variant c.[1361A>C; 1361A>C] in *HARS* was found by Puffenberger *et al*. A patient with an unidentified kind of Usher syndrome had compound heterozygous pathogenic mutations in *HARS* was identified by Tiwari *et al*.

USH2 is responsible for 70% USH cases in which *MYO7A* mutation is most prevalent that cause USH2.Many of the mutations have been reported in *MYO7A* gene that cause USH2. Cremers *et al*. reported two mutations c.[5227 C > T; c.5227 C > T] and c.[5581 C > T; 5581 C > T] in *MYO7A* gene. Neuhaus *et al*. reported many mutations in *MYO7A* gene, some of which are deletions, c.[3503 G > A; 6025delG], some are substitution, c.[3503 G > A; 5573 T > C] , c.[3718 C > T; 4814 C > A] .

Because mutation rate in *MYO7A* is most prevalent, so we targeted *MYO7A* gene to analyze mutation and polymorphism. DNA polymorphism refers to variations in the DNA sequence among individuals in a population. These variations can lead to changes in the structure and function of the resulting protein. For example, single nucleotide polymorphisms (SNPs) can alter a single amino acid in a protein and significantly change its activity or stability.

There are different mechanisms by which DNA polymorphisms can impact protein structure, including changing the codon usage, altering splicing patterns, disrupting the formation of specific protein domains or interactions, and altering the stability of the protein.

.In the given results, we find some novel polymorphisms. Polymorphisms usually do not change any amino acid or affect protein structure but if polymorphism occur in regulatory region, it can affect protein structure. Polymorphism g.29179T>G in exon 8 of RP13 family can change splice site and hence protein length that can further interrupt normal functioning of protein. Splice site change can lead to the stop codon

in transcript that translate into protein. These changes can decrease protein length that led to the improper structuring and folding. Polymorphism at position c.783T>C in exon 8 of RP30 family show no amino acid change.

Polymorphism in RP37 family at position g.29181G>A is homozygous that affect protein motifs. In silico analysis shows that this polymorphism can affect protein features by changing amino acid or splice site.

The impact of a splice site change on protein structure and function can be significant. If the change affects critical residues or domains in the protein, it can alter its three-dimensional structure and disrupt its function. For example, a change in the splice site can cause a misfolding of the protein, leading to its aggregation and degradation, or a loss of its ability to bind to other molecules. In some cases, a change in the splice site can lead to the production of a non-functional protein, or a protein with a different function to the normal protein.

In conclusion, all six families used in this study have usher symptoms with RP, hearing loss and vestibular disfunctioning. Due to lack of budget and government interest, Sanger's sequencing of only three selected exon 6,7 and 8 was done to analyze mutation and polymorphism but no any disease-causing variant was identified in this study. Sanger's sequencing is now considered as traditional method of genetic sequencing now as compared to high-Throughput Sequencing (HTS) techniques or Next-Generation Sequencing (NGS) methods. Genetic counselling is given to affected Pakistani families but study need new research innovations and negotiation.

References

Abbott, J. A., Guth, E., Kim, C., Regan, C., Siu, V. M., Rupar, C. A., . . . Robey-Bond, S. M. (2017). The usher syndrome type IIIB histidyl-tRNA synthetase mutation confers temperature sensitivity. *Biochemistry, 56*(28), 3619-3631.

Adato, A., Michel, V., Kikkawa, Y., Reiners, J., Alagramam, K. N., Weil, D., . . . Petit, C. (2005). Interactions in the network of Usher syndrome type 1 proteins. *Human molecular genetics, 14*(3), 347-356.

Ahmed, Z., Riazuddin, S., Riazuddin, S., & Wilcox, E. (2003). The molecular genetics of Usher syndrome. *Clinical genetics, 63*(6), 431-444.

Ahmed, Z. M., Riazuddin, S., Aye, S., Ali, R. A., Venselaar, H., Anwar, S., . . . Friedman, T. B. (2008). Gene structure and mutant alleles of PCDH15: nonsyndromic deafness DFNB23 and type 1 Usher syndrome. *Human genetics, 124*(3), 215-223.

Ahmed, Z. M., Riazuddin, S., Bernstein, S. L., Ahmed, Z., Khan, S., Griffith, A. J., . . . Wilcox, E. R. (2001). Mutations of the protocadherin gene PCDH15 cause Usher syndrome type 1F. *The American Journal of Human Genetics, 69*(1), 25-34.

Ahmed, Z. M., Riazuddin, S., Khan, S. N., Friedman, P. L., Riazuddin, S., & Friedman, T. B. (2009). USH1H, a novel locus for type I Usher syndrome, maps to chromosome 15q22-23. *Clinical genetics, 75*(1), 86-91.

Astuto, L., Bork, J., Weston, M., Askew, J., Fields, R., Orten, D., . . . Khan, S. (2002). CDH23 mutation and phenotype heterogeneity: a profile of 107 diverse families with Usher syndrome and nonsyndromic deafness. *The American Journal of Human Genetics, 71*(2), 262-275.

Astuto, L. M., Weston, M. D., Carney, C. A., Hoover, D. M., Cremers, C. W., Wagenaar, M., . . . Greenberg, J. (2000). Genetic heterogeneity of Usher syndrome: analysis of 151 families with Usher type I. *The American Journal of Human Genetics, 67*(6), 1569-1574.

Bahloul, A., Michel, V., Hardelin, J.-P., Nouaille, S., Hoos, S., Houdusse, A., . . . Petit, C. (2010). Cadherin-23, myosin VIIa and harmonin, encoded by Usher syndrome type I genes, form a ternary complex and interact with membrane phospholipids. *Human molecular genetics, 19*(18), 3557-3565.

Bitner-Glindzicz, M., Lindley, K. J., Rutland, P., Blaydon, D., Smith, V. V., Milla, P. J., . . . Shepherd, R. M. (2000). A recessive contiguous gene deletion causing infantile hyperinsulinism, enteropathy and deafness identifies the Usher type 1C gene. *Nature genetics, 26*(1), 56-60.

Blazejczyk, M., Sobczak, A., Debowska, K., Wisniewska, M. B., Kirilenko, A., Pikula, S., . . . Wojda, U. (2009). Biochemical characterization and expression analysis of a novel EF-hand Ca2+ binding protein calmyrin2 (Cib2) in brain indicates its function in NMDA receptor mediated Ca2+ signaling. *Archives of biochemistry and biophysics, 487*(1), 66-78.

Bolz, H., von Brederlow, B., Ramírez, A., Bryda, E. C., Kutsche, K., Nothwang, H. G., . . . Molina, O. P. (2001). Mutation of CDH23, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D. *Nature genetics, 27*(1), 108-112.

Bork, J. M., Peters, L. M., Riazuddin, S., Bernstein, S. L., Ahmed, Z. M., Ness, S. L., . . . Srisailpathy, C. S. (2001). Usher syndrome 1D and nonsyndromic

autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel cadherin-like gene CDH23. *The American Journal of Human Genetics, 68*(1), 26-37.

Chen, Z.-Y., Hasson, T., Kelley, P. M., Schwender, B. J., Schwartz, M. F., Ramakrishnan, M., . . . Corey, D. P. (1996). Molecular cloning and domain structure of human myosin-VIIa, the gene product defective in Usher syndrome 1B. *Genomics, 36*(3), 440-448.

Chishti, A. H., Kim, A. C., Marfatia, S. M., Lutchman, M., Hanspal, M., Jindal, H., . . . Mohandas, N. (1998). The FERM domain: a unique module involved in the linkage of cytoplasmic proteins to the membrane. *Trends in biochemical sciences, 23*(8), 281-282.

Cosgrove, D., & Zallocchi, M. (2014). Usher protein functions in hair cells and photoreceptors. *The international journal of biochemistry & cell biology, 46*, 80-89.

Ebermann, I., Phillips, J. B., Liebau, M. C., Koenekoop, R. K., Schermer, B., Lopez, I., . . . Bernd, A. (2010). PDZD7 is a modifier of retinal disease and a contributor to digenic Usher syndrome. *The Journal of clinical investigation, 120*(6), 1812-1823.

Elledge, H. M., Kazmierczak, P., Clark, P., Joseph, J. S., Kolatkar, A., Kuhn, P., & Müller, U. (2010). Structure of the N terminus of cadherin 23 reveals a new adhesion mechanism for a subset of cadherin superfamily members. *Proceedings of the National Academy of Sciences, 107*(23), 10708-10712.

Ernest, S., Rauch, G.-J., Haffter, P., Geisler, R., Petit, C., & Nicolson, T. (2000). Mariner is defective in myosin VIIA: a zebrafish model for human hereditary deafness. *Human molecular genetics, 9*(14), 2189-2196.

Eudy, J. D., Ma-Edmonds, M., Yao, S., Talmadge, C. B., Kelley, P. M., Weston, M. D., . . . Sumegi, J. (1997). Isolation of a novel human homologue of the gene coding for echinoderm microtubule-associated protein (EMAP) from the Usher syndrome type 1a locus at 14q32. *Genomics, 43*(1), 104-106.

Eudy, J. D., Weston, M. D., Yao, S., Hoover, D. M., Rehm, H. L., Ma-Edmonds, M., . . . Ayuso, C. (1998). Mutation of a gene encoding a protein with extracellular matrix motifs in Usher syndrome type IIa. *Science, 280*(5370), 1753-1757.

Friedman, T. B., Schultz, J. M., Ahmed, Z. M., Tsilou, E. T., & Brewer, C. C. (2011). Usher syndrome: hearing loss with vision loss. *Medical genetics in the clinical practice of ORL, 70*, 56-65.

Gumbiner, B. M. (2005). Regulation of cadherin-mediated adhesion in morphogenesis. *Nature reviews Molecular cell biology, 6*(8), 622-634.

Joensuu, T., Hämäläinen, R., Yuan, B., Johnson, C., Tegelberg, S., Gasparini, P., . . . Lehesjoki, A.-E. (2001). Mutations in a novel gene with transmembrane domains underlie Usher syndrome type 3. *The American Journal of Human Genetics, 69*(4), 673-684.

Kauffman, M. A., Moron, D. G., Consalvo, D., Bello, R., & Kochen, S. (2008). Association study between interleukin 1β gene and epileptic disorders: a HuGe review and meta-analysis. *Genetics In Medicine, 10*(2), 83-88.

Keats, B. J., & Savas, S. (2004). Genetic heterogeneity in Usher syndrome. *American journal of medical genetics Part A, 130*(1), 13-16.

Kim, E., Niethammer, M., Rothschild, A., Nung Jan, Y., & Sheng, M. (1995). Clustering of Shaker-type K+ channels by interaction with a family of membrane-associated guanylate kinases. *Nature, 378*(6552), 85-88.

Kimberling, W. J., & Möller, C. (1995). Clinical and molecular genetics of Usher syndrome. *Journal of the American Academy of Audiology, 6*(1), 63-72.

Kremer, H., van Wijk, E., Märker, T., Wolfrum, U., & Roepman, R. (2006). Usher syndrome: molecular links of pathogenesis, proteins and pathways. *Human molecular genetics, 15*(suppl_2), R262-R270.

Lagziel, A., Ahmed, Z. M., Schultz, J. M., Morell, R. J., Belyantseva, I. A., & Friedman, T. B. (2005). Spatiotemporal pattern and isoforms of cadherin 23 in wild type and waltzer mice during inner ear hair cell development. *Developmental biology, 280*(2), 295-306.

Maerker, T., van Wijk, E., Overlack, N., Kersten, F. F., McGee, J., Goldmann, T., . . . Kremer, H. (2008). A novel Usher protein network at the periciliary reloading point between molecular transport machineries in vertebrate photoreceptor cells. *Human molecular genetics, 17*(1), 71-86.

May-Simera, H., Nagel-Wolfrum, K., & Wolfrum, U. (2017). Cilia-The sensory antennae in the eye. *Progress in retinal and eye research, 60*, 144-180.

Mermall, V., Post, P. L., & Mooseker, M. S. (1998). Unconventional myosins in cell movement, membrane traffic, and signal transduction. *Science, 279*(5350), 527-533.

Michel, V., Booth, K. T., Patni, P., Cortese, M., Azaiez, H., Bahloul, A., . . . Lelli, A. (2017). CIB2, defective in isolated deafness, is key for auditory hair cell mechanotransduction and survival. *EMBO molecular medicine, 9*(12), 1711-1731.

Miller, K. A., Williams, L. H., Rose, E., Kuiper, M., Dahl, H.-H. M., & Manji, S. S. (2012). Inner ear morphology is perturbed in two novel mouse models of recessive deafness. *PLoS One, 7*(12), e51284.

Morgan, C. P., Krey, J. F., Grati, M. h., Zhao, B., Fallen, S., Kannan-Sundhari, A., . . . Barr-Gillespie, P. G. (2016). PDZD7-MYO7A complex identified in enriched stereocilia membranes. *Elife, 5*, e18312.

Nguyen, J. T., Porter, M., Amoui, M., Miller, W. T., Zuckermann, R. N., & Lim, W. A. (2000). Improving SH3 domain ligand selectivity using a non-natural scaffold. *Chemistry & biology, 7*(7), 463-473.

Nolen, R. M., Hufnagel, R. B., Friedman, T. B., Turriff, A. E., Brewer, C. C., Zalewski, C. K., . . . Brooks, B. P. (2020). Atypical and ultra-rare Usher syndrome: a review. *Ophthalmic genetics, 41*(5), 401-412.

Pakarinen, L., Karjalainen, S., Simola, K., Laippala, P., & Kaitalo, H. (1995). Usher's syndrome type 3 in Finland. *The Laryngoscope, 105*(6), 613-617.

Petit, C. (2001). Usher syndrome: from genetics to pathogenesis. *Annual review of genomics and human genetics, 2*(1), 271-297.

Puffenberger, E. G., Jinks, R. N., Sougnez, C., Cibulskis, K., Willert, R. A., Achilly, N. P., . . . Lawrence, J. J. (2012). Genetic mapping and exome sequencing identify variants associated with five novel diseases. *PloS one, 7*(1), e28936.

Reiners, J., Nagel-Wolfrum, K., Jürgens, K., Märker, T., & Wolfrum, U. (2006). Molecular basis of human Usher syndrome: deciphering the meshes of the Usher protein network provides insights into the pathomechanisms of the Usher disease. *Experimental eye research, 83*(1), 97-119.

Reisser, C. F., Kimberling, W. J., & Otterstedde, C. R. (2002). Hearing loss in Usher syndrome type II is nonprogressive. *Annals of Otology, Rhinology & Laryngology, 111*(12), 1108-1111.

Riazuddin, S., Belyantseva, I. A., Giese, A. P., Lee, K., Indzhykulian, A. A., Nandamuri, S. P., . . . Terrell, D. (2012). Alterations of the CIB2 calcium-and

integrin-binding protein cause Usher syndrome type 1J and nonsyndromic deafness DFNB48. *Nature genetics, 44*(11), 1265-1271.

Riazuddin, S., Nazli, S., Ahmed, Z. M., Yang, Y., Zulfiqar, F., Shaikh, R. S., . . . Javid, F. T. (2008). Mutation spectrum of MYO7A and evaluation of a novel nonsyndromic deafness DFNB2 allele with residual function. *Human mutation, 29*(4), 502-511.

Rosenberg, T., Haim, M., Hauch, A. M., & Parving, A. (1997). The prevalence of Usher syndrome and other retinal dystrophy-hearing impairment associations. *Clinical genetics, 51*(5), 314-321.

Schneider, M. R., & Kolligs, F. T. (2015). E-cadherin's role in development, tissue homeostasis and disease: Insights from mouse models: Tissue-specific inactivation of the adhesion protein E-cadherin in mice reveals its functions in health and disease. *Bioessays, 37*(3), 294-304.

Seki, N., Hattori, A., Hayashi, A., Kozuma, S., Ohira, M., Hori, T.-a., & Saito, T. (1999). Structure, expression profile and chromosomal location of an isolog of DNA-PKcs interacting protein (KIP) gene. *Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression, 1444*(1), 143-147.

Sheng, M., & Pak, D. T. (2000). Ligand-gated ion channel interactions with cytoskeletal and signaling proteins. *Annual review of physiology, 62*(1), 755-778.

Sheng, M., & Sala, C. (2001). PDZ domains and the organization of supramolecular complexes. *Annual review of neuroscience, 24*(1), 1-29.

Siemens, J., Kazmierczak, P., Reynolds, A., Sticker, M., Littlewood-Evans, A., & Müller, U. (2002). The Usher syndrome proteins cadherin 23 and harmonin form a complex by means of PDZ-domain interactions. *Proceedings of the National Academy of Sciences, 99*(23), 14946-14951.

Smith, R. J., Pelias, M. Z., Daiger, S. P., Keats, B., Kimberling, W., & Hejtmancik, J. F. (1992). Clinical variability and genetic heterogeneity within the Acadian Usher population. *American journal of medical genetics, 43*(6), 964-969.

Srisailapathy, C. S., & Mohanram, R. K. (2018). The tip link protein Cadherin-23: from hearing loss to cancer. *Pharmacological Research, 130*, 25-35.

Sudol, M. (1998). From Src Homology domains to other signaling modules: proposal of theprotein recognition code'. *Oncogene, 17*(11), 1469-1474.

Tsilou, E. T., Rubin, B. I., Caruso, R. C., Reed, G. F., Pikus, A., Hejtmancik, J. F., . . . Kaiser-Kupfer, M. I. (2002). Usher syndrome clinical types I and II: could ocular symptoms and signs differentiate between the two types? *Acta Ophthalmologica Scandinavica, 80*(2), 196-201.

Udovichenko, I., Vansant, G., & Williams, D. (2000). *Actin-based motility of baculovirus-expressed and native retinal myosin VIIa.* Paper presented at the Molecular Biology of the Cell.

van Wijk, E., van der Zwaag, B., Peters, T., Zimmermann, U., Te Brinke, H., Kersten, F. F., . . . Cremers, C. W. (2006). The DFNB31 gene product whirlin connects to the Usher protein network in the cochlea and retina by direct association with USH2A and VLGR1. *Human molecular genetics, 15*(5), 751-765.

Verpy, E., Leibovici, M., Zwaenepoel, I., Liu, X.-Z., Gal, A., Salem, N., . . . Keats, B. J. (2000). A defect in harmonin, a PDZ domain-containing protein expressed in the inner ear sensory hair cells, underlies Usher syndrome type 1C. *Nature genetics, 26*(1), 51-55.

von Brederlow, B., Bolz, H., Janecke, A., La O Cabrera, A., Rudolph, G., Lorenz, B., . . . Gal, A. (2002). Identification and in vitro expression of novel CDH23

mutations of patients with Usher syndrome type 1D. *Human mutation, 19*(3), 268-273.

Webb, S. W., Grillet, N., Andrade, L. R., Xiong, W., Swarthout, L., Della Santina, C. C., . . . Müller, U. (2011). Regulation of PCDH15 function in mechanosensory hair cells by alternative splicing of the cytoplasmic domain. *Development, 138*(8), 1607-1617.

Weil, D., El-Amraoui, A., Masmoudi, S., Mustapha, M., Kikkawa, Y., Lainé, S., . . . Zina, Z. B. (2003). Usher syndrome type IG (USH1G) is caused by mutations in the gene encoding SANS, a protein that associates with the USH1C protein, harmonin. *Human molecular genetics, 12*(5), 463-471.

Weil, D., Küssel, P., Blanchard, S., Lévy, G., Levi-Acobas, F., Drira, M., . . . Petit, C. (1997). The autosomal recessive isolated deafness, DFNB2, and the Usher 1B syndrome are allelic defects of the myosin-VIIA gene. *Nature genetics, 16*(2), 191-193.

Well, D., Blanchard, S., Kaplan, J., Guilford, P., Gibson, F., Walsh, J., . . . Weston, M. D. (1995). Defective myosin VIIA gene responsible for Usher syndrome type IB. *Nature, 374*(6517), 60-61.

Weston, M. D., Luijendijk, M. W., Humphrey, K. D., Möller, C., & Kimberling, W. J. (2004). Mutations in the VLGR1 gene implicate G-protein signaling in the pathogenesis of Usher syndrome type II. *The American Journal of Human Genetics, 74*(2), 357-366.

Wheway, G., Parry, D. A., & Johnson, C. A. (2014). The role of primary cilia in the development and disease of the retina. *Organogenesis, 10*(1), 69-85.

Xu, Z., Oshima, K., & Heller, S. (2010). PIST regulates the intracellular trafficking and plasma membrane expression of Cadherin 23. *BMC cell biology, 11*(1), 1-11.

Yan, J., Pan, L., Chen, X., Wu, L., & Zhang, M. (2010). The structure of the harmonin/sans complex reveals an unexpected interaction mode of the two Usher syndrome proteins. *Proceedings of the National Academy of Sciences, 107*(9), 4040-4045.

Yan, D., & Liu, X. Z. (2010). Genetics and pathological mechanisms of Usher syndrome. *Journal of human genetics, 55*(6), 327-335.

Yang, J., Liu, X., Zhao, Y., Adamian, M., Pawlyk, B., Sun, X., . . . Li, T. (2010). Ablation of whirlin long isoform disrupts the USH2 protein complex and causes vision and hearing loss. *PLoS genetics, 6*(5), e1000955.

Yang, J., Wang, L., Song, H., & Sokolov, M. (2012). Current understanding of usher syndrome type II. *Frontiers in bioscience: a journal and virtual library, 17*, 1165.

Zardadi, S., Razmara, E., Asgaritarghi, G., Jafarinia, E., Bitarafan, F., Rayat, S., . . . Garshasbi, M. (2020). Novel homozygous variants in the TMC1 and CDH23 genes cause autosomal recessive nonsyndromic hearing loss. *Molecular genetics & genomic medicine, 8*(12), e1550.

sZou, J., Luo, L., Shen, Z., Chiodo, V. A., Ambati, B. K., Hauswirth, W. W., & Yang, J. (2011). Whirlin replacement restores the formation of the USH2 protein complex in whirlin knockout photoreceptors. *Investigative ophthalmology & visual science, 52*(5), 2343-2351.

Turnitin Originality Report

Molecular genetic study of Usher Syndrome in a cohort of consanguineous families from the local population by Hussan Bilal .

From CL DRSML (CL DRSML)

- Processed on 13-Feb-2023 09:11 PKT
- ID: 2012710985
- Word Count: 12956

Similarity Index
15%
Similarity by Source

Internet Sources:
11%
Publications:
10%
Student Papers:
5%

sources:

1 3% match ()
Meg Whatley, Abbie Francis, Zi Ying Ng, Xin Ee Khoh, Marcus D. Atlas, Rodney J. Dilley, Elaine Y. M. Wong, "Usher Syndrome: Genetics and Molecular Links of Hearing Loss and Directions for Therapy", Frontiers in Genetics

2 1% match (student papers from 12-Jan-2010)
Submitted to Higher Education Commission Pakistan on 2010-01-12

3 1% match (student papers from 02-Mar-2012)
Submitted to The Robert Gordon University on 2012-03-02

4 1% match (Internet from 17-Jan-2023)
https://worldwidescience.org/topicpages/h/human+usher+syndrome.html

5 1% match (Internet from 05-Sep-2021)
https://web.archive.org/web/20190503103254if_/https://pdfs.semanticscholar.org/2027/153c2f19cd88f7f2a753b99c646433de805e.pdf

6 < 1% match ()
José M. Millán, Elena Aller, Teresa Jaijo, Fiona Blanco-Kelly, Ascensión Gimenez-Pardo, Carmen Ayuso, "An Update on the Genetics of Usher Syndrome", Journal of Ophthalmology

7 < 1% match (student papers from 23-Nov-2010)
Submitted to Higher Education Commission Pakistan on 2010-11-23

8 < 1% match (student papers from 09-Jun-2016)
Submitted to Higher Education Commission Pakistan on 2016-06-09

9 < 1% match (student papers from 30-Sep-2022)
Submitted to Higher Education Commission Pakistan on 2022-09-30

10 < 1% match (student papers from 28-Sep-2022)
Submitted to Higher Education Commission Pakistan on 2022-09-28

11 < 1% match (student papers from 09-Sep-2016)
Submitted to Higher Education Commission Pakistan on 2016-09-09

12 < 1% match (Internet from 17-Jan-2023)
https://worldwidescience.org/topicpages/t/targeting+retinal+photoreceptors.html

13 < 1% match (Christine Petit, " U S : From Genetics to Pathogenesis ", Annual Review of Genomics and Human Genetics, 2001)
Christine Petit, " U S : From Genetics to Pathogenesis ", Annual Review of Genomics and Human Genetics, 2001