USHER SYNDROME AND ITS GENETIC CHARACTERIZATION

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ABSTRACT: Usher syndrome is defined as an autosomal recessive disorder which is caused by a mutation in any one of at least 10 genes resulting in a combination of hearing loss and visual impairment. The loss of hearing is caused by a defect in the inner ear, whereas degeneration of the retinal cells called retinitis pigmentosa (RP), results in loss of vision. Based on severity and age when signs and symptoms appear, there are three clinical subtypes of Usher syndrome (characterized as Usher I, II, and III). People suffering from Usher I are deaf by birth and during the first decade of life, they begin to lose their vision. Balance difficulties are also exhibited by them. Patients with Usher II have hearing loss but they are not deaf by birth. They also lose their vision later on. They do not show problems with balance. People having Usher syndrome III lose their vision and hearing gradually but they are not born deaf; they may or may not show difficulties with balance. In this review, those mouse models for usher syndrome will be discussed in which homologue of humans was identified first and a model called, "mouse model" was based upon gene defects in the human beings.

Key words: Usher syndrome, retinitis pigmentosa, genes, deaf, hair lose, visual impairment, mouse model.

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INTRODUCTION

Usher syndrome (USH) is a syndrome categorized by the destruction of sensorineural audial and progressive retinitis pigmentosa (RP) which leads to night blindness [1,62].This autosomal recessive disorder Li was first reported by Charles Usher in 1914 [2]. USH accounts for 5% inherited deafness and 18% of RP cases [65]. This disease involving cilia dysfunction is the most common cause of inherited deaf-blindness in human with 3-8:100000 worldwide prevalence [64,65,66]

A syndrome is defined as a disorder that has more than one symptom or feature. Hearing impairment (HI) is usually classified on the basis of the defective part of the hearing organ, i.e., sensorineural and conductive. Conductive HI is due to the defects in the external or middle ear. It yields mild to moderate hearing faults and in most cases can be medically treated. Whereas the defect is situated all along the auditory pathway i.e. from cochlea to auditory cerebral cortex leads to sensorineural HI and results in a mild to intense HI. In most of the cases these problems cannot be completely resolved but yet the hearing aids or cochlear implants can be used for disease management [3].

One major and important symptom of the USH is RP, characterized by night blindness and peripheral vision failure because of the progressive retinal disintegration. With the progress of disease, a condition called "tunnel vision" arises in which only central vision remains and the field of vision narrows. Also, severe balance problems are associated with USH [4].

Clinical types of Usher syndrome: USH has three clinical types (USH1, USH2, and USH3) based upon the progression and severity of deafness, retinal degeneration and vestibular dysfunction [6]. Types 1 and 2 are the most common in the United States, and account for about 90 to 95% of the reported cases of USH [4].

There are various molecular causes associated with this syndrome. So far nine genes and 11 genetic loci have been identified to cause this disorder [7]. Which are:

- USH1 (USH type 1): MY07A, USH1C, CDH23, PCDH15, SANS
- USH2 (USH type 2): USH2A, VLGR1, WHRN
- USH3 (USH type 3): USH3A [4]

USH1: It is the most severe form of Usher syndrome [8]; characterized by constant vestibular dysfunction, intense loss of hearing at birth and progressive retinitis pigmentosa which begins in early adolescence resulting

in retarded motor development [6]. Children with USH1 have severe balance problems, and are profoundly deaf at birth; hearing aids are of no or little use for many of these children. Vision problems most often initiate with night blindness and progress rapidly to complete loss of vision. It can be caused due to mutation in any of the 6 different genes (MY07A, USH1C, CDH23, PCDH15, SANS) [9]. Six loci (USH1A-F) for USH1 have been tabled [10]. The genes which have shown mutation are mutated at 4 of these loci have now been recognized i.e. USH1C at USH1C, myosin VIIa (MYO7A) at USH1B, protocadherin 15 (PCDH15) at USH1F and cadherin 23 (CDH23) at USH1D [11,12]. CDH23 and MYO7A have also been observed to underlie non-syndromic forms of deafness [12]. The largest contribution to USH1 is made by mutations in myosin 7A (USH1B), whereas genes USH1D-USH1F (chromosome 10 region) are the second largest contributor identified so far to USH1 genetic load [13].

USH2: It is the most frequent form of USH, which is characterized by normal vestibular function, moderate to severe innate hearing destruction, and a later commencement of retinal degeneration [14]. Although the degree of hearing impairment varies, hearing aids can be beneficial to the majority of these patients which help them to communicate orally. In USH2, vision problems associated with the onset of RP are likely to develop more slowly than those in USH1, and are often not apparent until adolescence. USH2 is caused by the mutation in any of the three genes (USH2A, VLGR1, WHRN) [4].

USH3: It is the rarest type of USH, characterized by gradual loss of hearing and the age for the onset of retinal degeneration is also variable [15]. Hearing at birth is normal in Children with USH3. Most of the children with USH 3 have balance from normal to near normal, but a few of them may later on develop balance problems. Only gene USH3A is found to be associated with USH 3[4]. All these types showing in Table 1.

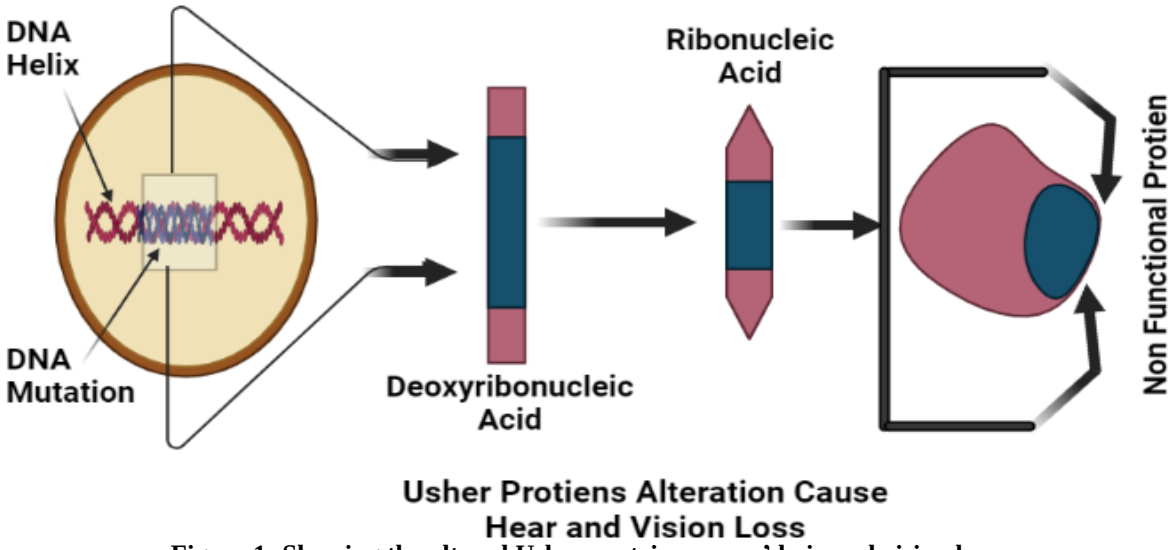

Figure 1: Showing the altered Usher proteins causes' hair and vision loss

Table 1: Showing USH has clinical types based upon the progression and severity of deafness, retinal degeneration and vestibular dysfunction.

Type	Hearing Loss (HL)	HL Onset	Retinitis Pigmentosa	Vestibular Response
USH1	Severe to profound HL	Congenital	Usually prepubertal	Altered
USH2	Moderate to severe	Congenital	Around puberty or post-pubertal	Normal
USH3	Moderate to severe	Progressive	Around puberty or post-pubertal	Altered

[14]

Usher Complex: The proteins crucial for the hair bundle morphogenesis are also concerned with USH, this protein complex is known as Usher complex. A variety of functions are performed by the members of Usher complex, that range from the cell adhesion molecules to the transmembrane protein receptors and motor proteins over scaffold proteins [6]. An outline of the different Usher genes along with their respective mouse models have been presented in the following Table 2.

Table 2: Showing different Usher genes along with their respective mouse models.

HL type	Usher Syndrome	Gene	Protein	Mouse mutant phenotype	Stereociliar phenotype
DFNA1 and DFNB2	USH1B	MYO7A	Myosin VIIa	Shaker 1	Splayed stereocilia, disorganized hair bundles
DFNB12	USH1D	CDH23	Cadherin 23	Waltze	Shorter stereocilia, disorganized hair bundles,
DFNB18	USH1C	USH1C	Harmonin	Deaf circler	Shorter stereocilia, disorganized hair bundles,
DFNB23	USH1F	PCDH15	Proto-cadherin 15	Ames waltzer	Splayed stereocilia, disorganized hair bundles
	USH1G	USHIG	SANS	Jakson shaker	Splayed and few shorter stereocilia, disorganized hair bundles
	USH2A	USH2A	Usherin	Ush2a knock out	Stereocilia missing from OHC in basal cochlear turn
	USH2C	USH2C	VLGRI	Ush2a knock out	Splayed stereocilia, disorganized hair bundles
DFNB31	USH2D	WHRN	Whirlin	Whirler	Rounded arrangement of shortened stereocilia
	USH3A	CLRN1	Clarinl	-	-

[16]

Mouse models: For many reasons it is often difficult to identify the genetic factors that are responsible for different forms of hearing loss in humans [17]. Large families with several hearing-impaired members are required for Genetic linkage analysis of hereditary hearing loss in humans [18]. Linkage analysis in small families with hearing impairment leads to difficulty in positional cloning [19].

Inner ear in humans is difficult to isolate as it is located deep within the temporal bone. Also, human cell lines exhibiting the characteristics of developing inner ear are not available, therefore to study various biological functions (the interactions between proteins expressed within the inner ear, their spatial and temporal expression patterns, their functions etc.), only primary cultures or model animals can be used [16].

Among model animals, mice are found to be an excellent model for the purpose of studying the genetics involved in hearing impairment. In the breeding colonies several circling mice have been observed worldwide. Inner ear mouse mutants are identified by a distinguished characteristic of Circling or head bobbing behaviour. These mutants also suffer from hearing impairments. Mice are small, with short gestation periods and are easy to handle as well as these are cost effective animal models. Inner ear of the mouse exhibits significant similarities with the inner ear of humans. Several imaging techniques like computed tomography (CT) scanning procedures and scanning electron microscopy have been optimized to study the inner ear of the mouse during the last two decades. For mice there are various molecular biological techniques that have been optimized therefore are available to create knock in/ knock out and transgenic mice that are widely used in research areas [20, 21]. Moreover, homologous recombination technology is applied solely on mice among mammals on a routine basis. Above all, there exist high resemblance between the mouse genome and the human counterpart as it has 80% similarity with the genome of humans [1].

When a gene is suspected to be responsible for hereditary hearing loss in humans, the same kind of mutations may be engineered in mice to prove this hypothesis. Gene's role can be revealed by comparing, 'knockout' mice produced by gene-targeted mutagenesis, with wild type mice. In addition, mouse models are used to identify new genes that are associated with inner ear development and normal hearing [22].

In this review, those mouse models for usher syndrome will be discussed in which homologue of humans was identified first and a model called, "mouse model" was based upon gene defect in the human beings [16].

Shaker 1 Mouse Model: It has been found that Several Myo7a mutations cause recessive loss of hearing in shaker 1 (sh1) mice and in humans. MYO7A mutations (DFNB2 and DFNA11) cause both recessive and dominant hearing loss, as well as Usher 1B syndrome [23]. One important transport molecule which is responsible for the transport of many of the other Usher complex proteins is considered to be Myo7a, formed by binding of several ligands with the tail domain of myosin 7A [24]. Unconventional myosins are made up of motor proteins which travel along the filaments of actin by

utilizing energy produced by the hydrolysis of ATP. Therefore, the cell adhesion complex is further strengthened by this dynamic alleged link to the actin cytoskeleton. As myosin VIIa is located, along the entire length of the stereocilia, thus, the role played by these myosin VIIa filaments is considered as an ankle link to provide strength due to which the organization of the stereocilia bundle is maintained [25].

In the 1920s shaker 1 mouse mutants were described for the first time. Firstly the defects are observed in inner ear neuroepithelia by using light microscopy, and then progressive degeneration of the sensory hair cells of the organ of Corti and saccule are seen. For a short period, shaker 1 mouse mutants exhibit some behavioral and physiological responses to loud sounds that are followed by the onset of auditory function within two weeks after birth [26].

It was observed in 1995 that Myo7a mutation leads to phenotype Shaker 1 [27]. Afterwards the alleles of Myo7a (Myo7a816SB and Myo7a6J) were identified. Different shaker 1 mutants have shown different phenotypes. The normal height gradient pattern of stereocilia is locked by the mutants of Myo7a6J and Myo7a816SB, this abnormal pattern is frequently seen in apical regions of the cochlea. Stereocillia of inner hair cells joined together to form giant fused stereocilia and were severely affected. As a result, Myo7a6J and Myo7a816SB both suffered from an intense loss of hearing. However, a less severe phenotype is exhibited by the original mutant of Shaker 1 (Myo7ash1). Stereocilia bundles remain normal to a certain extent, and the mutants show less severe impairment of hearing. A continuing loss of outer hair cells could also be noticed in these mice [16].

Hyperactivity characteristic, head tossing, circling and deafness is expressed by the mice which are homozygous for the natural mutation of shaker 1 (Myo7ash1). They show normal viability and a high breeding ability. They are often deaf and up to 4 weeks of age they can swim well on the surface of water but this ability is lost later on. By light microscopy, the degenerative changes are seen in the spiral ganglion, the organ of corti, stria vascularis in the cochlea, the vestibular ganglion in the vestibular labyrinth and also in the saccular macula [28].

Deaf Circler: The USHIC was first described in Louisiana State of USA, in its Acadian population. On the chromosome 11p USH1C gene is located which is responsible to encode a protein present at PDZ domain called harmonin. Twenty eight coding exons comprising 51 kb have been found to be present in the USH1C gene. Three subclasses of harmonin isoform have been reported which are formed by the alternate splicing of eight exons [9].

There are two spontaneous mutants of mouse that have been identified through the mutations in Ush1c that are named as deaf circler (dfcr) and deaf circler 2 Jackson (dfcr-2J). The dfcr mutants have a large deletion in both constitutive and alternatively spliced exons, while the mutation in dfcr-2J causes a frameshift in a single alternatively transcribed exon. In both mutants, severe balance dilemmas have been observed. Deafness was observed from the earliest stage in these mice and beginning from 3 months age a gradual hair cells loss was also reported. By the age of 8 months this condition became more intense and secondary to its loss of spiral ganglion cells was followed by it. Hair cell stereocilia analysis revealed that particularly outer hair cell's stereocilia were disorganized and splayed, even though inner hair cell's stereocilia were also affected [16]. Though clear vestibular and inner ear defects have been found in deaf circler mice, yet they are known to show normal viability of the photoreceptor cell across the entire normal ultrastructure and function of the rod photoreceptor synapse and retina [29].

Whirler mice: The mutations concerning WHRN gene have been reported [30] to be accountable for USH2D along with a non-syndromic loss of hearing (DFNB31) [31]. At the same time these mutations were indicated by a whirler, which is an automatic mouse mutant for whirlin [31]. From the earliest measured stage, whirler mice are deaf and exhibit head tossing behavior and circling. Significantly shorter IHC stereocilia have been found in these mice. Compared to controls, stereocilia of OHC were also shorter, but the effect was less pronounced. In addition, the rounded 'U' shape was seen in the hair bundles of OHCs instead of the normal 'V' pattern. During embryonic development these defects were previously observed and ultimately led to IHCs (by P80) and OHCs (by P60) degeneration [25]. By using the expression pattern of whirler mutant whirlin and study it was thought that whirlin may direct the growth of the stereocilia membrane with F actin [31]. Additionally, by Myo15a, whirlin appears to be distributed to the stereocilia tips, as by transfection experiments it was observed [32].

Jackson shaker mice: Two different spontaneous mutants referred respectively as Jackson Shaker (js) original allele and other as jsseal allele, carry mutations in Sans. Profound loss of hearing and circling behaviour has been exhibited in both mutants [33]. Incomplete differentiation of the hair cell of maculae and stereocilia of the OHCs was observed in Jackson shaker mice. Throughout the entire cochlea, regular V shaped configuration of the stereocilia is not shown by most of the OHCs. Classical, stair case configuration of stereocilia was not preserved also, in many hair cells of the saccular macula and utricular. Remaining hair cells

normally developed, only the stereocilia were affected [34].

In humans, IG Usher syndrome is triggered by SANS mutations [35]. A scaffold protein is SANS. It has been revealed through binding assays (in vitro) and colocalization SANS interferes directly with harmonin and myosin VIIa, while with Pcdh15 and Cdh23, no binding could be detected. Below the cuticular plate in OHCs and IHCs, SANS is mainly concentrated. Below the kinociliar basal body where the cuticular plate is thinner it is found, in particular [36]. Therefore, SANS is not an integral member of the inter stereocilia link complexes, rather it has a role in trafficking components of these complexes [37]. Hair cells, the sensory receptors of the inner ear, respond to mechanical forces originating from sounds and accelerations. An essential feature of each hair cell is an array of filamentous tip links, consisting of the proteins protocadherin 15 (PCDH15) and cadherin 23 (CDH23), whose tension is thought to directly gate the cell's transduction channels [69]. Proposed minimal machinery for tension generation showing in Figure 2.

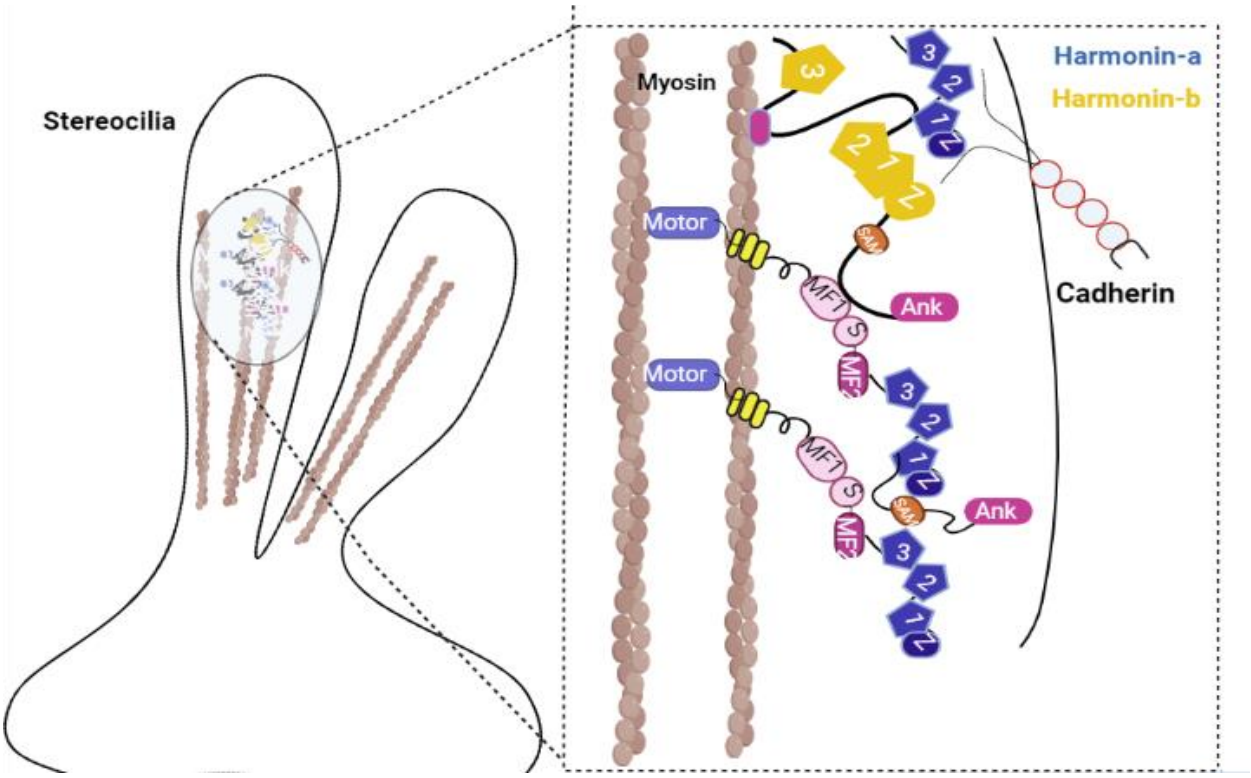

Figure 2: Showing proposed minimal machinery for tension generation

Waltzer Mouse Model: For Usher syndrome ID and DFNB12 related loss of hearing in humans, CDH23 is responsible [12]. Kinocilial link, Tip links and ankle links are associated with CDH23 [38,39]. At the lateral transient links and ankle between the adjacent membranes of stereocilia, CDH23 is localized. These lateral transient links and ankle are lacking in the mature coclea. In the upper part of the tip and ankles between the membranes of adjacent stereocilia, CDH23 is localized. These transient lateral links and ankles are lacking in mature coclea. To the upper part of the tip links, CDH23 are localized in mature cochlear hair cells. A spontaneous cadherin 23 mutant mouse is Waltzer. In Waltzer mice the loss of hearing has been observed and could be ascribed to hair cells degeneration at older ages and progressive hair bundle disorganization [40]. CDH23 is part of a transmembrane complex that incorporates stereocilia into the bundle indicated because splayed stereocilia is present in the Cdh23 deficient mice. In USH1 patients, stereocilia bundles disturbance and eventually deafness has been reported to be caused by flaws in the construction of this complex [16].

In the inner ear of the mouse two splice variants of Cdh23 were found. Both have PDZ binding motifs which can bind harmonin. A truncated cadherin 23 that lacks the extracellular domain was also reported (reviewed in circling behaviour, erratic movements and head tossing which in homozygotes appear from birth). At the time of birth heterozygotes appeared to be normal, but at older ages had a tendency to develop a progressive loss of hearing and had a higher sensitivity for loss of hearing, induced by noise [41]. Progressive disorganization of the hair bundle has been exhibited by Waltzer mouse mutants, which is first observed at day

18.5 of embryonic life (E18.5) at beginning of the bundle formation and as the hair cells mature it becomes more pronounced [42]. In addition, the kinocilium is misplaced. At an older age, stereocilia seem thicker and fused, leading to hair cell degeneration [25].

Homozygous waltzer mice in the circling mutants show the hyperactivity, deafness, head tossing and circling behaviour. Degeneration of the saccular macula, stria vascularis, and organ of Corti spiral ganglion are the abnormalities of the inner ear. Homozygous piebald mice exhibit irregular white coloured spotting, an amount of which is greatly affected by minor modifying genes. Homozygous piebald mice have dark eyes. Melanocytes are completely lacking in the white areas of the coat and in the choroid layer of the eye there is a reduction in the number of melanocytes [28].

Ames waltzer (av) mouse: A model for defects of inner ear connected with Usher syndrome type 1F is deaf circling Ames waltzer (av) mouse harbors a mutation in the protocadherin 15 (Pcdh15) gene [43]. In av mutants several reports of inner ear pathology have been reported, when av mutation in mice was first reported in 1961 [44], although in most reports cochlear abnormalities have been focused [45]. In the adults or at any time during postnatal development, no evidence of auditory function was found through the study of av mice (presumed as original alleles of av) physiology.

These findings have been found compatible with that of degenerative structural variations, in the stereocilia of the cells of cochlea and hair seen as early as P15. Though circling behavior was not displayed by the av mice displayed, so no anatomical vestibular apparatus abnormalities were found. In adult av mice, study of the saccular neuroepithelium under transmission electron microscopy failed to explore any ultrastructural defects in the sensory cells, neural elements or supporting cells, Therefore, in av mice the structural source for early onset of circling behavior is still not clear [46].

It is anticipated that Pcdh15 (Wild type) is a transmembrane protein just like other protocadherins. Vestibular neural function Vestibular evoked potential was considered with VsEPs (linear vestibular evoked potentials). These are the compound action potentials of the vestibular part of 8th cranial nerve and central relays of it which are produced by saccule and utricle in reaction to the stimuli linearly accelerated. Four alleles of controls and Ames waltzer, which are age matched heterozygote have been assessed: Pcdh15av-J (7 controls, 10 mutants), Pcdh15av-2J (2 controls, 3 mutants), Pcdh15av-Tg (5 controls, 10 mutants), and Pcdh15av-3J (5 controls, 10 mutants); results from Pcdh15av-Tg and Pcdh15av-3J were alike. By means of a digital camera swimming behavior was observed. In a glass aquarium each mouse was kept which was packed with tepid water. Horizontal bodyline swimming has been retained by normal mice at the surface and appears to be the surface of the water instantaneously. Swimming capacity decline has been exhibited when the mouse remains submerged or submerged often, disoriented, tumbling and turning beneath the surface of water.

One important suggestive of inner ear vestibular abnormality in mice is circling behaviour. In young animals in case of Ames waltzer, defect in structure is evident with significant vestibular abnormality has not been recognized, though vigorous circling behaviour is being shown by them. Whether the experiential phenotype might be a consequence of a central, instead of peripheral, vestibular abnormality this is an essential question which has been raised. Definitive evidence has been presented by this study that circling behaviour in Ames waltzer mutation is linked to severe functional abnormalities of peripheral vestibular receptor organs. Furthermore, results propose that vestibular abnormality may be the consequence of non-functional mechanosensory transduction channels in the cells of the ampullary and macular receptor [46].

There are several isoforms of human and mouse harmonin. These isoforms are represented in a tissue specific mode and differ from each other by the number and existence of binding sites for protein. The expression of the longest harmonin class b isoform is in the inner ear only [47]. Harmonin is a potent scaffold protein and it may contain domain called PST (Proline, Serine, Threonine, rich), Drosophila disc large tumor suppressor, 2 or 3 PDZ Postsynaptic density protein and Zonula occludens-1 protein that play their role in protein-protein interactions.

Another important scaffold protein is whirlin which contains PDZ domains. These scaffold proteins containing PDZ domains are usually located in sensory pathways where they play their role to synchronize the organization of signalling molecules into the complexes of macromolecules [48]. It is evident that whirlin and harmonin have the ability to bind to all other components of Usher complex which includes usherin, myosin 7a, VLGR1, protocadherin 15 and cadherin 23 with their PDZ containing domains. It designates that whirlin and harmonin play a central role in Usher complex formation of [49,36]. Non-syndromic hearing loss (DFNB18) [50,51] and USHER syndrome [52,47] both are caused by mutations in the USHIC gene that encodes for harmonin.

Two spontaneous mutants of mouse have been identified through mutations in Ush1c; deaf circler 2 and deaf circler Jackson. Severe deficits of balance were observed in both of these mutants. Furthermore, from the earliest measured stage, the mutant mice were deaf (at P 21). Conversely, progressive hair cells loss was also noticed beginning from the age of 3 months which became more severe by the age of 8 months. On this stage a secondary spiral ganglion cells loss was also

accompanied by hair cells loss. It is suggested by these data that in these mutants dysfunction of hair cells preceded hair cells loss. It has been shown by the analysis of hair cell stereocilia that OHCs' stereocilia were especially disorganized, though IHCs' stereocilia were also affected. The abnormalities which were observed at stereocilia of hair cells had already occurred at P21 and these abnormalities might be responsible for observed loss of hearing [29]. WHRN gene mutations account for non-syndromic loss of hearing (DFNB31) [31] and USH2D [30]. A whirlin spontaneous mouse mutant called the whirler was used simultaneously to describe these mutations [31]. From the earliest measured stage, the whirler mice are deaf and head tossing and circling behavior is exhibited by these mice. IHC stereocilia are significantly shorter in whirler mice. As compared to control, the stereocilia of OHC were also shorter in these mice, but the effect wasn't so obvious. In addition to this an abnormal rounded 'U' shape was seen in the hair bundles of OHCs instead of the normal 'V' pattern. Already, during embryonic development, these abnormalities were observed which ultimately resulted in IHCs degeneration by P80 and OHCs degeneration by P60 [25]. It was hypothesized from the whirler mutant study and the expression pattern of whirlin that stereocilia membrane and F-actin growth may be coordinated by whirlin [31]. Additionally, it was observed by transfection experiments that whirlin is delivered to the stereocilia tips by Myo15a [32].

Protocadherin 15 and Cadherin 23: Protocadherin 15 (PCDH15) and Cadherin 23 (CDH23) are related to cadherin family which are molecules cell adhesion. Both gene mutations result in loss of hearing. Mutually these molecules are considered to be vital for mechanotransduction. Usher syndrome 1D as well as human DFNB12 related loss of hearing is caused by CDH23 mutation [12], while Usher syndrome 1F and non syndromic DFNB23 loss of hearing are associated with human PCDH15 mutations [53].

There is an interaction between intracellular signalling proteins present in cytoskeleton and cytoplasmic domains of PCDH15 and CDH23 [54]. The occurrence of class I PDZ binding domains in PCDH15 and CDH23 makes them different from classical cadherins. These class I PDZ binding domains in PCDH15 and CDH23 enable these to unite harmonin and fix themselves to cytoskeleton of actin [53,55]. There are two complementary binding surfaces of PDZ in the cytoplasmic domain of CDH23 with which the two harmonin, PDZ domains interact. PCDH15 uses its PDZ binding domains to interact with harmonin through the same mechanism. In the stereocilia of hair cells found in ear, PCDH15 and CDH23 are expressed. PCDH15 is connected with kinocilium links and tip links [56], while CDH23 is associated with ankle links, tip links and kinocilia links [38,39].

At transient lateral links and ankle links present in between the neighboring stereocilia membranes, during the differentiation of the hair cell, CDH23 is localized. In mature cochlea, these ankle links and transient lateral links are not present [57]. In mature hair cells of cochlea, both PCDH15 and CDH23 are located in tip links. PCDH15 is localized to the lower part of the tip link, while CDH23 is confined to the upper part of the tip link. Homomersof PCDH15 and CDH23 are never observed; both of these molecules always form heteromeric complexes [41].

From the different mouse models of cadherin study, much of the knowledge was obtained about the role of PCDH15and CDH23 in the Usher complex. A spontaneous mutant of cadherin 23 in mice is Waltzer. In Waltzer mice, the loss of hearing is observed which may probably be due to a progressive disorganization in the hair bundle, in older ages it could be due to deterioration of hair cells [40]. The mice deficient in CDH23 may have divided stereocilia, this showed that CDH23 can be an important part of the transmembrane complex due to which the stereocilia are connected to a bundle. Any defect during formation of the complex results in the disruption of the stereocilia bundles eventually leads to loss of hearing (deafness) in the patients of USH1. A similar phenotype is displayed by Ames waltzer mice, which show a mutation in PCDH15. Degeneration of hair cells is resulted due to stereocilia disorganization in the vestibulum Wally and organs of corti [13]. It has been recommended that harmonin binding to PCDH15 ensures attaching of PCDH15 to core of actin all through development of hair bundle [49]. Hearing and balance involve the transduction of mechanical stimuli into electrical signals by deflection of bundles of stereocilia linked together by protocadherin 15 (PCDH15) and cadherin 23 'tip links'. PCDH15 transduces tip link tension into opening of a mechano-electrical transduction (MET) ion channel [68].

USH2A knock out: USH2A has been found to be responsible in about 70% of all US patients. On chromosome 1q, USH2A gene is present and which encodes a unique 171 kDa protein (usherin) having 1,551 amino acids in it. Usherin shows similarity with cell–cell adhesion proteins and extracellular matrix, and has both fibronectin type III domains and laminin epidermal growth factor. USH2A transcript is expressed principally in the cells of the outer nuclear layer of the retina and the cochlea. In USH2A at least 20 different mutations consisting of 21 exons are found, (in which first is noncoding), across a length of at least 105 kb. 2299delG is the most frequent mutation, in the United States and Europe which may be present in more than 20% of the USH2A as well as in some USH3 patients. In addition to

this, mutation in 2299delG has been reported in those patients whose prime abnormality is retinitis pigmentosa and who reported minimal loss of hearing, and another mutation of USH2A, in about 4.5% of nonsyndromic autosomal recessive retinitis pigmentosa, Cys759Phe was found [9].

A mice line was synthesized which harbors the disruption in gene Ush2a, which results in the removal of protein called Usherin (mice named Ush2a null mice). A spectrum of hearing and retinal defects closely resembling those of human patients of USH2A, including progressive degradation of photoreceptors and moderate, non-progressive loss of hearing, especially at frequencies of higher altitude has been reported to develop in Usherin null mice [46]. Until after the age of 10 this was not seen. In USH2A patients, as such the model imitates the hearing and visual deficits. Current applicability for gene therapy has been limited due to the large size of usherin gene i.e.171-kDa protein. To explore whether a cell based therapy might be a useful substitute in this condition, this model can be used [58]. Etiological mutations in USH2A, the most common cause of RP and USH, were found in 16.34% (n = 218) genetically solved IRD patients (Li *et al*., 2022).

Therapeutic Strategies: At present there is no effective treatment of Usher syndrome. However, research is going on to develop different molecular and cell based therapies. The most attractive target for the treatment of this syndrome is retina because of its immune privileged nature and it provides the accessibility for intraocular treatment. Currently, most of research is focused on gene replacement therapy by the use of viral vectors [59]. Different studies reported that after a subretinal injection, the expression of photoreceptors and myosin VIIa in the RPE cells of Myo7a Mutant mice is restored by using adeno-associated virus (AAV) vectors [60]. In another study, it was reported that a functional MYO7A was delivered in the retina of USH1B mouse model via lenti-virus based vector and it proved successful [61]. The human MYO7A gene carried in an equine infectious anemia virus based vector was injected into the retina of neonatal mutant mice which resulted in the restoration of photoreceptor function and protected the light induced degeneration of photoreceptor [62].

Cell based therapeutic strategies included a study in which transplantation of neural progenitor cells derived from forebrain was done into the retina at P80 of Ush2a knockout mice [58]. It resulted in prevention of vision deterioration and reverse of cone pigment mislocalization for 10 weeks only. Implantation of human NTC-201 cells contained capsules in the retina of patients with isolated USH3, USH2 and RP is being trailed [59]. Most USH patients benefit from CI. USH patients who undergo CI at younger ages generally achieve better hearing, speech, and cognitive outcomes. CI at older ages can still prove beneficial if appropriate auditory amplification is started at the right time. Further research is warranted to fill the gap in understanding regarding the gene mutations underlying the pathophysiology of USH that have favorable CI outcomes as well as the optimal time to perform CI [67]..

Conclusion: The first USH gene was discovered about 20 years ago, since then extensive progress has been made to understand the genetics of Usher syndrome. A lot of proteins having different cellular functions are encoded by USH genes. This review highlights functions of the genes and proteins involved in the pathogenesis of Usher syndrome and their interaction in the retina and inner ear which may serve as potential targets for effective treatment of this syndrome.

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