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Rebecca Levinson
Case Western Reserve University

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HEARING LOSS PHENOMENON IN USHER SYNDOME 1: PROTEIN PROFIING OF THE COCHLEA, USING PROTEOMIC METHODOLOGIES

-Rebecca Levinson-

Rebecca Levinson is a fourth year student studying biology and philosophy at CWRU. Outside of the classroom she is active in the University Program Board, and the National Residence Hall Honorary. After the conclusion of her undergraduate experience she plans to go to graduate school.

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ABSTRACT

Hearing loss usually results from the abnormal development of, or injury to, the "hair cells" in the cochlea (inner ear). Usher syndrome, a type of deafness, is characterized by the degeneration of hair cells due to genes involved in HHI (Geller et al, 2009). Mouse mutants that simulate Usher syndrome have served as an excellent model to understand the basis for HHI. Prior work has identified networks of proteins in the cochlea, specifically those associated with cellular degeneration as factors that contribute to Usher syndrome (Chance et al). Protein profiling, using gel-based approaches like 2D differential expression analysis (2D DIGE), has shed light on the possible roles of different proteins including cochlin (Chance et al). That data has suggested that Cochlin and its isoforms are very much involved in the mechanism of hearing loss (Chance et al). A "shotgun" approach of cleaving proteins into peptides prior to their analysis with liquid chromatography-mass spectrometry (LC-MS) technology is a more sensitive alternate approach. The goal is to (a) profile the Cochlear proteome (protein compendium) using proteomic methodologies such that we can decipher the role of cochlin and its related proteins and (b) isolate and characterize Cochlin precursor proteins such that more can be determined about its role in hearing loss.

INTRODUCTION

Usher syndrome is a degenerative disease that causes ear and eye problems in humans (NIDCD, 2008). There are several types of usher syndrome, USH1F, being the most common. Usher syndrome is responsible for about 3-6% of the cases of deafness in the United States (NIDCD, 2008). The most common eye degeneration from Usher syndrome is retinitis pigmentosa, or tunnel vision (HHIRR, 2009). Almost 4 out of every 100,000 babies are born with Usher syndrome (NIDCD, 2008). Babies with Usher syndrome are either born deaf or will become deaf in the first year of their life (Genetics Home Reference, 2009). Difficulty walking and sitting up often accompanies Usher syndrome, as balance is controlled by the inner ear.

There is significant research trying to identify the gene defects associated with Usher syndrome (Genetics Home Reference, 2009). Recently, it was determined that a mutation of the PCDH15 gene which codes for the protocadherin15 protein is important in Usher syndrome (Ahmed et al, 2008 and NCBI, 2009).

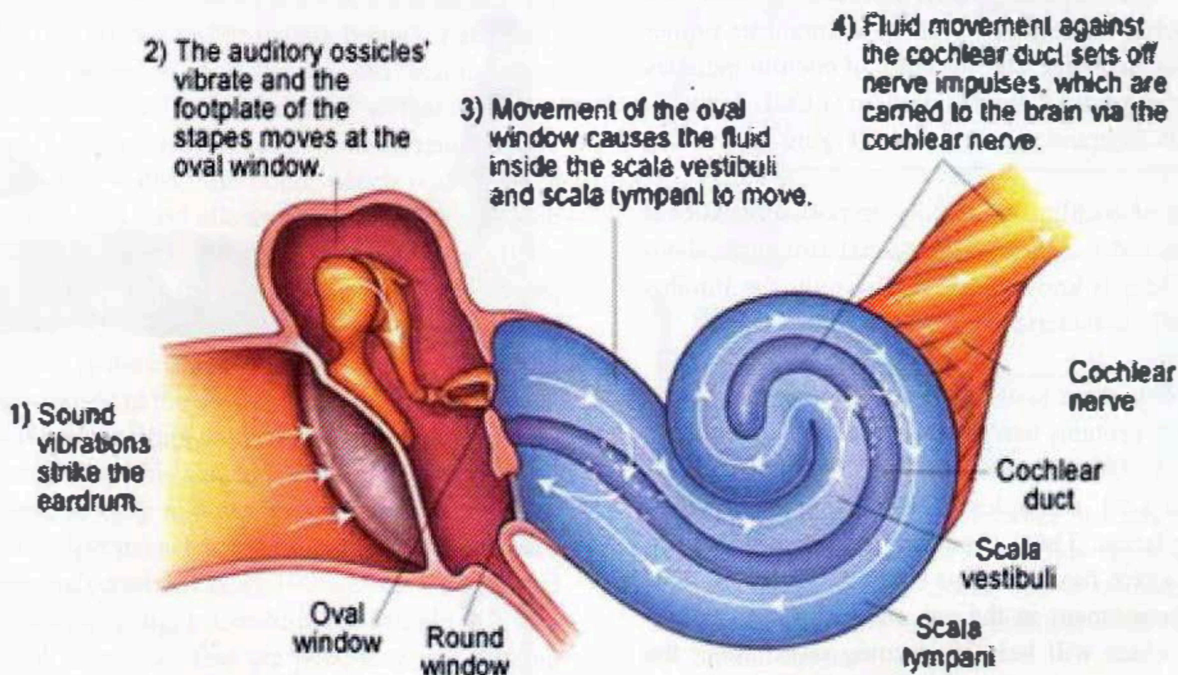

Figure 1: The cochlea is the part of the inner ear responsible for hearing (Inner Ear Anatomy, 2009).

The inner ear cochlea is made up of several types of cells, including hair cells, which play an important role in hearing. If these hair cells are damaged, it will affect the ability to hear. During the progression of Usher syndrome, the hair cells and other cochlear cells gradually begin to self-destruct by an unknown mechanism. Eventually a hole is left in the cochlea (Figure2).

The identification of this phenomenon prompted our approach, as the gene mutations have identifiable consequences in a specific tissue. A proteomic study on the tissue affected by the mutation would allow us to see the changes in the amount of various proteins in the cell during the progression of the disease, which can connect the histology with a specific molecular pathology (Chance et al). Proteomics is the study of the structure, abundance, interaction, and function of all proteins in a sample. The proteome is the collection of all proteins present in a tissue or organ and can change due to environmental conditions or health status. For example, the proteome of one mouse may dramatically change in a healthy mouse compared to one with a specific disease. There is only one genome for the cell, but the proteome can vary depending on age or environmental factors. We have chosen to profile the proteome because it will permit us to analyze many of the proteins in the inner ear including cochlin, one of the major auditory proteins. Profiling the proteome means creating a detailed list of which proteins are the most common in the cochlea, and which genes and processes these proteins are associated with. We also used a comparative method in our profiling, in which we looked at the difference in the proteomes between normal mice and those having Usher syndrome due to a mutation in PCDH15. This provides us with information about the specific proteins that are associated with deafness.

We discovered a large number of proteins that were present in the cochlea. After obtaining a general idea of the protein environment of the cochlea we chose to focus our attention on cochlin. Cochlin is hypothesized to be extremely important in the structure of the inner ear (Chance et al). It is a structural protein secreted into the intercellular matrix and is theorized to bind collagen and other structural proteins to provide a specific molecular archi-

tectural structure for the ear (Nagy et al. 2008). Cochlin is found in multiple isoforms, which theoretically affect the ability of cochlin to help the cochlea maintain its proper structure (Chance et al). The structure of cochlin includes a short signal peptide, a limulus domain (LCCL domain), and two vonWillebrand type A factors (Figure 3).

Our attention of cochlin was mainly on post-translational modifications and the three dimensional structure, about which very little is known. In cochlin, only the limulus domain is well characterized.

We can also determine post-translational modifications of major auditory proteins that are in one sample and not the other. Post-translational modifications are small changes to the amino acid, examples of which are methylation or phosphorylation. These changes are known to lead to changes in protein function. This could help give us clues about the environment in the ear under different conditions. These clues will help in learning more about the progression of the disease. Post-translational modifications can greatly affect the way proteins are structured by disrupting bond stability and placement, and therefore how they function.

MATERIALS AND METHODS

An Ames Waltzer (av) mouse was used as a model for Ushers syndrome. The av mouse has an induced mutation in the protocadherin15 gene, which causes deafness, making it a suitable model for human deafness. The cochleas were excised from the ears of a mouse with the induced mutation, and from a mouse without the mutation both at the age of 30 days. The cochlear tissue was stored in 300µL of 25mM Tris pH 8.8 buffer to allow for a minimal amount of protein degradation during storage and was then homogenized.

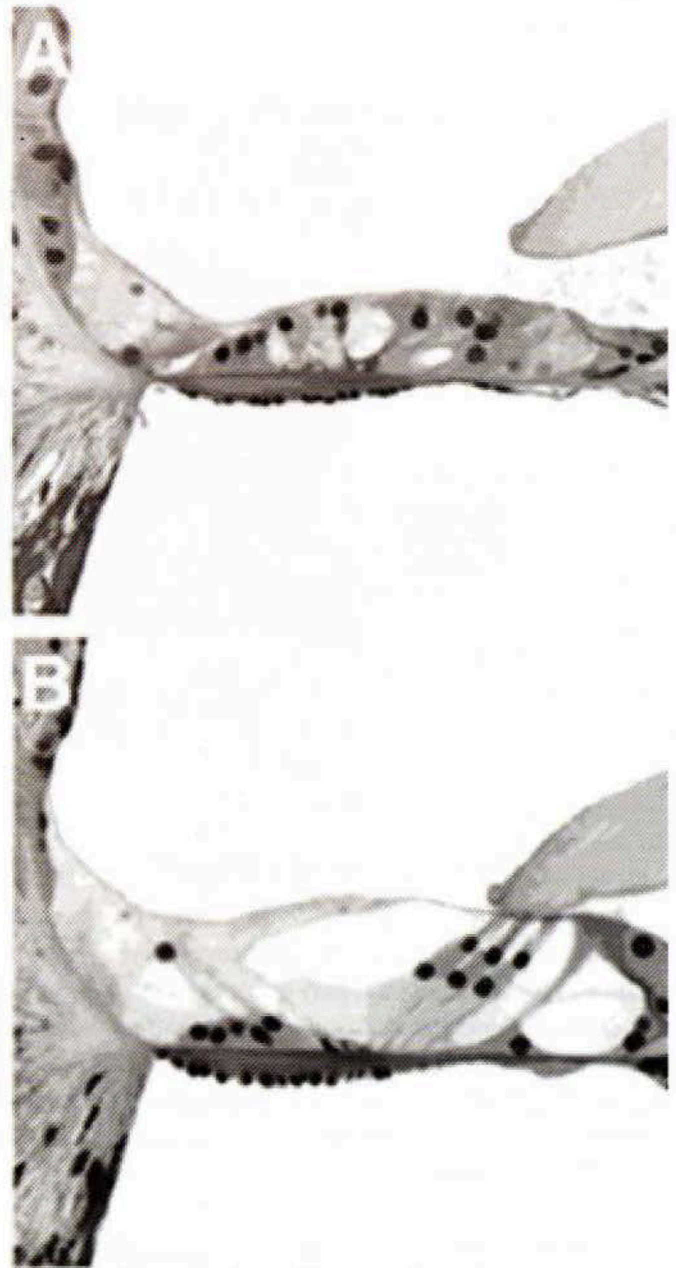

Figure 2: A comparison of organ of corti in a normal (A) and av (B) mouse (Chance et al).

Figure 3: The structure of cochlin with the known human mutations in the amino acid sequence (Robertson et al., 2008).

For the first approach, known as "shotgun" proteomics, 35µL of the homogenized tissue was used and diluted 1:1 with 25mM Tris pH 8.0 buffer (tissue lysate). 1µL of dithiothreitol was added to each sample, and then the samples were incubated for 10 minutes at 56° so that reduction could occur. The cysteine residues in proteins tend to bond with each other in a disulfide bond, which occurs when the protein is folded, and allows the protein to maintain its tertiary conformation. Reduction breaks the disulfide bonds, helping to unfold the protein. After the incubation, 8µL of 550µM Iodoacetamide was used for alkylation of the sulfur atoms in the cysteine residues before incubating at room temperature in the dark for 45 minutes. Alkylation makes it so that the sulfurs on the cysteine residues cannot bind back together. This forces the protein to stay in an unfolded form, leading to a more complete digestion and stronger mass spectrometry identification. The samples were precipitated (crashed) using 100% ethanol and stored in a -20° freezer overnight. Crashing the sample is a way of stopping all processes that are occurring and making the protein fall out of solution. A precipitate was formed after crashing, which was isolated, and then resolubilized in 100µL of 8M in 25mM Ammonium bicarbonate buffer pH 8.0. The reduction and alkylation were repeated before digesting the sample using a tryptic digest. A tryptic digest enzymatically cleaves protein using the enzyme trypsin. Trypsin cuts the peptide bond at the C-terminal end of each lysine and argenine, unless followed by a proline. We took the product of our digest, and performed a clean up using a C-18 chromatography column to remove any salts present that would affect the charges of the peptide. We then added .01% formic acid, and transferred to HPLC vials for nano LC-MS/MS.

In a second approach, we loaded a 4-20% gradient SDS-Page gel with 15µL of each sample cell protein extract. This gel separates the proteins by molecular weight, allowing the smallest proteins to travel the farthest through the gel, while the largest proteins travel the least distance. The SDS detergent binds the proteins and makes them negatively charged. A current is run through the gel to help the fragments move and settle throughout the gel. This gel ran at 120V and was then stained using coomasie blue dye, which helps visualize the proteins. The gel was destained and cut into bands. Each of these bands was digested separately using a tryptic digest. This gave us

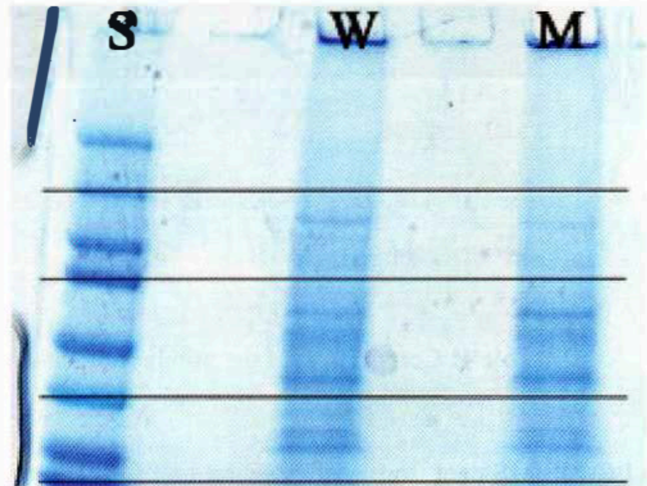

Figure 4: Light and fluorescence microscopy of electrospun poly(caprolactone) scaffolds seeded with green fluorescent protein expressing 3T3 fibroblasts via electrostatic spraying, showing the uniform distribution of cells throughout the matrix.

5 bands per sample (Figure 4). The five bands had molecular weights of 5-20kDa, 21-37kDa, 38-75kDa, 76-150kDa, 151-250kDa from the bottom of the gel to the top. Each of these digested bands had formic acid added, and was run though liquid chromatography tandem mass spectrometry (LC-MS/MS) and peptide separation.

Nano-LC-MS/MS is a set of instruments that allow a sample to be analyzed using a combination of liquid chromatography and mass spectrometry at a high resolution. When a sample is injected into the LC-MS/MS, it is pushed into a chromatography column where the peptides bind. Then a series of liquids are run through the column starting with inorganic and increasing in organic concentration. The peptides are released from the column based on their charge and chemical properties and leave the column entering the ionization chamber of the MS. A chromatograph is generated showing the intensity of a molecule versus the mass per charge (m/z) ratio of that molecule. The molecules are collided with gas molecules to break the peptide bonds. The change in the mass per charge ratio as peptides are removed gives a probable sequence. An MS2 is generated detailing the breakdown of the peptide.

	Wildtype Only	Mutant Only	Both	Total
Proteins	45	7	286	331
Peptides	789	232	867	1888

Table 1: Comparison of the number of proteins and peptides found in only one of the samples as well as those found in both.

The LC-MS/MS data from each preparation of each sample was run through a series of computer programs to identify the proteins present. The computer programs used were Mascot Daemon, Scaffold, and Babelomics. Mascot Daemon used the files generated from the mass spectrometry to generate a redundant list of proteins present in the sample. To avoid redundancies and increase statistical reliability, we analyzed the same files in Scaffold. Scaffold produced a list of protein IDs that identify which gene a protein comes from. These IDs can be put into Babelomics, a program that identifies the genes involved in specific pathways and creates maps indicating important types of processes.

To identify post-translational modifications, data from mouse deafness studies that had been prepared in a 2D DIGE method was used. 2D DIGE is a gel procedure, in which the protein fragments are separated by isoelectric point and then by mass (Chance, 2008). This was done because 2D DIGE allows certain modifications, such as phosphorylations to be seen more clearly. Bioinformatic analysis allowed the identification of modifications on specific amino acid residues. The chromatographs and MS/MS spectra associated with each of these modifications were analyzed, eliminating the spectra that did not indicate a real modification.

RESULTS

The protein we saw in the greatest quantity in both the diseased and control mouse in both of the preparations was cochlin. There were a total of 331 proteins in the control sample and a total of 293 proteins in the diseased sample (Table 1).

We found 45 proteins and 789 peptides in the wildtype samples only. There were 7 proteins and 232 peptides that appeared only in the mutant sample. 286 proteins and 867 peptides appeared in both samples. We found several post-translational modifications on cochlin. Those that appear to be the most important are the 362 Lysine, which we found can be modified by an acetylation or a methylation, the phosphorylation on the 126 threonine, and the methylation on the 545 argenine.

In the control sample, growth and cell maintenance protein groups were important, as determined by bioinformatic analysis, and the proteins necessary appeared in greater concentration. The bioinformatic analysis was done by David, a program that generated the gene ontology (GO) groups that each protein fits into, and identifies the most probable group given the processes occuring in the cell. When the data was analyzed, we generated a diagram of the biological processes found in each of the samples.

The wildtype sample had biosynthetic and cellular metabolic pathways that were not found in the mutant. The number of protein partners participating in the process may account for some of the discrepancy between mutant and wildtype protein identifications. The mutant had cellular developmental, nitrogen compound metabolic, maturation, and death processes not seen in the wildtype. In particular, the diseased sample, the cell death pathway had a much greater prevalence.

Cochlin was represented by 1.15% of all spectra in the control and 1.6% of spectra in the mutant. It was the protein in greatest abundance in each sample. We also found the coverage of the cochlin sequence by each of the samples (Figure 6). Coverage is the amount of the amino acid sequence of the protein that we could identify.

The coverage of cochlin was 78%, but there was difficulty in finding the N-terminus of the protein. In analyzing the MS/MS spectra we found several modifications on cochlin. We annotated the original spectra in each case (Figure 7), as well as using Mascot Daemon to add significance to our results.

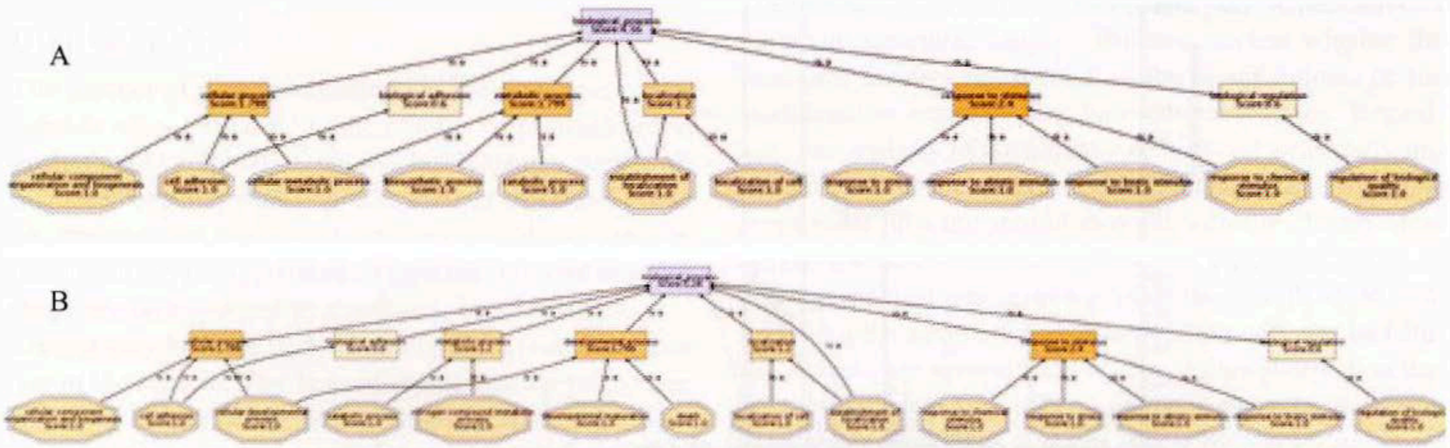

Figure 5: The biological processes represented by the proteins found in the cochlea of the wildtype mouse (A) and the processes represented by the mutant mouse (B).

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Gene Symbol= Coch Cochlin
Control 434/552 amino acids (78.6% coverage)
Diseased 436/552 amino acids (78.9% coverage)
MPSSRIPALC LGAWLLLLLL PRFARAEGAV PIPVTCFTRG LDIRKEKADV
LCPGGCSLEE FSVFGNIVYA SVSSICGAAV HRGVIGTSGG PVRVYSLPGR
ENYSSVDANG IQSQMLSRWS ASFAVTKGKS STQEATGRAV STAHPPSGKR
LKKTPEKKTG NKDCKADIAF LIDGSFNIGQ RRFNLQKNFV GKVALMLGIG
TEGPHVGLVQ ASEHPKIEFY LKNFTSAKDV LFAIKEVGFR GGNSNTGKAL
KHTAQKFFTA DTGVRKGIPK VVVVFIDGWP SDDIEEAGIV AREFGVNVFI
VSVAKPIPEE LGMVQDVAFN DKAVCRNNGF FSYHMPNWFG TTKYVKPLVQ
KLCTHEQMMC SKTCYNSVNI AFLIDGSSSV GDSNFRLMLE FVSNIAKTFE
ISDIGAKIAA VQFTYDQRTE FSFTDYNTKE NVLAVLANIR YMSGGTATGD
AIAFTVRNVF GPIRDSPNKN FLVIVTDGQS YDDVRGPAAA AHDAGITIFS
VGVAWAPLDD LRDMASKPKE SHAFFTREFT GLEPIVSDVI RGICRDFLES
QQ
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Green= Control & Diseased
Yellow= Control Only
Red= Diseased Only

Figure 6: Coverage of the cochlin sequence by sample.found in both.

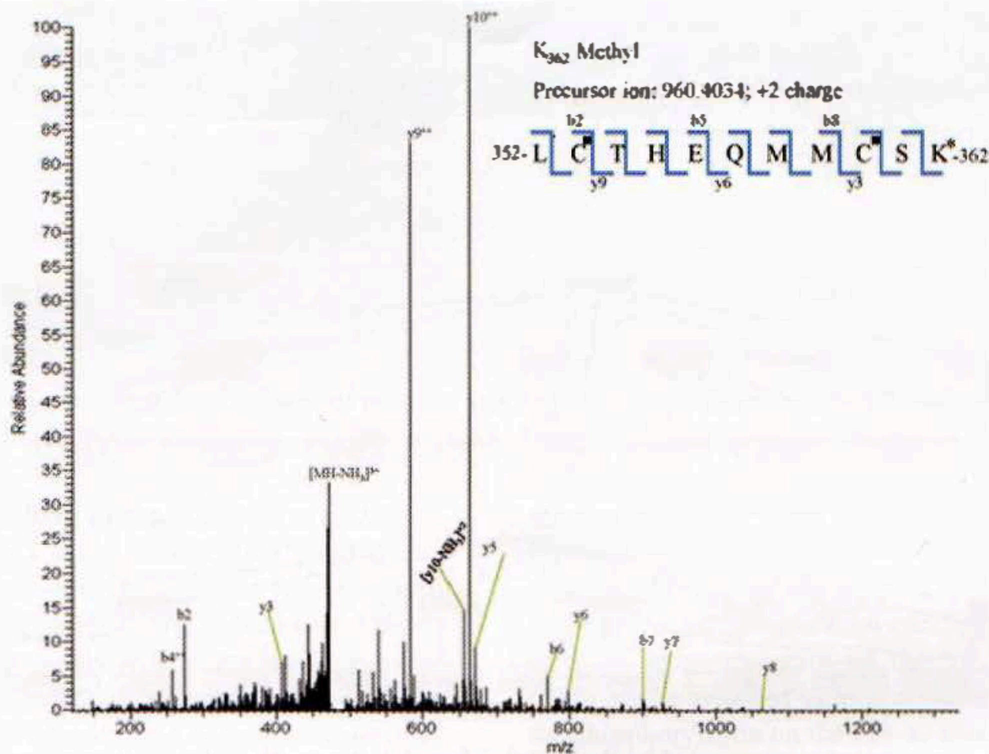

Figure 7: Sample MS2 spectra with notations to our results.

Peptide	Residue	Sequence	Approach
352-362	362	LCTHEQMMCSK*362	Gel Fractionation (Control)
182-192	182, 187, 192	R*182FNLQK■187NFVGK▲192	Gel Fractionation (Control)
158-181	159, 162, 175, 181	KT●159GNK▲162DCKADIAFLIDGS●175FNIGQR▲181	Shotgun (Control)
154-162	154, 162	T●154PEKKTGNK▲162	Shotgun (Control)
153-158	153, 157, 158	K■153TPEK●157K▲158	Gel Fractionation (Mutant)
352-362	362	LCTHEQMMCSK*362	Gel Fractionation (Mutant)
158-165	162	KTGNK*162DCK	Gel Fractionation (Mutant)
241-248	248	GGNSNTGK*248	Shotgun (Mutant)
542-552	545	GICR*545DFLESQQ	Gel Fractionation (Mutant)
154-162	154, 162	T●154PEKKTGNK▲162	Shotgun (Mutant)
154-165	158	TPEKK■158TGNKDCK	DIGE
119-129	126	WSASFAVT●126KGK	DIGE
352-362	362	LCTHEQMMCSK*	DIGE
■=Acetyl	▲=Dimethyl	*=Methyl ●=Phospho ♦=Trimethyl	

Table 2: Post-Translational modifications found on cochlin.

We attempted to correlate modifications to important structural features on the cochlin protein. This was done by looking at the structure of cochlin and the known mutation sites, and marking those that were within range to affect each other. We were left with three modifications that occurred near important structural sites of interest.

DISCUSSION

The number of proteins identified in the proteome is reasonable when compared to the number of proteins previously found by 2D DIGE approaches (Chance, 2008). We included "one hit wonder" proteins in our reporting. These are proteins that may have been represented only once in one of the sample repetitions. These are included because they may be important in a pathway, but the sample processing may have limited the amount of a protein we can see in the sample. The lack of representation of the one hit wonders may be due to our sample preparation affecting the protein and we would not want to discard important proteins or peptides due to flaws in our approach. This was also one of the reasons we used two complementary approaches. The shotgun digest and gel fractionation approaches treat the proteins in different ways. If one approach degrades a fragile protein, hopefully the other allows it to remain intact so it can be represented in the proteome. The proteins we expected to see most represented by both samples, cochlin, collagen, and various membrane proteins were highly represented. This is good because it tells us that our approach has not seriously destroyed the proteins. We cannot compare our results against previously published work, because the proteome of the cochlea has not yet been published. Following the completion of the wet lab work for this study, a new paper on how to reduce the remaining detergents in the sample, and therefore have a cleaner LC-MS/MS run, has been published (Wisniewski, 2009). Using this approach in the future and repeating the work done will add validity to our current results.

We looked at the sequence of cochlin after mapping it using Scaffold. This sequence was represented in the samples in a way that was consistent with what is known about cochlin. The N-terminus region was not represented in the mutant, which is consistent because cochlin has several different isoforms, mainly involving parts of the N-terminus being cleaved. In the normal mouse, a greater proportion of the N-terminus was represented, indicating that cochlin appears in normal mice in its more complete form. Neither of our preparations allowed us to see convincing coverage on the N-terminus that we hoped for, and this will hopefully also be increased by a new sample preparation protocol.

The modifications we found on cochlin may be indicative of important structural features. But it is unclear whether the structural features are disrupting the modifications, or the modifications are disrupting the structural features. Regardless, our analysis of cochlin showed several potentially important sites for the structure of the protein. The new sample preparation protocol should also aid with the identification of post-translational modifications as it will reduce the number of modifications resulting from the sample treatment. Enriching the sample for phosphorylation will also be helpful, as there are several known sites of phosphorylation that are thought to be important. A major obstacle to looking at post-translational modifications is that little is known about the importance of the methylation and acetylation modifications, which we found in several of our peptide chains.

A more in depth look at cochlin will help to reveal a lot, as it is so important in the ear, and is found in few other places in the body. Finding the binding partners of cochlin at different stages of degeneration will allow us to determine the environment in the cochlea at different times during the process. The binding partners will also be determined by the sequence of cochlin, and determining the cutting that leads to the sequences present during degeneration could be important in identifying factors in cochlear structural problems occurring during deafness. Using cochlin specific antibodies in an immune-precipitation approach will be a helpful next step, as it will allow us to affirm the cleavage points on the cochlin N-terminus.

The next steps in uncovering Usher syndrome are to do a micro-dissection and look at the protein changes in specific types of cells within the cochlea. Another logical step would be to look at the proteome of the eye during the course of degeneration. The similarities between the ear and the eye proteins will allow us to pinpoint what proteins are most significant in Usher syndrome 1 with a greater degree of confidence.

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APPENDIX I – PROTEOME OF COCHLEA OF AMES WALTZER MOUSE

- 11 kDa protein
- 29 kDa protein
- similar to ribosomal protein S2 isoform 2

Acan Aggrecan core protein
Acatl Acetyl-CoA acetyltransferase, mitochondrial
Aco2 Aconitate hydratase, mitochondrial
Acta1 Actin, alpha skeletal muscle
Actb Actin, cytoplasmic 1
Actn1 Alpha-actinin-1
Actn4 Alpha-actinin-4
Actr2 Actin-related protein 2
Actr3 Actin-related protein 3
Ahcy;ENSMUSG00000048538 Adenosylhomocysteinase
Ahnak AHNAK nucleoprotein isoform 1
Ahsg Alpha-2-HS-glycoprotein
Akr1a4 Alcohol dehydrogenase [NADP+]
Akr1b3 Aldose reductase
Alb Serum albumin
Aldh2 Aldehyde dehydrogenase, mitochondrial
Aldh7a1 aldehyde dehydrogenase 7 family, member A1 isoform a
Aldoa Fructose-bisphosphate aldolase A
Alpl Alkaline phosphatase, tissue-nonspecific isozyme
Anpep Aminopeptidase N
Anxa1 Annexin A1
Anxa2 Annexin A2
Anxa5 Annexin A5
Anxa6 annexin A6 isoform b
Apoa1 apolipoproteinA-1
Apod Apolipoprotein D
Apoe Apolipoprotein E
Ar Androgen receptor
Arhgdia Rho GDP-dissociation inhibitor 1
Arhgdia Rho GDP-dissociation inhibitor 1
Arpc1b Arpc1b protein
Arpc4 Actin-related protein 2/3 complex subunit 4
Atp1a1 Sodium/potassium-transporting ATPase subunit alpha-1
Atp1a2 Sodium/potassium-transporting ATPase subunit alpha-2
Atp1a3 Sodium/potassium-transporting ATPase subunit alpha-3
Atp1b1 Sodium/potassium-transporting ATPase subunit beta-1
Atp1b2 Sodium/potassium-transporting ATPase subunit beta-2
Atp1b3 Sodium/potassium-transporting ATPase subunit beta-3
Atp5a1 ATP synthase subunit alpha, mitochondrial
Atp5b ATP synthase subunit beta, mitochondrial
Atp5c1 ATP synthase subunit gamma, mitochondrial
Atp5d ATP synthase subunit delta, mitochondrial
Atp5f1 ATP synthase subunit b, mitochondrial
Atp5h ATP synthase subunit d, mitochondrial
Atp5o;LOC100047429 ATP synthase subunit O, mitochondrial
Bat1a Spliceosome RNA helicase Bat1
Bcat2 Branched-chain-amino-acid aminotransferase, mitochondrial
Bcat2 Branched-chain-amino-acid aminotransferase, mitochondrial
Bdh1 D-beta-hydroxybutyrate dehydrogenase, mitochondrial
Bgn Biglycan
Calr Calreticulin
Camp Putative uncharacterized protein
Canx Calnexin
Capza2 F-actin-capping protein subunit alpha-2
Capzb Isoform 2 of F-actin-capping protein subunit beta
Car2 Carbonic anhydrase 2
Car3 Carbonic anhydrase 3
Cbr1 Carbonyl reductase [NADPH] 1
Cct2 T-complex protein 1 subunit beta
Cct4 T-complex protein 1 subunit delta
Ceacam16 CEA-related cell adhesion molecule 16
Chi3l3 Chitinase-3-like protein 3
Ckb Creatine kinase B-type
Clic1 Chloride intracellular channel protein 1
Cltc Clathrin heavy chain 1
Cndp2 Cytosolic non-specific dipeptidase
Cnp Isoform CNPI of 2',3'-cyclic-nucleotide 3'-phosphodiesterase
Coch Cochlin
Col1a1 Isoform 1 of Collagen alpha-1(I) chain
Col1a2 Collagen alpha-2(I) chain
Col2a1 Isoform 2 of Collagen alpha-1(II) chain
Col4a2 Collagen alpha-2 (IV) chain
Col6a1 Collagen alpha-1(VI) chain
Col6a2 Collagen alpha-2(VI) chain
Col6a3 Col6a3 protein
Col9a2 Collagen alpha-2 (IX) chain
Col9a3 procollagen, type IX, alpha 3
Coro1a Coronin-1A
Cp Ceruloplasmin
Cs Citrate synthase, mitochondrial
Dars Aspartyl-tRNA synthetase, cytoplasmic
Ddost Dolichyl-diphosphooligosaccharide--protein glycosyltranferase 48 kDa subunit
Decr1 2,4-dienoyl-CoA reductase, mitochondrial
Dld Dihydrolipoyl dehydrogenase, mitochondrial
Dpysl2 Dihydropyrimidinase-related protein 2
Dpysl2 Dihydropyrimidinase-related protein 2

Eef1a1 Elongation factor 1-alpha 1
Eef1a2 Elongation factor 1-alpha 2
Eef1b2 Elongation factor 1-beta
Eef1g Elongation factor 1-gamma
Eef2 Elongation factor 2
EG241053 similar to ribosomal protein L12
EG268795 hypothetical protein isoform 2
EG545121 similar to 40S ribosomal protein S14 isoform 1
Eif4a1 Eukaryotic initiation factor 4A-I
Eif4a2 Isoform 1 of Eukaryotic initiation factor 4A-II
Emilin2 EMILIN-2
ENSMUSG00000072432 similar to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) isoform 1
Epyc Epiphycan
Es1 Liver carboxylesterase N
Espn Isoform 1 of Espin
Etfa Electron transfer flavoprotein subunit alpha, mitochondrial
Etfb Electron transfer flavoprotein subunit beta
Fbxo2 F-box only protein 2
Fga fibrinogen, alpha polypeptide isoform 2
Fgb Fibrinogen beta chain
Flna Isoform 1 of Filamin-A
Fn1 Fibronectin
Fth1 Ferritin heavy chain
Ftl1 Ferritin light chain 1
Gdi2 Isoform 1 of Rab GDP dissociation inhibitor beta
Glud1 Glutamate dehydrogenase 1, mitochondrial
Glul Glutamine synthetase
Gnb1 Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1
Gnb2l1 Guanine nucleotide-binding protein subunit beta-2-like 1
Got1 glutamate oxaloacetate transaminase 1, soluble
Got2 Aspartate aminotransferase, mitochondrial
Gpd2 Glycerol phosphate dehydrogenase 2, mitochondrial
Gpi1 Glucose-6-phosphate isomerase
Gsn Isoform 1 of Gelsolin
Gsta4 Glutathione S-transferase A4
Gstm1 Glutathione S-transferase Mu 1
Gstp2 Glutathione S-transferase P 2
H1f0 Putative uncharacterized protein
H2afj Histone H2A.J
H2afy Isoform 1 of Core histone macro-H2A.1
Hadha Trifunctional enzyme subunit alpha, mitochondrial
Hadhb Trifunctional enzyme subunit beta, mitochondrial
Hba-a2;Hba-a1 hemoglobin alpha, adult chain 2
Hbb-b1 Beta-globin
Hhatl Protein-cysteine N-palmitoyltransferase HHAT-like protein
Hist1h1a Histone H1.1
Hist1h1b Histone H1.5
Hist1h1c Histone H1.2
Hist1h1d Histone H1.3
Hist1h1e Histone H1.4
Hist1h2bh Histone H2B type 1-H
Hist2h2ab;Hist2h2ac Histone H2A type 2-C
Hist2h3c1;Hist2h3b;Hist1h3d;Hist1h3e;Hist1h3c;Hist1h3b;Hist1h3f;
Hist2h3c2 Histone H3.2
Hist3h2a Histone H2A type 3
Hk1 Isoform HK1-SA of Hexokinase-1
Hnrnpa2b1 Isoform 3 of Heterogeneous nuclear ribonucleoproteins A2/B1
Hnrnpa3 Isoform 1 of Heterogeneous nuclear ribonucleoprotein A3
Hnrnpd Isoform 2 of Heterogeneous nuclear ribonucleoprotein D0
Hnrnpf Isoform 1 of Heterogeneous nuclear ribonucleoprotein F
Hnrnpu Putative uncharacterized protein
Hsd17b10 Hydroxysteroid (17-beta) dehydrogenase 10
Hsp90aa1 Heat shock protein HSP 90-alpha
Hsp90ab1 MCG18238
Hsp90b1 Endoplasmin
Hspa12a Heat shock 70 kDa protein 12A
Hspa5 78 kDa glucose-regulated protein
Hspa8 Heat shock cognate 71 kDa protein
Hspa9 Stress-70 protein, mitochondrial
Hspd1 Isoform 1 of 60 kDa heat shock protein, mitochondrial
Hspd1 Isoform 1 of 60 kDa heat shock protein, mitochondrial
Hspg2 perlecan
Ibsp Bone sialoprotein 2
Idh2 Isocitrate dehydrogenase [NADP], mitochondrial
Idh3a Isoform 1 of Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
Impa1 Impa1 protein
Iqgap1 Ras GTPase-activating-like protein IQGAP1
Iqgap2 Ras GTPase-activating-like protein IQGAP2
Kpnb1 Importin subunit beta-1
Krt1 Keratin, type II cytoskeletal 1
Krt10 keratin complex 1, acidic, gene 10
Krt14 Keratin, type I cytoskeletal 14
Krt15 keratin 15
Krt17 Keratin, type I cytoskeletal 17
Krt2 Keratin, type II cytoskeletal 2 epidermal
Krt42 Keratin, type I cytoskeletal 42
Krt5 Keratin, type II cytoskeletal 5
Krt6a Keratin, type II cytoskeletal 6A
Krt72 Keratin, type II cytoskeletal 72
Krt73 Keratin, type II cytoskeletal 73

Krt75 Keratin, type II cytoskeletal 75
Krt78 keratin Kb40
Lamb1-1 laminin B1 subunit 1
Lamb2 Laminin, beta 2, isoform CRA_a
Lamc1 Laminin subunit gamma-1
Lcn2 Neutrophil gelatinase-associated lipocalin
Lcp1 Plastin-2
Ldha L-lactate dehydrogenase A chain
Ldhb L-lactate dehydrogenase B chain
Lgals1 Galectin-1
Lmna Isoform C of Lamin-A/C
Lmnb1 Lamin-B1
LOC100044223;Eno1;EG433182;EG103324 Alpha-enolase
LOC100045958;Pura Transcriptional activator protein Pur-alpha
LOC100046213;Hist1h2bl;Hist1h2bj;Hist1h2bn;Hist1h2bf Histone H2B type 1-F/J/L
LOC100048522 similar to Cofilin-1
LOC675192;EG668182 hypothetical protein
Lrrc59 Leucine-rich repeat-containing protein 59
Lta4h Leukotriene A-4 hydrolase
Ltf Putative uncharacterized protein
Mapk1 Mitogen-activated protein kinase 1
Mbp Isoform 4 of Myelin basic protein
Mdh1 Malate dehydrogenase, cytoplasmic
Mdh2 Malate dehydrogenase, mitochondrial
Mgp Matrix Gla protein
Mpo Myeloperoxidase
Mpz Myelin P0 protein
Msn Moesin
mt-Co2 Cytochrome c oxidase subunit 2
Myh11 Isoform 1 of Myosin-11
Myh9 Myosin-9
Myl6 Isoform Smooth muscle of Myosin light polypeptide 6
Ndp Norrin
Ndufa8 NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8
Ndufs1 NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
Ndufv1 NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial
Nefl Neurofilament light polypeptide
Nefm Neurofilament medium polypeptide
Ngp neutrophilic granule protein
Nid1 Nidogen-1
Oat Ornithine aminotransferase, mitochondrial
Ogdh Isoform 4 of 2-oxoglutarate dehydrogenase E1 component, mitochondrial
OTTMUSG00000000274 similar to ribosomal protein
P4hb Putative uncharacterized protein
Padi2 Protein-arginine deiminase type-2
Pafah1b2 Platelet-activating factor acetylhydrolase IB subunit beta
Pcbp1 Poly(rC)-binding protein 1
Pdhb Pyruvate dehydrogenase E1 component subunit beta, mitochondrial
Pdia3 Protein disulfide-isomerase A3
Pdia4 protein disulfide isomerase associated 4
Pdia6 Putative uncharacterized protein
Pebp1 Phosphatidylethanolamine-binding protein 1
Pfkm 6-phosphofructokinase, muscle type
Pfn1 Profilin-1
Pgam1 Phosphoglycerate mutase 1
Pgd 6-phosphogluconate dehydrogenase, decarboxylating
Pgk1 Phosphoglycerate kinase 1
Pgm1;Pgm2 Phosphoglucomutase-1
Phb Prohibitin
Phb2 Prohibitin-2
Pkm2 Isoform M2 of Pyruvate kinase isozymes M1/M2
Plp1 Isoform 1 of Myelin proteolipid protein
Plxnd1 plexin D1
Pnp1 Purine nucleoside phosphorylase
Ppia Peptidyl-prolyl cis-trans isomerase
Ppib Peptidyl-prolyl cis-trans isomerase B
Prdx1 Peroxiredoxin-1
Prdx2 Peroxiredoxin-2
Prdx5 Isoform Mitochondrial of Peroxiredoxin-5, mitochondrial
Prdx6 Peroxiredoxin-6
Prss2 Anionic trypsin-2
Prx Prx protein
Psat1 Phosphoserine aminotransferase
Ptgds Prostaglandin-H2 D-isomerase
Pygb Glycogen phosphorylase, brain form
Pzp Alpha-2-macroglobulin
Rab10 Ras-related protein Rab-10
Rab14 Ras-related protein Rab-14
Rab5c Ras-related protein Rab-5C
Rab7 Ras-related protein Rab-7a
Rac2 Ras-related C3 botulinum toxin substrate 2
Rph3al Isoform 2 of Rab effector Noc2
Rpl10l 60S ribosomal protein L10-like
Rpl11 60S ribosomal protein L11
Rpl14 60S ribosomal protein L14
Rpl15 60S ribosomal protein L15
Rpl3 60S ribosomal protein L3
Rpl4 60S ribosomal protein L4

Rpl5;OTTMUSG00000022843 60S ribosomal protein L5
Rpl6 60S ribosomal protein L6
Rpl7 60S ribosomal protein L7
Rpl7a;EG666669 60S ribosomal protein L7a
Rplp0 60S acidic ribosomal protein P0
Rpn1 Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1
Rpn2 Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2
Rps3 40S ribosomal protein S3
Rps3a 40S ribosomal protein S3a
Rps4x 40S ribosomal protein S4, X isoform
Rps7 40S ribosomal protein S7
Rpsa;LOC100045332 40S ribosomal protein SA
S100a8 Protein S100-A8
S100a9 Protein S100-A9
S100b Protein S100-B
Sdha Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
Sept7 cell division cycle 10 homolog
Serpina1c Alpha-1-antitrypsin 1-3
Serpina1d Alpha-1-antitrypsin 1-4
Serpinh1 Serpin H1
Skp1a S-phase kinase-associated protein 1
Slc12a2 solute carrier family 12, member 2
Slc25a11 Mitochondrial 2-oxoglutarate/malate carrier protein
Slc25a12 Calcium-binding mitochondrial carrier protein Aralar1
Slc25a3 Phosphate carrier protein, mitochondrial
Slc25a4 ADP/ATP translocase 1
Slc25a5 ADP/ATP translocase 2
Slc3a2 CD98 heavy chain
Slc4a1 Isoform Erythrocyte of Band 3 anion transport protein
Slc4a11 Sodium bicarbonate transporter-like protein 11
Sod1 Superoxide dismutase [Cu-Zn]
Spna2 Isoform 2 of Spectrin alpha chain, brain
Spnb2 Isoform 2 of Spectrin beta chain, brain 1
Spp1 Osteopontin
Ssb Lupus La protein homolog
Taldo1 Transaldolase
Tcp1 T-complex protein 1 subunit alpha A
Tecta Isoform 1 of Alpha-tectorin
Thbs1 Thrombospondin 1
Tkt Transketolase
Tln1 Talin-1
Tpi1 Triosephosphate isomerase
Tpm3 Isoform 2 of Tropomyosin alpha-3 chain
Tpm4 Tropomyosin alpha-4 chain
Trf Serotransferrin
Tuba1a Tubulin alpha-1A chain
Tuba1b Tubulin alpha-1B chain
Tuba4a Tubulin alpha-4A chain
Tubb2c Tubulin beta-2C chain
Tubb5 Tubulin beta-5 chain
Uba1 Ubiquitin-like modifier-activating enzyme 1
Uba52;Ubc;Ubb;OTTMUSG00000004411;2810422J05Rik hypothetical protein LOC666586
Uqcrc1 Cytochrome b-c1 complex subunit 1, mitochondrial
Uqcrc2 Cytochrome b-c1 complex subunit 2, mitochondrial
Vcl Vinculin
Vcp Transitional endoplasmic reticulum ATPase
Vdac1 Isoform Pl-VDAC1 of Voltage-dependent anion-selective channel protein 1
Vdac2 Voltage-dependent anion-selective channel protein 2
Vdac3 Voltage-dependent anion channel 3
Vim Vimentin
Ywhab Isoform Long of 14-3-3 protein beta/alpha
Ywhae 14-3-3 protein epsilon
Ywhag 14-3-3 protein gamma
Ywhaq Isoform 1 of 14-3-3 protein theta
Ywhaz 14-3-3 protein zeta/delta