The genetic dissection of Myo7a gene expression in the retinas of BXD mice

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Purpose: Usher syndrome (US) is characterized by a loss of vision due to retinitis pigmentosa (RP) and deafness. US has three clinical subtypes, but even within each subtype, the severity varies. Myosin VIIA, coded by Myo7a, has been identified as one of the causal genes of US. This study aims to identify pathways and other genes through which Myo7a interacts to affect the presentation of US symptoms.

Methods: In this study, we used the retinal tissue of BXD recombinant inbred (RI) mice to examine the expression of Myo7a and perform genetic mapping. Expression quantitative trait locus (eQTL), single nucleotide polymorphism (SNP), and gene correlation analysis were performed using GeneNetwork. Gene set enrichment analysis was performed using WebGestalt, and gene network construction was performed using the Gene Cohesion Analysis Tool.

Results: We found Myo7a to be cis-regulated, with varied levels of expression across BXD strains. Here, we propose a genetic network with 40 genes whose expression is highly correlated with Myo7a. Among these genes, six have been linked to retinal diseases, three to deafness, and five share a transcription factor with Myo7a. Gene ontology and pathway analysis revealed a strong connection among ion channel activity, Myo7a, and US.

Conclusions: Although Myo7a is a causal gene of US type I, this gene works with many other genes and pathways to affect the severity of US. Many of the genes found in the genetic network, pathways, and gene ontology categories of Myo7a are related to either deafness or blindness. Further investigation is needed to examine the specific relationships between these genes, which may assist in the treatment of US.

The human unconventional myosin VIIa (MYO7A, Gene ID 4647, OMIM 276903) gene is located on chromosome 11q13.5 and encodes the protein myosin VIIA, which is part of the unconventional myosins group. These proteins interact with actin and facilitate molecular transport within cells. Myosin VIIA is primarily expressed in the cochlear and vestibular neuroepithelia of the inner ear and in the retinal pigment epithelium (RPE) and photoreceptors. In the retina, myosin VIIA supports and maintains nourishment of the RPE. Myosin VIIA normally carries small sacs of pigment called melanosomes within the RPE, which are necessary for normal vision. Myosin VIIA is also found in other parts of the retina, where this protein most likely carries proteins and molecules important to vision, and is involved in the phagocytosis of disk membranes involved in normal sight [1]. Mutations in Myo7a have been known to cause Usher syndrome (US), a disorder affecting the ear and the retina.

US is an autosomal recessive genetic disorder that causes loss in vision, hearing, and vestibular function. Patients with US often develop inner ear defects and degeneration of retinal cells (retinitis pigmentosa, or RP). Combined, these symptoms lead to complete or partial loss of sight or hearing or both. The expression of US is highly variable in different individuals. The clinical and genetic heterogeneity of US shows that this trait is complex, with primary causal genes interacting with other coexpression genes to produce the clinical presentation. Globally, mutations in MYO7A are the most common cause of US type 1, accounting for anywhere between 29% and 75% of cases [2-8]. These mutations may also account for some cases of type 2 [7,9] and the atypical type 3 [10]. Even within type 1, there are wide phenotypic variations, and there has been much hypothesizing on the genotype–phenotype relationships between the various mutations in MYO7A and the diversity of clinical presentations. To date, our understanding of why such wide heterogeneity exists in US cases remains poor, although there is some evidence that null mutations may
result in milder phenotypes [11]. Mutations in MYO7A almost always present as autosomal recessive, with either true homozygotes or compound heterozygotes being affected with the disease. However, there are specific instances of autosomal dominant inheritance where interaction with the wild-type protein produces a dominant-negative effect. In addition, there is now evidence that other genetic factors may affect the penetrance of mutations in MYO7A [12,13], and this may account for some of the observed variability in the phenotype. Recombinant inbred (RI) mice are a powerful tool for systems genetics analysis. The current largest panel of these strains—the BXD family—consists of more than 100 strains derived from a cross between the C57BL/6J strain (B6), which has no aberrant ocular phenotype, and the DBA/2J strain (D2), which harbors mutations in several genes that result in some aberrant ocular phenotypes. For example, after 6 months of age, most D2 mice develop iris depigmentation and increased intraocular pressure (IOP) followed by damage to the optic nerve [14]. Mutations in two genes are already known to contribute to pigment dispersion syndrome (PDS) and pigment glaucoma (PG) in the D2 mouse: tyrosinase-related protein 1 gene (Tyrp1), which causes iris stromal atrophy, and glycoprotein neuromedin B-associated gene (Gpnmb), which is associated with pigment dispersion [15]. The mutations in Tyrp1 and Gpnmb and the phenotypes of PDS and PG from D2 mice segregate among BXD progeny. The BXD strains that harbor mutations in Tyrp1 and Gpnmb, such as BXD55, BXD62, BXD66, etc., develop iris depigmentation [16]. Jablonski’s group identified a gene network and modifiers of Tyrp1 and Gpnmb that contribute to phenotypes of PDS and PG using BXD mice and a systems genetics method [16-18]. Currently, BXD lines have been used extensively in genetic and genomic studies of the eye and central visual system to identify the genetic basis of ocular phenotypes, including molecular expression phenotypes and the regulating gene, in our case, the regulation of Myo7a expression [17-20].

Mice with a Myo7a mutation are deaf and have abnormalities in their RPE [21,22]. There is a Myo7a mutation in one BXD parental strain (the D2 mouse), and there is no mutation in the other BXD parental strain (the B6 mouse). We found the expression of Myo7a in the retina has significant differences between D2 and B6 mice, which provides an excellent opportunity to study the mechanism through which Myo7a causes US by using retina expression data of the BXD population. The purpose of this investigation was to combine the power of BXD RI strains of mice with systems genetics approaches using retinal tissue to identify a genetic network for Myo7a, determine the genetic regulation of Myo7a, and explore genes functionally related to Myo7a and pathways through which MYO7A induces US.

METHODS

Mice: A total of 222 mice from 55 strains (52 BXD strains, two parental strains, and their F1 hybrid strain; Appendix 1) were used for this study. During strain selection, we excluded BXD strains that carry the Tyrp1b mutation and the GpnmbR150X mutation, which result in pigment dispersion glaucoma. We also excluded BXD strains that carry the rd16 mutation and strains that have abnormally high Gfap levels previously observed in our retina expression data. Almost all animals were young adults between 60 and 100 days of age balanced for sex. Animals were weaned at 25 days of age and housed in same-sex cages with two to five mice per cage until the day of euthanasia. Animals had free access to standard laboratory chow and water and were maintained on a 12 h:12 h light-dark cycle. Room temperature ranged from 20 to 24 °C.

All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Tennessee Health Science Center (Memphis, TN) and Emory University (Atlanta, GA). Mice were handled in a manner consistent with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, the Public Health Service Policy on Humane Care and Use of Laboratory Animals).

Tissue and sample processing: The retinal tissue and RNA were prepared according to our published protocols [23]. Mice were euthanized with rapid cervical dislocation. Two retinas per mouse were immediately removed and immersed in 1 ml of 160 U/ml RiboLock for 1 min at room temperature and then transferred to Hank’s Balanced Salt solution with RiboLock in 50 µl RiboLock (RiboLock RNase #EO0381 40 U/µl 2500 U, Thermo Fisher Scientific, Waltham, MA). The RNA was isolated using a QiaCube (QiaGen, Hilden, Germany) and the in-column DNase procedure. The quality and purity of RNA were checked using an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) system to assess the relative quantities of 18S and 28S RNA, as well as the RNA integrity. The gene expression data were collected using the Affymetrix Mouse Gene 2.0 ST array based on the manufacturer’s protocol (Thermo Fisher Scientific). This array was designed with a median of 22 unique probes per transcript that measure gene expression at almost every exon and 3′-untranslated region (UTR) and provide comprehensive transcriptome coverage with more than 30,000 coding and non-coding transcripts.
**Quantitative RT–PCR.** The expression of Myo7a in the retina of the wild-type and mutated BXD mice was verified using quantitative reverse transcription-PCR (qRT-PCR). We performed qRT-PCR as previously described [24]. Briefly, total RNA was extracted from the retinas of the B6, D2, and eight BXD strains (four strains with wild-type and four strains with a mutation at the Myo7a gene). Five samples were collected from the B6 mice, five samples were collected from the D2 mice, and three samples were collected from each BXD strain. The total RNA from each individual sample was transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA). Next, a 10-µl PCR reaction mixture was prepared by mixing 4.5 µl of diluted cDNA, 0.25 µl of 10 µM forward primer, 0.25 µl of 10 µM reverse primer and 5 µl 2 × SYBR Green PCR Master Mix (Life Technologies). Quantitative PCR was performed using a StepOne PCR system (Life Technologies) with the following program: 95 °C for 10 min, 40 cycles of 95 °C for 15 s followed by 60 °C for 1 min. At the completion of cycling, melting curve analysis was performed to establish the specificity of the PCR product: 95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s. *Hprt* (Gene ID 15452, OMIM 308000), a housekeeping gene, was used as an internal control. The primer sequences for mouse transcripts were as follows: *Hprt*, Forward, 5′-GAA AGA CTG GCT CGA GAT GTC ATG-3′; reverse 5′-CAC ACA GAG GGC CAC AAT GT-3′; *Myo7a* forward 5′-CCG CCC AGC AAC ATC CT-3′; reverse 5′-CTT GCG GGA CTG CAG AAA C-3′. The relative expression of the *Myo7a* gene was analyzed with the ΔΔCT method with *Hprt* used as the reference gene for normalization as previously described [24]. Briefly, the threshold cycle (CT) values for *Myo7a* gene was determined by automated threshold analysis using a StepOne Real-Time PCR System and normalized to the CT value of the reference gene *Hprt* to obtain ΔCT (*Myo7a* gene) = CT(*Myo7a* gene)− CT(*Hprt* gene). Then *Myo7a* gene expression in each mouse strain relative to that in a reference sample C57BL/6J was calculated as 2−ΔΔCT=2−[ΔCT(*Myo7a* gene in each mouse strain)−ΔCT(*Myo7a* gene in C57BL/6J)].

**Data processing:** Affymetrix CEL files were processed using the Robust Multichip Array (RMA) method and the default set in Affymetrix Expression Console Software [25]. We normalized the RMA expression data using modified Z-scores as previously described [26]. Briefly, we calculated the log base 2 of the RMA values, computed the Z scores for these RMA values for each array, multiplied the Z scores by 2, and added an offset of 8 units to each value. This transformation resulted in a set of Z-like scores for each array with a mean of 8, a variance of 4, and a standard deviation of 2. The advantage of this modified Z score is that a twofold difference in expression corresponds approximately to 1 unit. Finally, we computed the arithmetic mean of the values for the set of microarrays for each strain (an average of four samples per strain), and uploaded this data set into our GeneNetwork (GN) website for future analysis and public access (DoD CDMRP Retina Affy MoGene 2.0 ST (May15) RMA Gene Level).

**Heritability calculations:** The heritability of the expression level of *Myo7a* was calculated using broad sense heritability that compares the genetic variation between strains to the environmental variance within the strains [27]. The formula used for the heritability calculation was 0.5 Vg / (0.5 Vg + Ve), where Vg is the genetic variance (variances of the strain means), and Ve is the environmental variance. The factor of 0.5 in this ratio was applied to adjust for the twofold increase in the additive genetic variance among the inbred strains relative to the outbred populations [20].

**eQTL mapping and SNP analysis:** Expression quantitative trait locus (eQTL) mapping was performed using the WebQTL module on GeneNetwork using our published methods [23, 26,28]. This methodology uses regression analysis to determine the relationship between differences in a trait and differences in alleles at markers across the genome. Simple interval mapping was performed to identify potential eQTLs that regulate *Myo7a* expression levels and estimate the significance at each location using known genotype data for those sites. Composite interval mapping was also performed to control for genetic variance associated with major eQTLs and therefore identify any secondary eQTLs that may have been otherwise masked. Each analysis produced a likelihood ratio statistic (LRS) score, providing us with a quantitative measure of confidence of linkage between the observed phenotype—in this case, variation in expression level of *Myo7a*—and known genetic markers. The genome-wide significance for each eQTL was established using a permutation test that compared the LRS of our novel site with the LRS values for 1,000–10,000 genetic permutations [29]. Sequence variability between B6 and D2 was determined using the single nucleotide polymorphism (SNP) variant browser link on GeneNetwork.

**Genetic correlation and partial correlation analysis:** We computed the Pearson product–moment correlations of the strain means between the expression of *Myo7a* and the expression of all the other probe sets across the mouse genome to produce sets of genetically correlated genes. To identify true biologic correlates of *Myo7a*, we also performed partial correlation analyses to remove linkage disequilibrium by controlling genetic variation near *Myo7a* (locus rs8281437 at 105.2 Mb of chromosome 7). Then we performed a literature correlation to rank the list of genes using the Semantic Gene Organizer (SGO) software that automatically extracts...
gene–gene relations from titles and abstracts in MEDLINE citations [30]. All genetic correlations, partial correlations, and literature correlations were computed using the tools on GeneNetwork.

**Gene set enrichment analysis and gene network construction:**
Genes with a statistically significant genetic correlation and a partial correlation with Myo7a were selected and uploaded to Webgestalt for gene enrichment analysis [31]. The p values generated from the hypergeometric test were automatically adjusted to account for multiple comparisons using the Benjamini and Hochberg correction [32]. The categories with an adjusted p value (adj P) of less than 0.05 indicated that the set of submitted genes is statistically significantly over-represented in those categories.

The gene network was constructed and visualized using the Cytoscape utility through the Gene-set Cohesion Analysis Tool (GCAT). Nodes in the network represent genes, and edges between two nodes represent the cosine score of latent semantic indexing (LSI) that determines the functional coherence of gene sets is larger than 0.6. The significance of the functional coherence is evaluated by the observed number of gene relationships above a cosine threshold of 0.6 in the LSI model. The literature p value (LP) is calculated using Fisher’s exact test by comparing the cohesion of the given gene set to a random gene set [30].

**RESULTS**

**Myo7a expression levels in the retinas of B6, D2, and BXD mice, and heritability:**
There was a high level of variation in the expression level of Myo7a among the BXD strains. The average expression level of Myo7a in all BXD strains was 8.69±0.09 (mean ± SE). The range included a high of 9.63±0.06 for BXD6 and a low of 7.69±0.02 for BXD84 (Figure 1), a more than 3.8-fold change among the BXD strains. In the B6 parental strain, Myo7a had an average expression level of 9.22±0.02. In the D2 parental strain, Myo7a had an average expression level of 7.92±0.09. There was statistically significant difference in the expression of Myo7a between B6 and D2 (p<0.01). Similarly, BXD lines with the B6 allele at the Myo7a locus had higher Myo7a expression levels while those with the D2 allele at the Myo7a locus had lower expression levels (9.12±0.38 and 7.92±0.31, respectively; p<0.0001; Figure 2). To validate Myo7a expression from the array, we performed quantitative RT–PCR for the B6, D2, and 8 BXD strains (four strains with wild-type (BXD16, 43, 65b, and 29) and four strains with a mutation in the Myo7a gene (BXD86, 60, 87, and 09)). This analysis confirmed the statistically significant difference in the expression of Myo7a between the B6 and D2 mice and between the B6 allele and the D2 allele at the Myo7a locus among the BXD strains (p<0.0001; Figure 3). The heritability of Myo7a expression levels was 0.83, which suggested that a genetic factor is a major contributor to variation in expression. This heritable variation enables us to identify a genetic locus that controls the expression levels of Myo7a in BXD mice.

**eQTL mapping and sequence variants of Myo7a:** Simple interval mapping for Myo7a revealed a highly significant eQTL with an LRS of 86.1 at chromosome 7, the location of the gene itself (Figure 4). Composite interval mapping revealed no secondary loci that modulate Myo7a expression levels. Thus, Myo7a is defined as cis-regulated eQTL, suggesting the variation in the transcript expression levels is caused by sequence variants in or near Myo7a. We found a total of 764 SNPs in Myo7a between the BXD parental strains using new open access sequence data resources at GeneNetwork, which include all of the current available sequence variances of BXD mice. Most of those SNPs are located in introns. Three are located in the 3′-UTR or 5′-UTR. Thirty-four SNPs are located at coding areas, three of which are non-synonymous SNPs (Table 1). All SNPs located at the UTR and coding areas are listed in Appendix 2.

**Gene function enrichment:**
The 1,569 probe sets found to correlate significantly with Myo7a expression after genetic and partial correlations (p<0.05) were uploaded to Webgestalt. Among them, 495 probe sets were mapped to multiple Entrez gene ID or could not be mapped to any Entrez gene ID, resulting in 1,074 probe sets that can unambiguously map to 1,048 unique Entrez gene IDs. These probe sets were then used for enrichment analysis. The most significant enrichments in the molecular function category are “cation channel activity,” “metal ion transmembrane transporter activity,” “voltage-gated channel activity,” and “gated channel activity” (Appendix 3). The most significant enrichments in the biologic process category are “ion transmembrane transport,” “ion transport,” “membrane repolarization,” and “regulation of ion transmembrane transport” (Appendix 4). The most significant enrichments in the cellular component category are “axon part,” “cell periphery,” “plasma membrane,” “dendrite,” and “neuron projection” (Appendix 5). Gene pathway analysis showed that the genes are significantly enriched in “Calcium signaling,” “Neuroactive ligand-receptor interaction,” “Chemokine signaling,” and “Axon guidance” pathways (Table 2).

**Genetic network:** The strength of correlation among genes with which Myo7a is correlated can be evaluated using a coexpression network. To identify known biologic relations among coexpressed genes, we performed a literature correlation analysis after the genetic and partial correlation analysis.
Figure 1. Expression of *Myo7a* in the retina for the B6 and D2 parental strains, F1 hybrids, and 52 BXD lines. Expression for each strain (four mice per strain) was generated using the RMA method and the Affymetrix Expression Console Software and normalized using modified Z-scores. The x-axis denotes the strain while the y-axis denotes the mean expression given in a log2 scale. Each bar shows the mean expression values ± standard error of the mean (SEM).

Figure 2. The mean expression of *Myo7a* of BXD strains with the B allele and the D allele at the *Myo7a* locus. Among the 55 strains used, 33 have the B allele at the *Myo7a* locus, 19 have the D allele, and three are heterozygous. The values on the y-axis denote normalized expression levels on a log2 scale. There is a statistically significant difference in the expression of *Myo7a* among the strains carrying the B versus D alleles (p<0.0001).
The top 50 genes (literature correlation coefficient >0.37) were uploaded to GCAT for functional coherence analysis and gene network construction. Five genes could not be found in the GCAT database, four genes had no functional relationship with the other genes in the network, and one gene was a Riken clone. These ten genes were excluded from the gene coherence analysis. The remaining 40 genes (Appendix 6) showed significant functional cohesion with a literature p value of 1.693507e-17 (Figure 5). Multiple resources, including Chillibot, GeneCards, and PubMed, were used to determine

Figure 3. mRNA expression of Myo7a in the retina for the B6 and D2 strains and eight selected BXD lines. The x-axis denotes the strain while the y-axis denotes the fold change in the Myo7a gene for the nine strains relative to the B6 strain using the ΔΔcT method. Each bar shows the mean relative expression values ± standard error of the mean (SEM). n=3–5; p<0.0001.

Figure 4. Genetic mapping of Myo7a. A statistically significant expression quantitative trait locus (eQTL) is present at the location of Myo7a itself (the triangle on the x-axis) on chromosome 7, making it a cis-eQTL. The left y-axis provides the likelihood ratio statistic (LRS) score in blue, and the right y-axis provides the additive effect. The red and green lines show the effect of the D or B allele on trait values, respectively. The upper x-axis shows the location by chromosome, and the lower x-axis shows the location in megabases. The two horizontal lines across the plot make the threshold for genome-wide statistically significant (p<0.05, red or upper line) and suggestive (p<0.63, gray or lower line) thresholds.

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whether members of the Myo7a coexpression network had been previously associated with retinitis pigmentosa or deafness, the main symptoms of US. In addition to Myo7a, some genes in this network (Fzd4, Sema4a, Trex1, Sumf1, Trim32, and Ccde28b) are already known to be related to retinitis pigmentosa or other retinal diseases. Several genes (Fgf3, Psap, and Ank1) are related to deafness. Furthermore, some genes in the network (Ank1, Ddr1, Neb, Numbl, and Sema4a) share the same transcription factor (AP4) with Myo7a.

**DISCUSSION**

We analyzed the differential expression of Myo7a in the retinal tissue of BXD RI mice and found Myo7a to be cis-regulated, indicating that it is regulated by itself or genes close to it. We present a genetic network for Myo7a that

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**Table 1. Nonsynonymous SNPs that are located within Myo7a gene.**

| SNP ID          | Chr | Mb   | Alleles | Domain | Function     | B6 | D2 |
|-----------------|-----|------|---------|--------|--------------|----|----|
| wt37–7-105239564 | 7   | 105.2396 | T/C     | Exon 14 | Nonsynonymous | T  | C  |
| wt37–7-105246235 | 7   | 105.2462  | C/T     | Exon 6  | Nonsynonymous | C  | T  |
| wt37–7-105255574 | 7   | 105.2556  | A/G     | Exon 3  | Nonsynonymous | A  | G  |

**Table 2. The significant enriched KEGG pathway.**

| Pathway Name                                      | N of genes | adjusted P Value |
|---------------------------------------------------|------------|------------------|
| Calcium signaling pathway                         | 20         | 0.0092           |
| Neuroactive ligand-receptor interaction            | 26         | 0.0129           |
| Chemokine signaling pathway                       | 19         | 0.0129           |
| Axon guidance                                     | 14         | 0.0387           |

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Figure 5. Myo7a gene network graph created using the Gene-set Cohesion Analysis Tool described in the Methods section. These genes may be functionally related. Gene symbols are located at nodes in circles, and the lines interconnecting the nodes are based on literature correlations. The literature p value for these genes is 1.693507e-17. This computation was based on the 40 terms that most frequently co-occur with Myo7a.
involves genes that we found were related to retinal diseases and deafness via literature reports. Finally, we present gene enrichment categories and pathways through which Myo7a and its genetic network interact to affect US phenotypes.

We created a genetic network using the top 40 correlated genes with Myo7a. In this network, Fzd4, Sema4a, Trex1, Sumf1, Trim32, and Ccdc28b are known to be associated with retinal diseases. We propose that these genes could interact with Myo7a to affect the severity of US.

Retinitis pigmentosa is a highly heterogeneous disease characterized by degeneration of rod photoreceptors and often presents with night blindness, progressive peripheral visual field loss, and blindness [33]. US and Bardet-Biedl syndrome present with retinitis pigmentosa. US and Bardet-Biedl syndrome account for 1,200 pathogenic mutations out of 3,100 mutations in more than 50 genes known to cause non-syndromic RP [34]. From the gene network we constructed, Trim32 and Ccdc28B are key players in Bardet-Biedl syndrome and may also play a part in the presentation of the severity of RP in US. Tripartite motif-containing protein 32 (Trim32) is known to cause Bardet-Biedl syndrome, an autosomal recessive disorder characterized by vision loss due to retinal tissue degeneration, obesity, polydactyly, and mental retardation [35]. Coiled-coil domain-containing protein 28B (Ccdc28B) colocalizes and interacts with several proteins involved with Bardet-Biedl syndrome [36]. Ccdc28B is a modifier gene, meaning that a hypomorphic mutation in Ccdc28B affects the expressivity of Bardet-Biedl syndrome, but in itself the gene is not a cause of the syndrome [37].

Semaphorin-4a (Sema4a) is a transmembrane protein that plays a role in retinal development [36,38]. Sema4a participates in cell–cell communication in the immune system and is present in the brain and eye. It is mainly found in the RPE and the inner retina when the photoreceptor outer segments lengthen. Mice with Sema4a deficiency have been shown to have compromised function of rod and cone photoreceptors, as well as severe retinal degeneration, thinner retinal vessels, and depigmentation using fundus photography [39]. Sema4a is also implicated in cone rod dystrophy [40].

Three more genes from the network (Fzd4, Trex1, and Sumf1) are also reported to be involved in retinal diseases. Frizzled-4, or Fzd4, has been linked to familial exudative vitreoretinopathy (FEVR) which, similar to retinitis pigmentosa, is a hereditary vitreoretinal disorder that affects the blood vessels in the eye and can lead to blindness [41,42].

Three prime repair exonuclease 1 (Trex1) is a 3’-5’ exonuclease that affects the vasculature of the retina. Retinal vasculopathy with cerebral leukodystrophy (RVCL) is a genetic condition affecting the central nervous system that leads to loss of vision, strokes, and dementia, and is caused by mutations in the TREX1 (Gene ID 11277, OMIM 606609) gene [43]. Ophthalmologic findings show capillary dropouts leading to loss of central vision, prominent juxtafoveolar capillary obliteration, and telangiectasis, as well as transient ischemic strokes, cognitive dysfunction, and personality disorders [44].

Mutations in sulfatase-modifying factor 1 (Sumf1) are the primary cause of multiple sulfatase deficiency (MSD), which is a metabolic abnormality featuring a reduction in several sulfatase functions. MSD is often characterized by neurodegeneration, coarse facial features, nystagmus, and neuropathy [45]. MSD also often occurs with eye abnormalities, such as retinal degeneration [46]. Furthermore, SUMF1 (Gene ID 285362, OMIM 607939) activates arylsulfatase I (ARSI; Gene ID 340075, OMIM 610009), which is preferentially expressed in the human RPE cell line ARPE-19 and is a candidate gene for affecting inherited eye diseases [47].

All of these genes are associated with retinal pathology and are good candidates for explaining the variety of ocular symptoms not only between US subtypes but also within each type. However, further investigation is needed to elucidate the relationship between each gene and Myo7a.

Although other genes in the Myo7a gene network have not been reported to be involved in retinal diseases thus far, they could be novel genes that work with Myo7a to affect symptom severity of US, especially genes that have been regulated by the same transcription factor as Myo7a. The transcription binding site analysis showed AP4 to regulate Myo7a along with Ank1, Ddr1, Neb, Numbl, and Sema4a.

Replication initiator 1 (Repin1), also known as AP4, is a transcription factor that binds the promoter sites of Myo7a, Ddr1, Nebl, Sema4a, Neb, and Ank1. As discussed earlier, Sema4a is related to retinal development, and defects in Ankyrin 1 (Ank1) are implicated in hearing loss [47,48]. Discoidin domain receptor family, member 1 (Ddr1) is involved in the regulation of cell growth, differentiation, and metabolism. Deletion of the Ddr1 gene is associated with a severe decrease in auditory function, and Ddr1 mutant mice have alterations in the organ of Corti [49]. Ddr1 also lies near several deafness loci linked to autosomal recessive or dominant non-syndromic hearing loss [50,51]. Numb-like protein, or Nebl, is expressed in the rat auditory sensory epithelium [52]. Nebulin, or Neb, is an actin-binding protein that promotes the contractile function of sarcomeres. A nonsynonymous variant of Neb may be involved in glaucoma, characterized by degeneration of retinal ganglion cells, in basset hounds [53]. Because Repin1 is the transcription factor for Repin1, it could be a key regulator of retinal diseases.
for these genes, each of which is related to either the retina or hearing, it may also play a role in deaf-blindness, although there are not many previous studies linking Repin1 to either symptom.

Gene enrichment analysis was used to identify gene ontology (GO) categories in which Myo7a and its correlated genes were over-represented and the pathways through which they interact. Many of the enrichment categories in molecular function and biologic process that were over-represented were related to transmembrane ion channel activity, and the most significant enrichments in cellular components were neuron-specific. Furthermore, one pathway revealed to be significant in the present pathway analysis was “neuroactive ligand-receptor interaction” and “axon guidance.” This is not surprising, because hearing involves transmitting sound vibrations to the cochlear and vestibular labyrinths, where mechanoelectrical transduction carries neural signals for integration, processing, and feedback. Myo7a is responsible for unconventional myosin found in stereocilia. Stereocilia contain tip links, thought to be mechanical springs needed to open the ion channels leading to depolarization [54]. Single hair cell measurements of mice with mutations in Myo7a have shown that when the stereocilia are not stimulated by sound, the gating springs are slack and transduction channels closed, as opposed to a maintained tension on the gating springs that keep some of the channels open even at rest in normal hair cells. Transducer currents can be measured in these mutant mice, which reflects cation flow into hair cells through transduction channels, but only with strong deflection of stereocilia bundles [55]. Myo7a is heavily implicated in the hair cells and transduction channels in the ear.

Gene pathway analysis further revealed “calcium signaling” to be a significant pathway in Myo7a interactions, supported by our GO analysis category “cation channel activity.” Myo7a has been reported to be a Ca²⁺/calmodulin-regulated gene and has altered expression when Ca²⁺ homeostasis was altered. Since Myo7a is expressed in rods and cones, changes in its expression may reflect differences in Ca²⁺ homeostasis in rods versus cones [56]. Although the exact nature of how Myo7a interacts with calcium signaling is still not clear, our gene ontology results support our pathway findings.

These gene ontology and pathways presented imply that many of Myo7a’s interactions with correlated genes are involved in ion channel activity, as well as neurons. Further investigation is needed to elucidate the exact nature of these relationships, and how Myo7a, its genetic network, and pathways all interact to affect the severity of expression of US. Conclusion: The expression of US is highly variable, and symptoms that manifest may differ in severity, even within the same US category. Myo7a is a causal gene of US type I, but many other factors interact with this gene to modify disease severity. Here, we propose a gene network, gene ontology categories, and pathways that interact with Myo7a. We found several genes in the genetic network to be related to deafness and blindness, and several others shared the same transcript factor with Myo7a. We further examined these relationships using gene ontology and pathway analysis and found that many of these genes interact with ion channel activity and neuronal activity, which is crucial in vision and hearing. Further investigation is needed to elucidate the nature and mechanisms of these relationships, which may assist in the treatment of symptoms of US.

APPENDIX 1. THE LIST OF STRAINS WITH LOG2 TRANSFERRED EXPRESSION VALUE OF MYO7A IN RETINA.

To access the data, click or select the words “Appendix 1”

APPENDIX 2. SNPS THAT ARE LOCATED UTR AND CODING REGIONS WITHIN MYO7A GENE.

To access the data, click or select the words “Appendix 2”

APPENDIX 3. THE SIGNIFICANT ENRICHMENTS IN MOLECULAR FUNCTION CATEGORY.

To access the data, click or select the words “Appendix 3”

APPENDIX 4. THE SIGNIFICANT ENRICHMENTS IN BIOLOGICAL PROCESS CATEGORY.

To access the data, click or select the words “Appendix 4”

APPENDIX 5. THE SIGNIFICANT ENRICHMENTS IN CELLULAR COMPONENT CATEGORY.

To access the data, click or select the words “Appendix 5”

APPENDIX 6. THE LIST OF GENES THAT ARE USED TO CONSTRUCT MYO7A GENE NETWORK.

To access the data, click or select the words “Appendix 6”

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