

# Novel compound heterozygous nonsense variants, p.L150\* and p.Y3565\*, of the *USH2A* gene in a Chinese pedigree are associated with Usher syndrome type IIA

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**Abstract.** Usher syndrome refers to a group of genetically and clinically heterogeneous autosomal recessive diseases with retinitis pigmentosa (RP) and hearing deficiencies. The association between Usher syndrome-causative genes and resultant Usher syndrome phenotypes in patients are highly variable. In the present study, a Chinese family with Usher syndrome was recruited, and targeted next-generation sequencing, Sanger sequencing and segregation analysis were performed. The expression profiles and functional effects of the pathogenic variants of *USH2A* identified were analyzed. Novel nonsense compound heterozygous variants, c.T449G (p.L150\*) and c.T10695A (p.Y3565\*), were identified in the *USH2A* gene, which showed co-segregation with the disease phenotype causing Usher syndrome type IIA in the recruited Chinese pedigree. The p.L150\* variant was predicted to produce a truncated protein which lacked almost all the functional domains of *USH2A*, whereas the p.Y3565\* variant is located in one of the fibronectin type 3 domains, resulting in the loss of several fibronectin type 3 domains at the C-terminus of *USH2A* by producing the truncated protein. It was shown that *Ush2a*

mRNA expression levels were higher in the retina compared with those in the eye tissues (lens, sclera and cornea), uterus, ovary, breast, testis, spleen, kidney, liver, intestine, brain, skeletal muscle and blood. Additionally, the protein structure was shown to be highly conserved by comparing *Homo sapiens* *USH2A* to eight other species. To the best of our knowledge, the present study is the first to identify two novel pathogenic variants, c.T449G (p.L150\*) and c.T10695A (p.Y3565\*), in the *USH2A* gene in a patient with Usher syndrome type IIA, thereby expanding the known spectrums of *USH2A* causative mutations. The present discovery may assist in understanding the molecular pathogenesis underlying the development of RP and Usher syndrome type IIA, and in the development of diagnostic, therapeutic and genetic counseling strategies in patients with Usher syndrome type IIA disease.

## Introduction

Usher syndrome is a group of genetically and clinically heterogeneous autosomal recessive diseases with progressive retinitis pigmentosa (RP) and sensorineural hearing deficiencies, including Usher syndrome types I, II and III (1–4). Patients with Usher syndrome type II (*USH2*) present with mild hearing impairments but normal vestibular responses, and type II is the most common type amongst the three types (1). Usher syndrome type II is genetically heterogeneous, including *USH2A*, *USH2C* and *USH2D*. Usher syndrome type IIA (*USH2A* locus) (OMIM, 276901) is the result of mutations of the *USH2A* gene (OMIM, 608400) (5), whereas *USH2C* (OMIM, 605472) is the result of mutations of the *ADGRV1* gene (OMIM, 602851) (1,6) or by digenic mutations of both *ADGRV1* and *PDZD7* genes (OMIM, 612971) (7) and *USH2D* (OMIM, 611383) can be caused by mutations of the whirlin (*WHRN*) gene (OMIM, 607928) (8). As Hmani-Aifa *et al* (9) identified mutations of the adhesion G-protein coupled receptor V1 gene in an Usher II syndrome pedigree which had been previously mapped to the *USH2B* locus of chromosome 3p23-p24.2, the designation for the *USH2B* locus was withdrawn.

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Aliases for the *USH2A* gene include *Usherin*, *USH2*, *US2*, *Usher Syndrome 2A*, *Usher Syndrome Type-2A Protein*, *Usher Syndrome Type Iia Protein*, *DJ1111A8.1* and *RP39*. This gene maps to chromosome 1q41, encoding a protein 5,202 amino acids in length that is comprised of a pentaxin motif, laminin epidermal growth factor (EGF) motifs and numerous fibronectin type III domains (10). Different isoforms have also been identified (5,10-12). The protein is localized in the basement membrane and serves a vital role in the development and homeostasis of the inner ear and retina (2). Mutations of the *USH2A* gene have been identified in patients with Usher syndrome type IIA and non-syndromic RP (13). Eudy *et al* (5) was the first to identify *USH2A* mutations among patients with Usher syndrome type IIA.

The association between the variants in the Usher syndrome-causative genes and the resultant Usher syndrome phenotypes in patients are highly variable (1,3,14). To the best of our knowledge, novel *USH2A* mutations in patients with Usher syndrome type IIA and association between the genotypes and phenotypes have not been well documented in the Chinese population. In the present study, by using *targeted* next-generation sequencing (TGS), Sanger sequencing and segregation analysis, novel nonsense, compound heterozygous mutations in the *USH2A* gene were identified in a Chinese pedigree with Usher syndrome.

## Materials and methods

**Pedigree and clinical assessment, blood collection, and DNA isolation.** The M147 pedigree consisted of a proband and four family members from Sichuan, China (Fig. 1A). The proband was a 35-year-old female, and her parents, husband and son were 71, 60, 36 and 5-years-old, respectively. The blood samples of the proband were collected in June 2013, and TGS analysis was performed in December 2016. The blood samples of the four family members were collected in March 2019 for segregation analysis in our laboratory. For detailed clinical assessments, clinical history and ophthalmic examinations were performed with the proband, including funduscopy, fundus photographs and fundus fluorescent photographs as previously described (15). Audiometric testing in the proband was also performed using pure-tone audiometry with different frequencies (0.25, 0.5, 1, 2, 4 and 8 kHz) (1). Written informed consent from the participants or guardians was obtained, and the study conformed to the Declaration of Helsinki. Blood samples were taken, and DNA was isolated from the family members (16,17). DNA from blood samples was also taken from 200 healthy controls with an average age of 40.6 years-old (age ranges: 20-66; sex distribution: 2:3 female: Male) from Luzhou city between December 2016 and February 2017 without hearing and vision problems. The DNA integrity was verified by running the DNA sample on a 0.8% agarose gel. Written informed consent was obtained from the patients and healthy controls.

**TGS.** TGS analysis was performed on the proband M147 DNA, according to the manufacturer's protocol (Illumina, Inc.) as described previously (18-20). Library construction, TGS and data analysis were performed according to the manufacturer's protocols and as described previously (19,21).

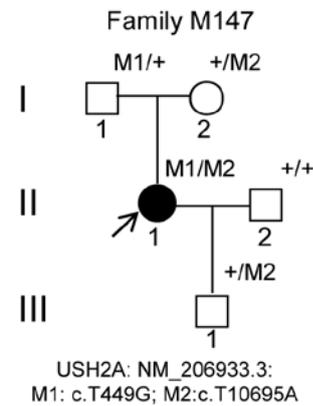


Figure 1. An M147 pedigree with Usher syndrome type IIA. Normal individuals are shown as a clear circle (female) or squares (males). The filled circle indicates the proband (II: 1, arrow) with the compound heterozygous mutation of the *USH2A* gene: NM\_206933.3: c.T449G, c.T10695A. *USH2A*, usherin.

**Sanger validation and segregation analysis.** PCR amplification was performed for mutation validation. The primer pairs M147-USH-T10695A and M147-M201-USH were designed using the Primer3 program (<http://bioinfo.ut.ee/primer3-0.4.0/>) with genomic DNA sequences containing the NM\_206933.3: c.T449G and c.T10695A, respectively, of the *USH2A* gene (Table I). PCR amplification was performed using the aforementioned primer pairs and the amplified PCR products were then sequenced using the Sanger method on an ABI-3500DX sequencer (Thermo Fisher Scientific, Inc.) (22) using M147-USH-T10695A-L and M147-M201-USH-L primers (Table I). Control samples from unrelated, ethnically matched individuals were also amplified using the primer pairs M147-USH-T10695A and M147-M201-USH and sequenced using the primers M147-USH-T10695A-L and M147-M201-USH-L. Segregation analysis in the M147 family was performed based on the sequencing results.

**Prediction of protein structure and bioinformatics analysis.** The homologs of the *USH2A* gene (NM\_206933.3) in humans were analyzed using HomoloGene ([ncbi.nlm.nih.gov/homologene?Db=homologene&Cmd=Retrieve&list\\_uids=66151](http://ncbi.nlm.nih.gov/homologene?Db=homologene&Cmd=Retrieve&list_uids=66151)).

Conserved domains of the protein structure of *USH2A* (NP\_996816.2) were searched using the NCBI conserved domain database ([ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi](http://ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi)) (23-25).

**RNA-sequencing (RNA-seq) profile, RNA isolation and reverse transcription-PCR (RT-PCR).** To determine tissue specificity in humans, *USH2A* gene expression profiles were analyzed using RNA-seq data which was performed on normal samples from 95 human individuals representing 27 different tissues using: <https://www.ncbi.nlm.nih.gov/gene/7399/?report=expression>. The above RNA-seq data were collected from a project called HPA RNA-seq normal tissues (BioProject no. PRJEB4337).

Total RNA was extracted from tissues in mice using TRIzol<sup>®</sup> reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Experiments involving mice followed the international, national and institutional guidelines for the care and use of laboratory animals (26,27,28) and was reviewed and approved

Table I. Sequences of *USH2A* PCR primers.

Primer name	Sequence, 5'-3'	Size, bp	Mutation site
M147-USH-T10695A	F: AGGAACTGCTTGAGACAGCAA R: CTGAACCCCTTTTCCCAGAG	346	c.T10695A
M147-M201-USH	F: AGGCCTCAGTAGCTGCATCA R: TTGGGGAAACAACCTGGAAGA	321	c.T449G
RT-ush2a	F: CGCTCTGCCTCTCCTCTCTA R: TTTATTGGAGGCTGCAAACC	320	N/A
RT-actin-m	F: TGTTACCAACTGGGACGACA R: TCTCAGCTGTGGTGGTGAAG	392	N/A

F, forward; R, reverse; N/A, not available. RT, reverse transcription.

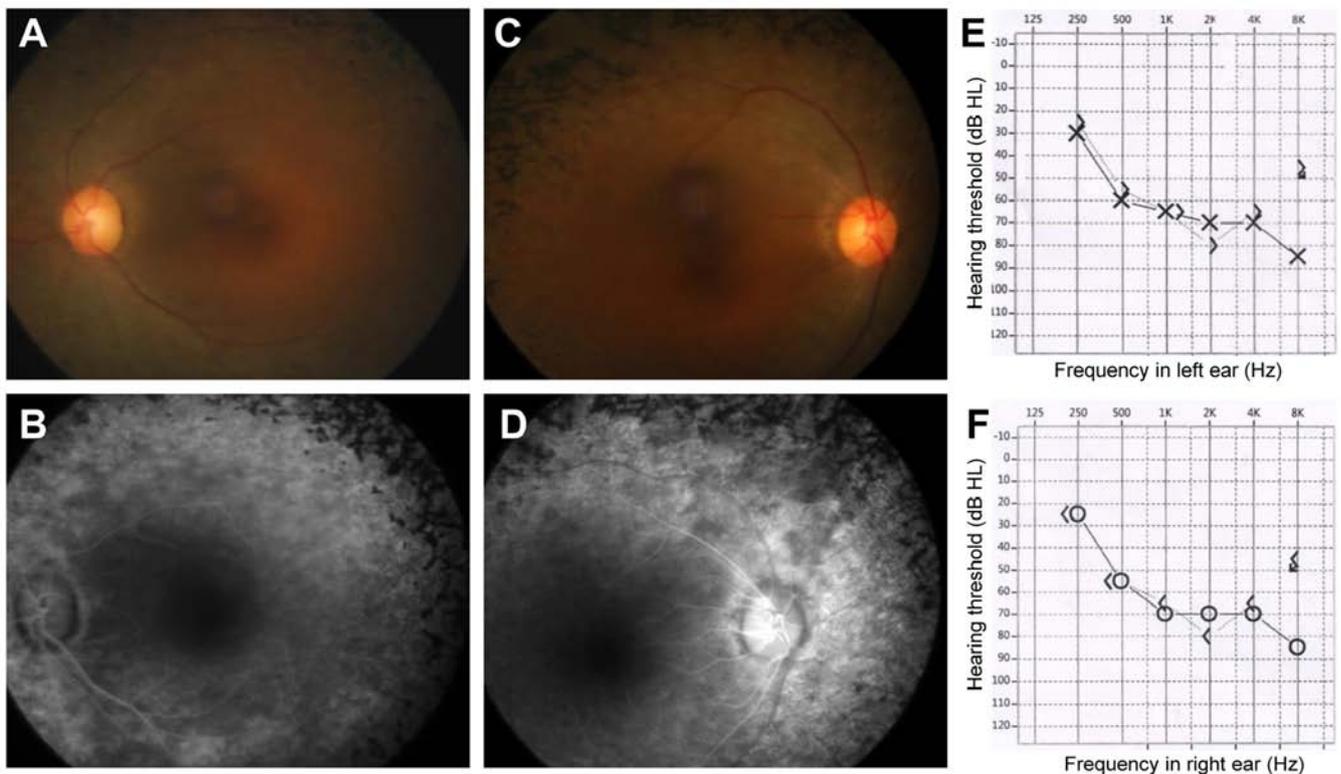


Figure 2. Representative retinal phenotypes and ear audiograms of proband II: 1. (A-D) Representative fundus photographs and fundus fluorescent photographs in patient II: 1 of both eyes. (E and F) Physiological audiograms of both ears of proband II:1.

by the Ethics Committee of Southwest Medical University, Sichuan, China. Male and female BALB/c mice (8 weeks old, ~21 g), were purchased from SPF (Beijing) Biotechnology Co., Ltd, China, and housed individually at room temperatures (18-22°C) with 40-60% and with a 12-h light/dark cycle. All mice were negative for pathogens. Mice received food and water *ad libitum*. At least five healthy mice for eye tissues and one healthy mouse for other tissues were used for RNA sampling. Retinal tissue at developmental stages, including whole embryo eye at 12.5 and 20.5 days before birth, and 2 weeks, 1 month, 2 and 3 months after birth, were used. Pentobarbital sodium (200 mg/kg of body weight) was intraperitoneally injected to mice for euthanasia. Death was

verified as absence of vital signs, including no heartbeat, dilated pupils or cervical dislocation after anesthesia. First strand cDNA was then synthesized from 1 µg total RNA with the ReverTra Ace<sup>®</sup> qPCR RT kit (cat. no. FSQ-201; Toyobo Life Science) according to the manufacturer's instructions and semi-quantitative RT-PCR was performed as described in our previous study (29,30). Briefly, the PCR reaction system consisted of 5 µl 2X Taq PCR MasterMix (Tiangen Biotech Co.), 1 µl 2.5 µM RT primers, 1 µl RT products and 3 µl double distilled H<sub>2</sub>O. Amplification reactions were performed using the Applied Biosystems<sup>®</sup> Veriti<sup>®</sup> 96-Well Thermal Cycler PCR machine (Thermo Fisher Scientific, Inc.) with the following steps: Initial denaturation at 95°C for 90 sec, 28-30 cycles

Table II. Characteristics of *USH2A* variants and disease-causing effects in the proband.

Gene	Exon	Variation			Status	ExAC status
		Nucleotide	Protein	Type of mutation		
USH2A	2	c.T449G	p.L150*	Nonsense	Heterozygous	Novel
USH2A	54	c.T10695A	p.Y3565*	Nonsense	Heterozygous	Novel

USH2A, usherin; c, variant at the cDNA level; p, variant at the protein level; ExAC, Exome Aggregation Consortium; \*, stop codon.

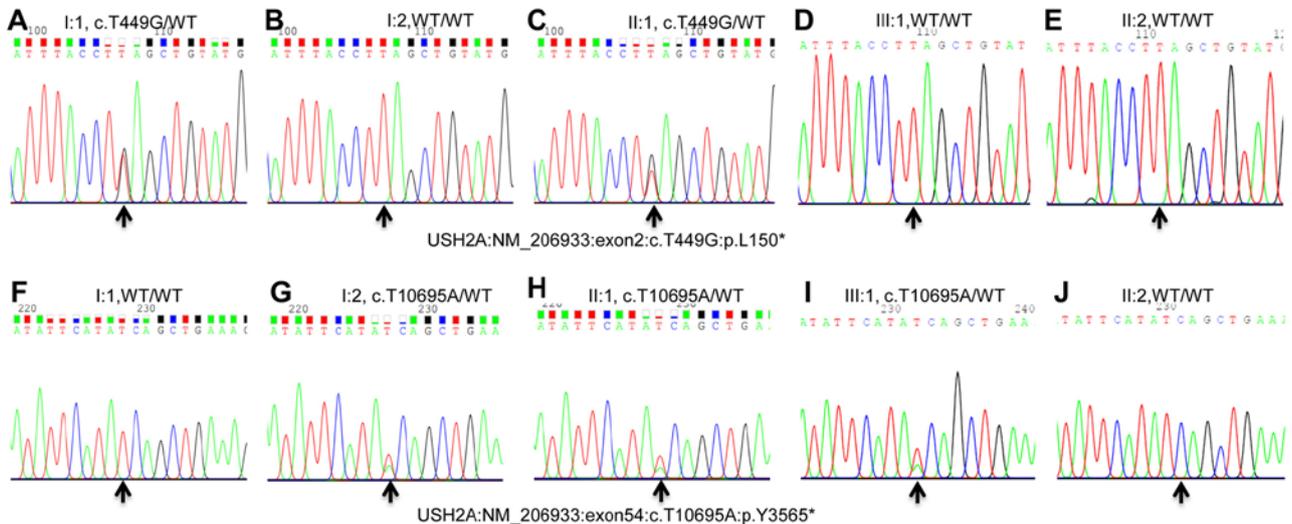


Figure 3. Pyrogram profiles for variant verification using Sanger sequencing. (A-E) Sequencing results of I: 1 (heterozygous mutant type), I: 2 (WT), II: 1 (heterozygous mutant type), III: 1 (WT), and II: 2 (WT) of variant c.T449G. (F-J) Sequencing results of I: 1 (WT), I: 2 (heterozygous mutant type), II: 1 (heterozygous mutant type), III: 1 (heterozygous), and II: 2 (WT) of variant c.T10695A. The arrows indicate the mutation position of NM\_206933.3: c.T449G or c.T10695A in the *USH2A* gene. *USH2A*, usherin; WT, wild-type.

of denaturation at 94°C for 30 sec, annealing at 72°C for 30 sec, extension at 72°C for 30 sec and a final extension step at 72°C for 5 min. Primer pairs for RT-ush2a (RT-ush2a-L and RT-ush2a-R) targeting the mouse *Ush2a* gene, and the mouse  $\beta$ -actin gene which served as a control, are listed in Table I. RT-PCR products were separated on a 1.2% agarose gel in triplicate, and the gels were visualized by 0.5  $\mu$ g/ml ethidium bromide (EB) staining. Densitometry was performed using ChemiDoc XR (version 5.2, Bio-Rad Laboratories, Inc.) (17,31).

## Results

**Proband and clinical characteristics.** The patient (Fig. 1A, II: 1) was a 35-year-old Chinese female. She suffered from night blindness since adolescence at age 12 and was first diagnosed with RP at the Yibin local county hospital 7 years ago. Fundus examinations showed bony spicule pigmentation and attenuated retinal vessels in both eyes, characteristic of a typical RP phenotype (Fig. 2A-D). The retinal pigment epithelium were atrophied, and electroretinography results displayed no amplitude reactions (data not shown). The proband had a right naked eye vision of 0.6 and a left naked eye vision of 0.7, and a visual acuity with correction of 0.8 in the right eye and 0.8+ in the left eye. The logMAR or Snellen measurements were not

available for this patient. The proband did not claim to exhibit hearing loss. The proband was characterized as RP. Based on genetic diagnostic results, pure tone audiometry testing was performed and found 65 dB hearing losses of both ears, indicating a moderate-to-severe binaural sensorineural hearing loss across all frequencies (Fig. 2E, left ear; Fig. 2F, right ear). Both the proband's parents and her son was normal. Thus, the proband was diagnosed with possible Usher syndrome type IIA.

**Results of TGS and co-segregation analysis.** Compound heterozygous mutations (c.T449G) in exon 2 and (c.T10695A) in exon 54 in the *USH2A* gene (NM\_206933.3) were identified, leading to the presence of early stop codons (from TTA to TGA and from TAT to TAA, respectively) at amino acid positions 150 (p.L150\*) and 3565 (p.Y3565\*), respectively, in the *USH2A* protein (NP\_996816.2; Fig. 1A, II: 1). Thus, both *USH2A* variants (c.T449G, p.L150\*) and (c.T10695A, p.Y3565\*) resulted in the production of truncated proteins, which were hypothesized to affect protein function. The characteristics of the *USH2A* variants and disease-causing effects of the proband are shown in Table II. Hence, these nonsense mutations (c.T449G, p.L150\*) and (c.T10695A, p.Y3565\*) in the *USH2A* gene affected protein function and supported the diagnosis of Usher syndrome type IIA. Both variants were revealed to be novel by

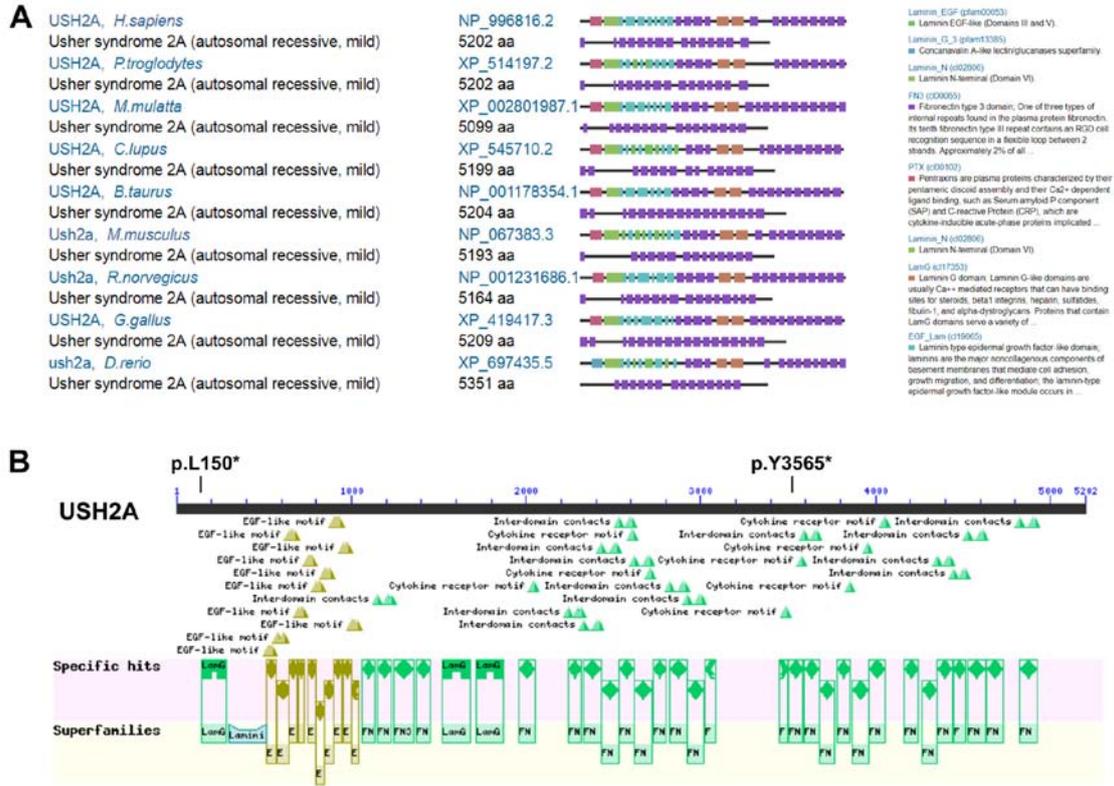


Figure 4. USH2A comparison, structure and mutant locations between different species. (A) Conservation analysis of USH2A in the indicated species. (B) USH2A domains and mutant positions. Variants p.L150\* and p.Y3565\* of USH2A are indicated panel B. USH2A, usherin; aa, amino acids. EGF, epidermal growth factor; FN, fibronectin.

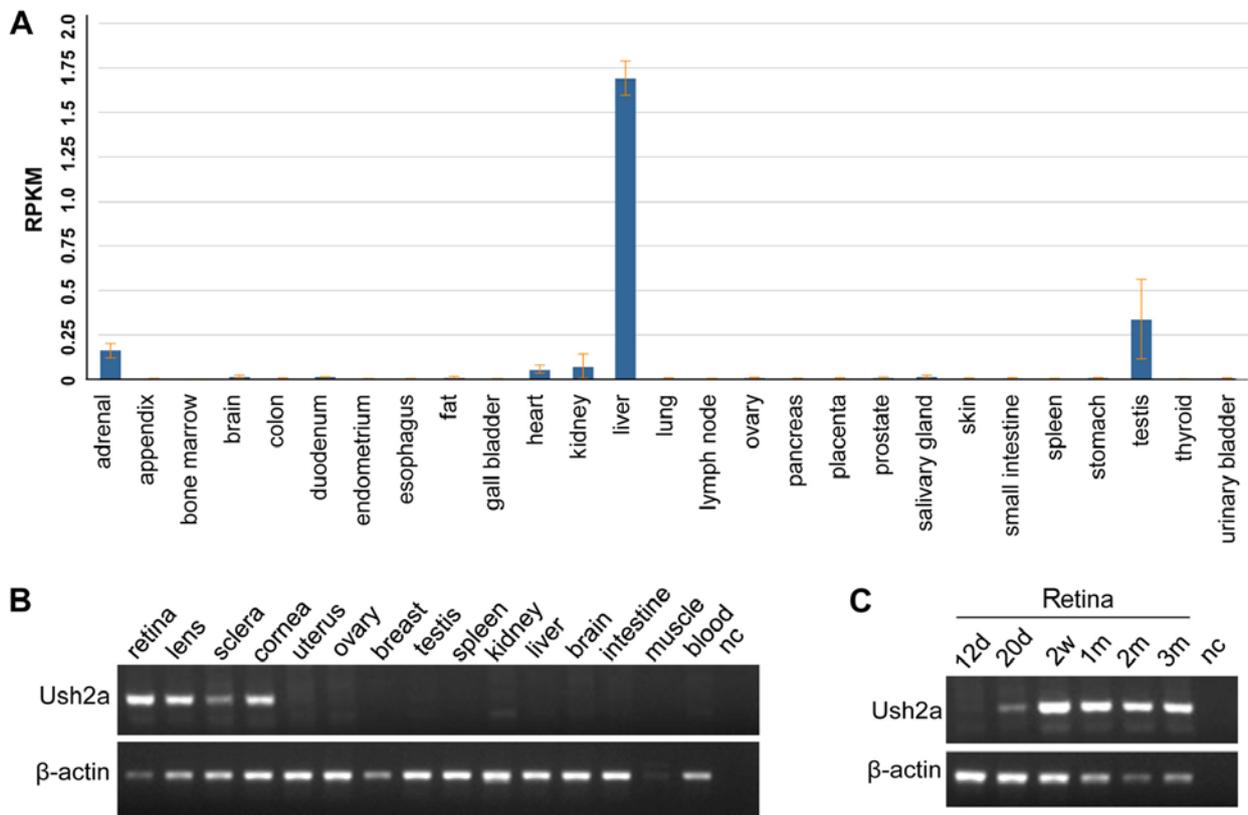


Figure 5. mRNA expression of *USH2A* in human tissues and *Ush2a* in mouse tissues. (A) *USH2A* mRNA expression in human tissues. The source of the data was derived from the link: <https://www.ncbi.nlm.nih.gov/gene/7399/?report=expression>. (B) *Ush2a* mRNA levels in the indicated mouse tissues. (C) *Ush2a* mRNA levels at the indicated developmental time periods in retinal tissue in mice. Whole embryo eyeballs were obtained at 12.5 days (12d) and 20.5 days (20d). d, days; w, weeks; m, months; nc, no DNA template; muscle, skeletal muscle; RPKM, reads per kilobase of transcript per million mapped reads.

Table III. Expression of *USH2A* mRNA in human tissues.

Tissue	Number of samples	RPKM values	Counts
Adrenal	3	0.163±0.040	37,820
Appendix	3	0.005±0.002	984
Bone marrow	4	0.001±0.001	355
Brain	3	0.017±0.008	4,671
Colon	5	0.005±0.004	4,064
Duodenum	2	0.015±0.001	2,072
Endometrium	3	0.005±0.0020	1,114
Esophagus	3	0.004±0.002	1,592
Fat	3	0.011±0.007	2,513
Gall bladder	3	0.004±0.002	1,546
Heart	4	0.06±0.0230	30,633
Kidney	4	0.076±0.068	21,041
Liver	3	1.691±0.096	469,141
Lung	5	0.006±0.004	2,714
Lymph node	5	0.003±0.004	1,827
Ovary	2	0.009±0.004	2,706
Pancreas	2	0.006±0.002	1,498
Placenta	4	0.005±0.005	2,596
Prostate	4	0.009±0.006	2,782
Salivary gland	3	0.015±0.009	7,066
Skin	3	0.006±0.003	2,279
Small intestine	4	0.007±0.004	2,300
Spleen	4	0.003±0.003	1,410
Stomach	3	0.009±0.003	2,563
Testis	7	0.341±0.222	348,396
Thyroid	4	0.003±0.002	1,543
Urinary bladder	2	0.006±0.004	1,432

RPKM, reads per kilobase of transcript per million mapped reads; *USH2A*, usherin.

searching the databases for Exome Aggregation Consortium (<https://gnomad.broadinstitute.org/>) and HGMD (<http://www.hgmd.cf.ac.uk/ac/index.php>) (Table II).

Sanger sequencing was performed to confirm the variants and analyze co-segregation (Fig. 3). The mutations (c.T449G, c.T10695A) in the *USH2A* gene were validated to be compound heterozygous in the proband (Fig. 3C and H; pedigree II: 1), of which c.T449G was inherited from her father (Fig. 3A and F; pedigree I: 1) and c.T10695A was inherited from her mother (Fig. 3B and G; pedigree I: 2). The proband's son was revealed to be heterozygous c.T10695A with a normal phenotype (pedigree III: 1; Fig. 3D and I), and the proband's husband had a normal phenotype and with wild-type alleles (Fig. 3E and J; pedigree II: 2). Therefore, these mutations in the *USH2A* gene and the mutations were co-segregated with this clinical phenotype in this family. Both c.T449G and c.T10695A variants were absent in the blood samples of 200 normal ethnically matched controls. Taken together, this discovery highlighted co-segregation of the variants in this family and pinpointed their roles in the pathogenesis of Usher syndrome type IIA.

*Functional effects of the variants c.T449G (p.L150\*) and c.T10695A (p.Y3565\*) in USH2A.* By comparing of *Homo sapiens* (*H. sapiens*) *USH2A* protein to eight other species, including *Pan troglodytes*, *Macaca mulatta*, *Canis lupus*, *Bos taurus*, *Rattus norvegicus*, *Mus musculus*, *Gallus gallus* and *Danio rerio*, it was shown that *USH2A* is highly orthologously conserved (Fig. 4A). *USH2A* protein in *H. sapiens* contains a lamG-like jellyroll fold domain, laminin EGF domains, laminin-type EGF-like domains, laminin G domains, laminin N-terminal (Domain VI) and many fibronectin type 3 domains (Fig. 4A and B). The c.T449G (p.L150\*) variant is located in the lamG-like jellyroll fold domain (aa.146-aa.283), leading to the production of a truncated protein which had lost almost all the functional domains (Fig. 4B); whereas the c.T10695A (p.Y3565\*) variant is located in one of the fibronectin type 3 domains (aa. 3503-aa. 3586), leading to the production of a truncated protein, that lost several fibronectin type 3 domains at the C-terminus of *USH2A* (Fig. 4B). Together, these results showed that the *USH2A* pathogenic compound heterozygous variants c.T449G (p.L150\*) and c.T10695A (p.Y3565\*) may have caused Usher syndrome type IIA disease.

*mRNA expression profiles of USH2A and Ush2a.* *USH2A* expression analysis in humans indicated that *USH2A* mRNA expression was highest in the liver, with a reads per kilobase of transcript per million mapped reads value of 1.691±0.096, followed by the testis, and was low or very low in other tissues (Fig. 5A). RNA-seq values in different tissues are presented in Table III. No human eye tissues and developmental retinal stage tissues were available. Thus, the *Ush2a* expression in mice was studied. *Ush2a* mRNA expression levels were highly expressed in the retina and other indicated tissues including the lens, sclera and cornea (Fig. 5B); whereas expression was not detected in the tissues, uterus, ovary, testis, breast, spleen, kidney, liver, brain, intestine, skeletal muscle, and blood. *Ush2a* was also highly expressed in the latter four different developmental retinal stages following birth (Fig. 5C). These results suggested that *USH2A* may serve a vital role in retinal/eye function based on the very high levels of *Ush2a2* expression in the retinal tissue and its highly ubiquitous expression in other eye tissues.

## Discussion

The association between the variations in Usher syndrome-causative genes and the resultant Usher syndrome diseases or phenotypes in patients are highly variable; the genotype/phenotype associations are also divergent (1,3,4). Since Eudy *et al* (5) first identified three mutations in the *USH2A* gene in patients with Usher syndrome type IIA with RP and hearing loss, additional *USH2A* mutations have been shown to be associated with Usher syndrome type IIA (10-12,32-39). Patients with autosomal recessive RP (arRP; RP39; OMIM, 613809) without hearing loss were also identified to possess *USH2A* mutations, highlighting the complexity of genotype/phenotype associations in this disease (4). For example, Rivolta *et al* (40) identified *USH2A* mutations in patients with arRP without hearing loss. Zlotogora (41) reviewed examples and identified arRP patients without hearing loss who possessed mutations in

the *USH2A* gene. Compound pathogenic mutations in the *USH2A* gene in Chinese RP families were also recently identified (42).

In the present study, using TGS, Sanger sequencing and co-segregation analysis, compound heterozygous pathogenic nonsense mutations, c.T449G (p.L150\*) and c.T10695A (p.Y3565\*), were identified in the *USH2A* gene in a Chinese pedigree. *USH2A*, very large G-protein coupled receptor 1 (VLGR1), *USH1* and electroneutral sodium bicarbonate exchanger 1 (NBC3) are co-expressed in the synaptic terminals in both retinal photoreceptors and inner ear hair cells of mice and rats. The scaffold proteins harmonin, *USH2A*, VLGR1 and NBC3 interact with each other to assemble a multiprotein complex (43). *USH2* and NBC3 proteins are interaction partners in a network in the retina and inner ear (43,44). These p.L150\* and p.Y3565\* truncated *USH2A* mutations are hypothesized to affect complex formation (45), thereby elucidating the genetic roles of these *USH2A* mutant alleles in Usher syndrome type IIA.

The patient examined in the present study was first diagnosed as a nonsyndromic RP as she did not claim to suffer from hearing loss. Based on gene diagnostic results, pure tone audiometry testing was performed which presented binaural sensorineural deafness. Thus, *USH2A* mutations with Usher syndrome type IIA and an association of genotype/phenotype have been successfully linked in the patient of the studied family. Pater *et al* (46) recently reassigned the diagnosis of Usher syndrome by identifying novel *USH2A* splicing variants. To the best of our knowledge, the *USH2A* variants c.T449G (p.L150\*), c.T10695A (p.Y3565\*) are novel, thereby extending the spectrum of known mutations associated with this disease.

By comparing the of *H. sapiens* *USH2A* to eight other species, it was demonstrated that the *USH2A* protein is highly conserved. *Ush2a* mRNA expression levels in mice were demonstrated to only be highly expressed in the retina, lens, sclera and cornea from the eye tissues, consistent with a previous study (5), suggesting that *USH2A* serves vital roles in the functions of retina/eye. In addition to its high expression in the retina, *USH2A* in humans, rat and mice is also expressed in the cochlea (5,47). *USH2A* was found to a likely component of the interstereocilia ankle which links the inner ear sensory cells (12). Taken together, the present study showed that the *USH2A* compound heterozygous variant, c.T449G (p.L150\*) and c.T10695A (p.Y3565\*), resulted in Usher syndrome type IIA.

As a rare disorder and the most common inherited form of combined visual and hearing impairment, up to 14 genes (*MYO7A*, *CDH23*, *USH1C*, *PCDH15*, *USH1G* and *CIB2* for USH I, *USH2A*, *ADGRV1* and *WHRN* for USH II, *CLRN1* and *HARS* for USH III, and *PDZD7*, *CEP250* and *C2orf71* for either type I, II, or III of USH) are associated with Usher syndrome, with *USH2A* being the most prevalent worldwide (2,48,49). With genetic diagnosis for Usher syndrome, repairing specific mutations using CRISPR/Cas9 editing system may now be possible. For example, Fuster-García *et al* (48) investigated gene editing to target the mutation in fibroblasts of a USH patient bearing the c.2299delG homozygous variation, highlighting the potential of the CRISPR editing system for the treatment of Usher syndrome.

In conclusion, the present study is the first to identify the novel compound heterozygous variants c.T449G (p.L150\*)

and c.T10695A (p.Y3565\*) in the *USH2A* gene, which caused Usher syndrome type IIA in the examined patient. These mutations expand upon the library of known mutations associated with Usher syndrome. TGS provides a useful gene diagnostic approach (50). The present discovery may assist in understanding the molecular pathogenesis of RP and Usher syndrome type IIA, and in the development of strategies for the prevention, diagnosis, therapy and genetic counseling of patients with Usher syndrome type IIA. Additionally, the recruitment of more patients with Usher syndrome is an aim of future studies.

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### Availability of data and materials

The data generated using high-throughput sequencing were not submitted to any public databases due to the containing information that may compromise the proband's privacy but are available from the corresponding author on reasonable request.

### Authors' contributions

JuF designed and conceptualized the study. JiF and JC performed DNA extraction, PCR amplification, sequencing and data analysis. HL, QZ and CD recruited the clinical patients and were in charge of the clinical assessments. JuF, MAK and JP analyzed the data, wrote and revised the manuscript. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Southwest Medical University. The protocol and procedures employed for mice experiments were ethically reviewed and approved by the Ethics Committee of Southwest Medical University.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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