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CEP78 is mutated in a distinct type of Usher syndrome

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Abstract

Background—Usher syndrome is a genetically heterogeneous disorder featured by combined visual impairment and hearing loss. Despite a dozen of genes involved in Usher syndrome having been identified, the genetic basis remains unknown in 20–30% of patients. In this study, we aimed to identify the novel disease-causing gene of a distinct subtype of Usher syndrome.

Methods—Ophthalmic examinations and hearing tests were performed on patients with Usher syndrome in two consanguineous families. Target capture sequencing was initially performed to screen causative mutations in known retinal disease-causing loci. Whole exome sequencing (WES) and whole genome sequencing (WGS) were applied for identifying novel disease-causing genes. RT-PCR and Sanger sequencing were performed to evaluate the splicing-altering effect of identified *CEP78* variants.

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Contributors MX, XC, CZ and RC designed the study. QF, XS, ZY, YL, HL, ZS, HL, LY, FZ collected clinical data. KW and YL performed Next-generation sequencing (NGS). MX and XC analysed the NGS data. QF, MX, XC, RS and RC drafted the manuscript. All authors revised the manuscript.

Competing interests None declared.

Patient consent Obtained.

Ethics approval Institutional Review Board of Baylor College of Medicine.

Results—Patients from the two independent families show a mild Usher syndrome phenotype featured by juvenile or adult-onset cone–rod dystrophy and sensorineural hearing loss. WES and WGS identified two homozygous rare variants that affect mRNA splicing of a ciliary gene *CEP78*. RT-PCR confirmed that the two variants indeed lead to abnormal splicing, resulting in premature stop of protein translation due to frameshift.

Conclusions—Our results provide evidence that *CEP78* is a novel disease-causing gene for Usher syndrome, demonstrating an additional link between ciliopathy and Usher protein network in photoreceptor cells and inner ear hair cells.

INTRODUCTION

Primary cilium is a microtubule-based sensory organelle originating from centrioles. It widely exists in multiple cell types of human body. Primary cilium plays important roles in various cell signalling processes, thus essential for normal development and maintenance of tissue functioning.[12] The cilia proteome is highly heterogeneous and dynamic, containing dozens of proteins involved in ciliary transport, structural maintenance, as well as a number of tissue-specific cargo proteins. Correspondingly, mutations in these ciliary proteins cause a wide range of genetic disorders collectively referred to as ciliopathy. Defects in primary cilia lead to a series of clinical phenotypes such as retinal dystrophy, hearing loss, neurodevelopmental defects and kidney disease.[3]

Usher syndrome is a genetic disorder characterised by combined retinitis pigmentosa and sensorineural hearing loss. It is caused by disruption of protein components in the supramolecular Usher protein network. There is growing evidence showing that this protein network is associated with cilia proteome.[4] In retina photoreceptor cells, the Usher protein complex has been found to loca-lise at the periciliary region, which is critical for structural maintenance and ciliary transport.[5] In addition, some specific interactions between Usher protein components and ciliary proteins were also identified, including DFNB31-RPGR and USH1G-CEP290.[46] Usher syndrome has a prevalence ranging from 3.2 to 6.2 in 100 000,[7–9] and it can be classified into three major categories, namely USH I, II and III, depending on the age of onset and severity. Until now, 13 genes have been associated with Usher syndrome (RetNet, the Retinal Information Network).[10] However, mutations in these genes can only account for 70–80% of cases,[11–13] suggesting additional disease-causing loci are yet to be identified.

In this study, by whole exome sequencing (WES) and whole genome sequencing (WGS), we identified mutations in *CEP78*, a ciliary gene,[14] in two independent consanguineous families presenting a distinct Usher syndrome phenotype featured by cone–rod dystrophy and progressive sensorineural hearing loss. One allele affects the canonical splicing acceptor site of exon 14 and the other allele locates adjacent to the exon 10 splicing donor site. RT-PCR and Sanger sequencing confirmed that the two mutations cause splicing defects and lead to premature stop codons. Therefore, these results linked a new gene to Usher syndrome and provided more hints for understanding Usher/ciliary protein network in photoreceptor cells and inner ear hair cells.

METHODS

Clinical diagnosis

The patients in Family 1 were ascertained at the Department of Ophthalmology, Peking Union Medical College Hospital, Beijing, China. They underwent detailed ophthalmic evaluation, including best correct visual acuity (BCVA), slit-lamp bio-microscopy, dilated indirect ophthalmoscopy, fundus photography, visual field tests (Octopus, Interzeag, Schlieren, Switzerland), optical coherence tomography (OCT) (Heidelberg HRT II, Heidelberg, Germany) and electroretinograms (ERGs) (RetiPort ERG system, Roland Consult, Wiesbaden, Germany) using corneal 'ERGjet' contact lens electrodes. Auditory examinations including pure tone and speech audiometry were conducted by otolaryngologists. The patients in Family 2 were recruited at the Department of Ophthalmology, Ningxia Eye Hospital, Ningxia People's Hospital. They also underwent complete ophthalmic examinations, including assessments of BCVA, slit-lamp examination, funduscopy, colour vision test, visual field tests (Humphrey Perimetry, ZEISS, Germany), ERG, OCT and fundus fluorescein angiography (FFA) examinations. Genomic DNA was isolated from peripheral leucocytes using QIAamp DNA Blood Midi Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. This study adhered to the Tenets of the Declaration of Helsinki.

Target capture sequencing, WES, WGS and bioinformatic analysis

Precapture Illumina libraries were generated as in previous literature.[15–17] The targeted DNA was captured, washed and recovered using Agilent Hybridization and Wash Kits (Agilent Technologies, Santa Clara, California, USA). The genes included in target capture panel were listed in previous literature.[18] WES was performed by capturing the DNA with NimbleGenSeqCap EZ Hybridization and Wash Kit. Captured DNA libraries were sequenced on Illumina HiSeq 2000 (Illumina, San Diego, California, USA). For WGS, the DNA was prepared using the TruSeq Nano DNA HT Sample Prep Kit (Illumina) and the sequencing was performed on Hiseq X Ten (X10) platform (Illumina). After the sequencing step, the reads were obtained and aligned to human hg19 genome using Burrows-Wheeler Aligner (BWA) V.0.6.1.[19] Base quality recalibration and local realignment were done by the Genome Analysis Tool Kit V.3.6. Atlas-SNP2 and Atlas-Indel2 were used for variant calling.[20] Variant frequency data were obtained from a series of public and internal control databases, including Exome Aggregation Consortium (ExAC) database, CHARGE Consortium,[21] ESP-6500[22] and 1000 Genome Project.[23] The consanguineous nature of these two families and the absence of disease pheno-types in other generations strongly suggest an autosomal recessive inheritance model; thus, variants with a minor allele frequency higher than 1/200 were filtered out. Synonymous and deep intronic (distance >10 bp from exon–intron junctions) variants were also excluded from the following analysis. Annotate Variation (ANNOVAR) (V.11/12/2014) and dbNSFP suite (V.2.9, contains SIFT, PolyPhen-2, LRT, MutationTaster, MutationAssessor, etc) were used to annotate protein-altering changes. Known retinal disease-causing alleles were detected based on the Human Gene Mutation Database (HGMD) Professional database (V.11/15/2014).

RT-PCR and Sanger sequencing

Total RNAs were extracted from the periphery blood samples of the patients and unaffected control subjects, using the QIAamp RNA Blood Mini Kit (QIAGEN). cDNA were synthesised from 8 mg total RNA using GoScript Reverse Transcription System (Promega, Madison, USA) according to manufacturer's protocol. RT-PCR primers were designed on Primer 3 software (http://bioinfo.ut.ee/primer3-0.4.0/): RT-exon-9F (5′-GTGTCTGGTTTCTTGCCGTG-3′); RT-exon-11R (5′-GGCACTTCATGAACAC TCTCT-3′); RT-exon13F (5′-GCCCTTCATGCACAGTCATT-3′); RT-exon16R (5′-ACAGGAAAGGAGTCGAGAGG-3′). The 50 μL reaction system contained 40 ng cDNA, 10 pmol of each primer and 25 μL 2×Taq PCR Master Mix (TransGen Biotech, Beijing, China). DNA amplifications were performed with denaturing at 95°C for 5 min, followed by 33 cycles of a denaturing step at 95° C, an annealing step at 60°C and an extension step at 72°C, each for 30 s. A final extension step at 72°C was performed for 7 min. After purification, amplicons were sequenced using forward and reverse primers on an ABI 3730 Genetic Analyzer (ABI, Foster City, California, USA). Sequences were assembled and analysed using Lasergene SeqMan software (DNASTAR, Madison, Wisconsin, USA).

RESULTS

Clinical findings

Family 1 is a consanguineous family from Fujian Province of South China with Han Chinese ancestry. The proband is a 33-year-old woman suffering from progressive vision loss for over 20 years. She currently had a visual acuity of counting finger oculus dexter (OD) and hand motion oculus sinister (OS). She also failed colour vision test and presents photophobia. Progressive sensorineural hearing loss was noticed in recent years (figure 1R). The proband has an affected elder sister at the age of 41, with over 30 years of progressive vision loss. She has a visual acuity of 20/1000 OD and 20/400 OS. Photophobia, impaired colour vision and recent hearing loss were also presented. The clinical findings in ERG tests, colour fundus photos, fundus autofluorescence and OCT are similar in two patients: both of them show moderately reduced rod responses and remarkably reduced cone responses in ERG tests (figure 1M–Q). Funduscopy results showed greyish retinal pigments mottling along the retinal vessels with attenuated retinal arteries (figure 1A, B and online supplementary figure S2A, B). The fundus autofluorescence demonstrated annular hypofluorescence patches around the posterior pole with an inner hyperfluores-cent ring around the macular region (figure 1I, J and online supplementary figure S2I, J). OCT results revealed reduced thickness of macular region and disappeared ellipsoid zone (figure 1E, F and online supplementary figure S2E, F). In addition, the proband also presented dilated retinal veins with retinal haemorrhage, probably confounded by her hypertension (170/110 mm Hg). The two patients were diagnosed as a distinct subtype of Usher syndrome, with an autosomal recessive inheritance due to the consanguinity of this family.

In Family 2, consanguineous marriage was reported, while no family history of visual problem was recorded. The proband developed night blindness since early childhood. She also noticed hypochromatopsia and mild hearing loss since age 8 (figure 1S). She suffered from visual field decrease and central vision loss since age 10. At her latest visit at age 35,

her BCVAs dropped to 20/125 OD and 20/200 OS. Hypochromatopsia was confirmed by colour vision test. Funduscopy features include attenuated vessels, waxy optic disc and pigment deposits in the mid-peripheral retina (figure 1C, D). Macular region was also involved. Consistent with the fundus photography, FFA also revealed aberrant vascular arcades and speckled changes of increased fluorescence in the mid-peripheral retina (figure 1K, L). Attenuated outer nucleus layer, retinal pigment epithelium (RPE) and loss of ellipsoid zone were suggested by OCT presentations (figure 1G, H). Both scotopic and photopic ERG responses were abolished (see online supplementary figure S1A–C). She has an affected brother with similar ophthalmo-logical findings (see online supplementary figure S2, C, D, G, H, K, L) as well as hearing loss. According to these abnormalities, the patients in Family 2 were also clinically diagnosed as an atypical Usher syndrome phenotype.

Genetic findings

To identify the causative mutation underlying this phenotype, we initially performed target capture sequencing of known retinal disease genes on the proband of Family 1 with high quality (mean coverage: 160.65×; regions with >20× coverage: 95.74%). However, no candidate causative variants were identi-fied, suggesting the possibility that the patient carries mutations in a new disease-causing locus. Therefore, WES was performed on both patients. A mean WES coverage of 86.21× and 99.86× for the proband and her affected sister was obtained, respectively. Upon filtering, 397 and 411 variants were remained and 9 biallelic variants were shared by both patients (see online supplementary table S1). After variant exclusion and prioritisation, one *CEP78* homozygous variant was identified as the top candidate due to its possible loss-of-function nature and the functional link between CEP78 and cilia[142425] (see online supplementary table S1). This variant (NM_032171, c. 1254 +5G>A, p.?) is near the splice donor site of exon 10/intron 10 junction. It is extremely rare in control population with a frequency of 1/30 990 in the ExAC population database. Although this variant is not at the canonical splicing site, two computational methods that predict the splicing-altering effect (AdaBoost and random forests)[26] give scores of 0.603 and 0.528, respectively, suggesting that the variant is likely to affect the pre-mRNA splicing of *CEP78* transcript. Sanger sequencing was performed to validate the variant and confirm the cosegregation of this variant with disease phenotype in this family (figure 2A and online supplementary figure S3). For Family 2, WGS was performed on the proband as described and a mean coverage of 30× was achieved. A total of 352 rare protein-altering variants were remained after filtering and we also focused on homozygous variants due to the consanguinity (see online supplementary table S2). Interestingly, among them, a homozygous variant that affects the canonical splicing acceptor site of exon 14 (c.1629–2A>G, p.?) of *CEP78* was identified. It was not observed in any public control databases. Sanger sequencing also confirmed the genotype–phenotype cosegregation in this family (figure 2A and online supplementary figure S3). Genetic screening in additional 71 unsolved patients with Usher syndrome did not identify additional cases with biallelic *CEP78* mutations.

To further demonstrate that the two identified *CEP78* variants are *bona fide* splicing-disrupting, we collected the patient blood and performed RT-PCR with Sanger sequencing. In Family 1, we found that in both patients the 46 bp-long exon 10 was completely lost in

CEP78 mRNA (figure 2B). For Family 2, the proband has a 10 bp deletion of *CEP78* exon 14 (figure 2C), while a heterozygous carrier in this family shows wild-type/mutant hybrid sequence. Therefore, we confirmed that the two variants indeed affect the pre-mRNA splicing *in vivo* and they both lead to frameshifts and premature stop codons. Based on the Sanger sequencing results, the two variants are reannotated as c.1254+5G>A (p.R403Sfs*7) and c.1629–2A>G (p.G545Pfs*6).

DISCUSSION

CEP78 was originally identified as a centrosomal protein in a proteomic study.[27] In planarians, RNAi knockdown of *Smed-cep78* led to centriole-anchoring defects and slow locomotion of animals.[24] Furthermore, *CEP78* knockdown in human RPE1 cells causes a significant reduction of ciliated cell number,[24] suggesting the functionally conserved and essential role of CEP78 in ciliogenesis. Further characterisation showed that CEP78 localises at the distal end of centriole and the leucine-rich repeats are critical for its localisation.[25] The CEP78 localisation data are consistent with the functional linkage between centriole distal end and primary ciliogenesis.[2829]

Additionally, some evidence have shown the potential involvement of CEP78 in retinal and hearing abnormalities: CEP78 was identified to interact with cilia protein CEP250,[30] and the latter is a known Usher syndrome protein.[31] In another study, researchers tried identifying crucial genes which show significantly altered expression in cochlea after noise-induced hearing loss and *CEP78* was among the 15 genes with more than five-fold upregulation. These results suggest *CEP78* as a strong candidate disease-causing gene in the families we investigated.

Currently, the involvement of Usher protein network in cilia formation and function remains largely elusive.[4] The phenotypic overlapping between *CEP78* and other Usher syndrome genes would provide more clues for understanding the physical and genetic interactions within this protein network. Specifically, *CEP78*-associated phenotype has an age of onset similar to or even milder than Usher syndrome type III, suggesting the role of CEP78 in structural and functional maintenance instead of during early development. On the other hand, *CEP78* disruption distinctively affects cone photoreceptors earlier, while in all other Usher syndrome types, rod photoreceptor functions are initially compromised.[32] This indicates a specialised role of CEP78 in cone photoreceptors.

In summary, our results identified a novel ciliary gene *CEP78* involved in Usher syndrome. Further studies on additional cases and animal models would help to describe the *CEP78*-related phenotype more precisely and reveal the ciliary involvement in Usher protein network in photoreceptor cells and inner ear hair cells, thus pushing forward more efficient disease management and treatment for Usher syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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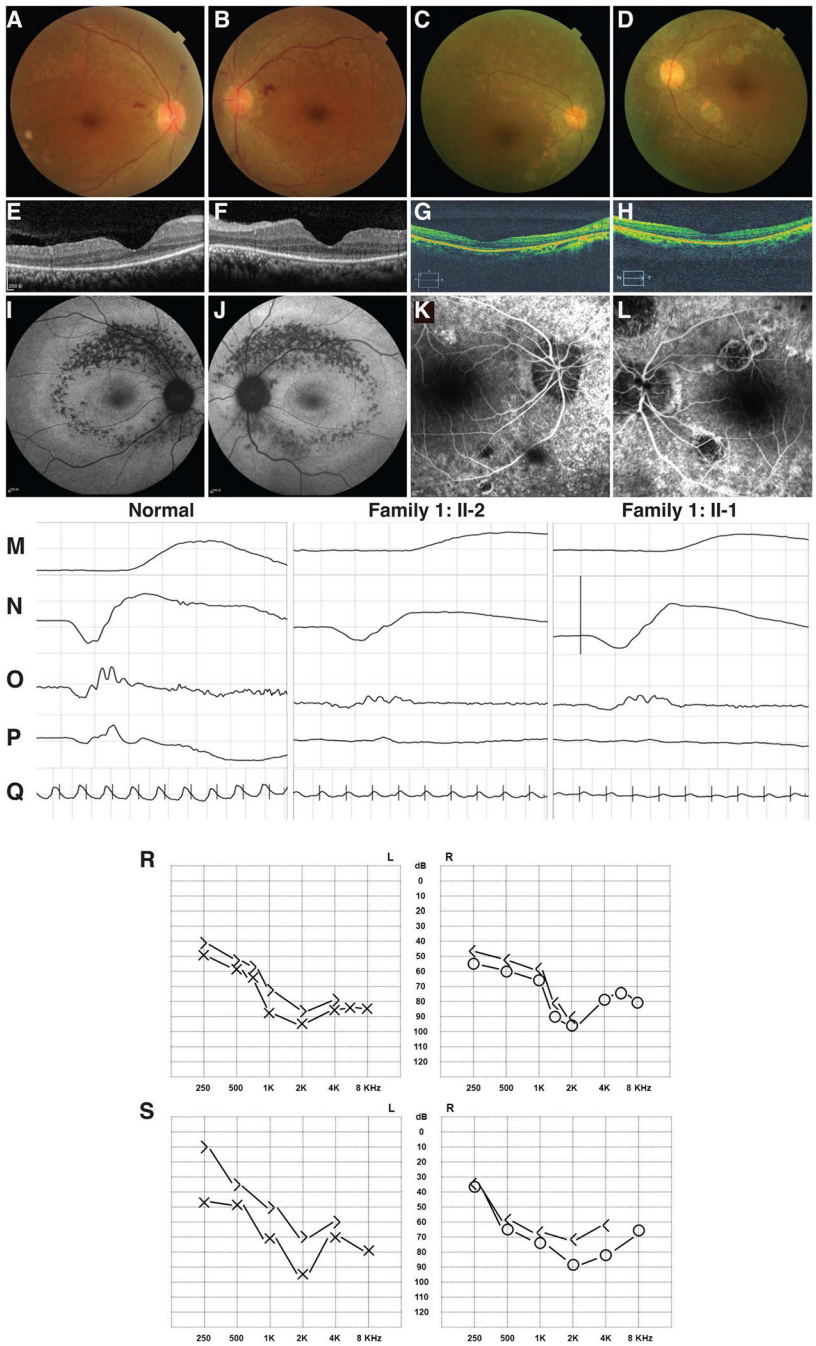

Figure 1.

Clinical manifestations of the patients with atypical Usher syndrome. Fundus images of Family 1: II-2, OD (A) and OS (B), Family 2: V-1, OD (C) and OS (D). Optical coherence tomography images of Family 1: II-2, OD (E) and OS (F), Family 2: V-1, OD (G) and OS (H). Fundus autofluorescence results of Family 1: II-2, OD (I) and OS (J). Fundus fluorescein angiography of Family 2: V-1, OD (K) and OS (L). The results of scotopic electroretinograms (ERGs) 0.01 (M), scotopic ERG 0.3 (N), oscillatory potential ERG (O), photopic ERG 3.0 (P) and flicker ERG 30 Hz (Q) of normal control (left panel), Family 1:

II-2 (middle panel) and Family 1: II-1 (right panel). Audiogram images of Family 1: II-2 (R) and Family 2: V-1 (S). In the audiogram images, cross or circle labels indicate air-conduction hearing, and right angle labels indicate bone-conduction hearing.

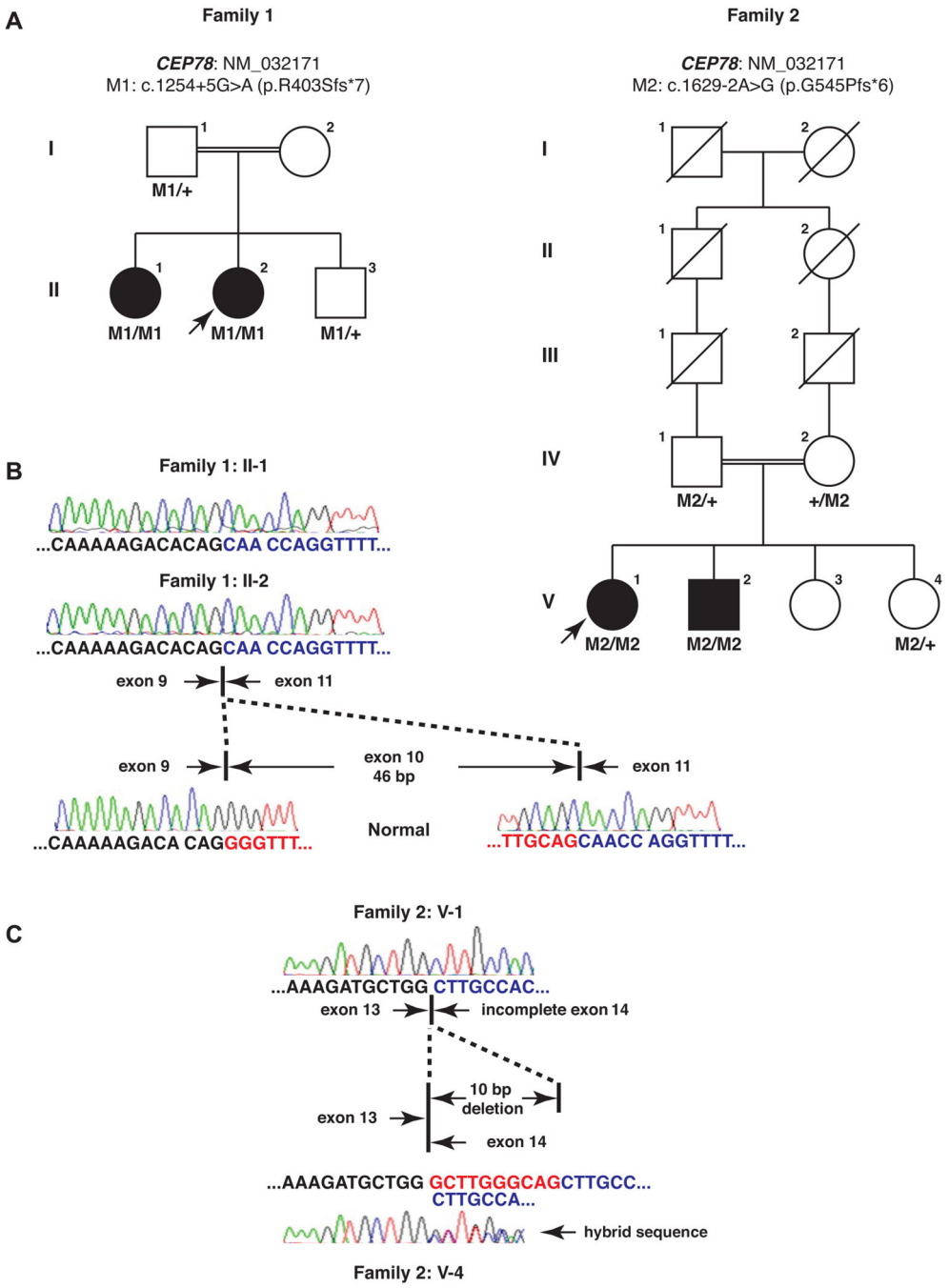

Figure 2.

Genetic findings in the patients with Usher syndrome. (A) *CEP78* mutations were identified in two unrelated consanguineous families. The variants cosegregate with the disease phenotype. Arrows indicate probands. (B) RT-PCR and Sanger sequencing confirmed the splicing-altering effect of the variant c.1254+5G>A in Family 1. In two patients' mRNA, the 46 bp exon 10 is completely skipped compared with normal control. (C) RT-PCR and Sanger sequencing confirmed the splicing-altering effect of the variant c.1629–2A>G in Family 2.

In the probands' mRNA, a 10 bp-long region in *CEP78* exon 14 was deleted, while in the unaffected heterozygous carrier, wild-type/mutant hybrid sequence was observed.