A Novel Frameshift Mutation of the USH2A Gene in a Korean Patient with Usher Syndrome Type II

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Case Report

INTRODUCTION

Usher syndrome type II (USH2) is the most common form of Usher syndrome, characterized by moderate to severe hearing impairment and progressive visual loss due to retinitis pigmentosa. It has been shown that mutations in the USH2A gene are responsible for USH2. The authors herein describe a 34-year-old Korean woman with the typical clinical manifestation of USH2; she had bilateral hearing disturbance and progressive visual deterioration, without vestibular dysfunction. Molecular genetic study of the USH2A gene revealed a novel frameshift mutation (c.2310delA; Glu771LysfsX17). She was heterozygous for this mutation, and no other mutation was found in USH2A, suggesting the possibility of an intronic or large genomic rearrangement mutation. To the best of our knowledge, this is the first report of a genetically confirmed case of USH2 in Korea. More investigations are needed to delineate genotype-phenotype correlations and ethnicity-specific genetic background of Usher syndrome.

Keywords. Usher syndrome type II, USH2A, Mutation, Frameshift, Korea

CASE REPORT

A 34-year-old female was referred to our hospital because of bilateral hearing disturbance since childhood. She complained of
a mild hearing difficulty but had not been wearing hearing aids. She had been also suffering from night blindness since adolescence and loss of visual field since age 17. Her visual deterioration had been progressive and she was almost blind at the time of presentation. She did not complain of vertigo. The family history was unremarkable. Physical examination revealed no obvious abnormalities of the external ear or tympanic membrane. For audiologic evaluation, pure tone audiogram (PTA), impedance audiogram, and auditory brainstem evoked response were performed. PTA showed bilateral down-sloping moderate sensorineural hearing loss (Fig. 1A). The threshold of auditory brainstem response was 60 dB HL on both sides. Electronystagmography with a bithermal caloric test demonstrated non specific findings. Temporal bone computed tomography and magnetic resonance imaging showed no specific findings. On ophthalmologic examinations, the lens and the cornea were intact, but the fundoscopic examination revealed bony spicule pigmentation with attenuated retinal vessels in both eyes, the typical finding of RP (Fig. 1B, C). The electroretinogram showed non-detectable waveforms in scotopic and photopic condition in both eyes. Taken together, she was clinically diagnosed as having USH2. The detailed clinical presentation of the patient was previously described by Boo et al. [5].

Molecular genetic analysis
After obtaining written informed consent from the patient, genomic DNA was extracted from peripheral blood leukocytes. We performed polymerase chain reaction (PCR) and direct sequencing of all the 71 coding exons and their flanking intronic sequences of the USH2A using primer pairs designed by the authors. Direct sequencing was performed on the ABI Prism 3100 Genetic Analyzer with the BigDye Terminating Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA). Sequence variations were analyzed with reference to the wild type sequence (GenBank accession No. NM_206933.1) using the Sequencher program (Gene Codes Corp., Ann Arbor, MI, USA). For the protein sequence, reference sequence NP_996816.2 was used. The sequence variation detected was described according to the recommendations by the Human Genome Variation Society, having the A of the ATG translation starting codon as +1 at the cDNA level and the corresponding methionine as +1 at the protein level.

As a result, we detected a heterozygous single nucleotide deletion in the exon 13 of the USH2A, which was expected to result in a frameshift leading to premature termination at the 787th codon (c.2310delA; p.Glu771LysfsX17) (Fig. 2). A review of the literature and the database revealed that this variation was a novel mutation. We also confirmed that the mutation was not observed in 200 control chromosomes.

DISCUSSION
USH2 is known to account for more than half of the Usher syndromes. USH2 could be easily distinguished from USH1 by the severity and audiometric configuration of the hearing loss and by the preserved vestibular function. USH2 is characterized by moderate to severe sensorineural hearing loss with a high frequency sloping configuration. Vestibular response to a caloric stimulus is typically normal. By contrast, the audiograms in USH1 show no detectable hearing across all frequencies, although there may be some residual hearing at the low frequencies.

The USH2A (OMIM 608400) is located in 1q41 and was first described as comprising 21 exons over 259 kb of genomic DNA [6]. However, in up to 40 to 70% of patients with USH2, only a single mutation was detected in these 21 exons of the USH2A gene [7]. The existence of additional exons, suggested by the presence of larger transcripts, was confirmed in 2004 when van Wijk et al. [8] identified 51 novel exons at the 3′-end of the USH2A. A long open reading frame extends from exon 2 to 72, encoding a putative protein of 5,202 amino acids. The functional significance of the long isoform of the USH2A was shown by

Fig. 1. Pure tone audiogram of the patient shows moderate hearing loss with a descending pattern (A). The fundoscopic finding of both eyes showed typical pigmentary degeneration with multiple bone spicule pigments in over the entire retina, attenuated retinal vessels, and a waxy yellowish optic disc. (B) Right, (C) Left. Reprinted from Boo et al. [5] with permission.
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the presence of pathological mutations in several of the 51 novel exons in patients with USH2. Up to date, more than 70 different mutations of USH2A have been reported in patients with USH2 from various ethnicities, with some founder mutations [9]. Small deletion mutations account for ~26% of all mutations reported. In the present study, we identified a novel small deletion mutation in exon 13 of USH2A (c.2310delA; p.Glu771LysfsX17). We speculate that the frameshift transcripts from the mutant allele in the patient were subjected to nonsense-mediated mRNA decay, leading to a deficiency of USH2A protein, and thereby, the disease phenotype. Of note, we could only detect one mutation, suggesting the presence of the other mutation that cannot be detected on direct sequencing, even involving all coding exons and flanking sequences. Baux et al. identified 34 distinct mutations in the USH2A gene associated with USH2, using the same molecular genetic technique as in our patient. They observed one patient who was homozygous for a mutation, 22 who were compound heterozygous for two different mutations, and 2 patients (8%) who were heterozygous for only one mutation [10]. This study, along with our patient described herein, suggests that there would be other types of USH2A mutations that cannot be detected by direct sequencing. The possibilities include mutations occurring in the promoter region, other intronic regions, or in the 3’- or 5’-untranslated region. Large genomic rearrangement mutations (deletion or insertion/duplication) can also be considered. Indeed, one patient in the Baux et al.’s series had a large deletion mutation involving at least exon 22, confirmed by semiquantitative PCR [10]. Recent studies suggest that USH2A protein is integrated into a protein network formed by other Usher-causing proteins [11]. Therefore, another possibility could be that the other mutation might lie in an exon that has not been characterized yet. Finding the other mutation underlying the disease in such patients will give new insight into the molecular pathogenesis of USH.

In summary, we described a novel heterozygote frameshift mutation in the USH2A gene in a Korean woman with USH2. This is the first report of a genetically confirmed case of USH in Korea. To better understand the genetic background of USH2 in the Korean population and thereby to establish an optimized strategy of molecular genetic diagnosis and genotype-phenotype correlations, mutation data from more cases are needed.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Sadeghi M, Cohn ES, Kelly WJ, Kimberling WJ, Tranebjaerg L, Moller C. Audiological findings in Usher syndrome types IIa and II (non-IIa). Int J Audiol. 2004 Mar;43(3):136-43.
2. Sankila EM, Pakarinen I, Kaariainen H, Aittomaki K, Karjalainen S, Sistonen P, et al. Assignment of an Usher syndrome type III (USH3) gene to chromosome 3q. Hum Mol Genet. 1995 Jan;4(1):93-8.
3. Ahmed ZM, Riazuddin S, Khan SN, Friedman PL, Riazuddin S, Friedman TB. USH1H, a novel locus for type I Usher syndrome,
maps to chromosome 15q22-23. Clin Genet. 2009 Jan;75(1):86-91.
4. Bernal S, Meda C, Solans T, Ayuso C, García-Sandoval B, Valverde D, et al. Clinical and genetic studies in Spanish patients with Usher syndrome type II: description of new mutations and evidence for a lack of genotype–phenotype correlation. Clin Genet. 2005 Sep;68(3):204-14.
5. Boo SH, Park DJ, Han CS. A case of type 2 usher syndrome. Korean J Otorhinolaryngol-Head Neck Surg. 2008 Sep;51(9):833-7.
6. Eudy JD, Weston MD, Yao S, Hoover DM, Rehm HL, Ma-Edmonds M, et al. Mutation of a gene encoding a protein with extracellular matrix motifs in Usher syndrome type IIa. Science. 1998 Jun;280(5370):1753-7.
7. Pennings RJ, Te Brinke H, Weston MD, Claassen A, Orten DJ, Weekamp H, et al. USH2A mutation analysis in 70 Dutch families with Usher syndrome type II. Hum Mutat. 2004 Aug;24(2):185.
8. van Wijk E, Pennings RJ, te Brinke H, Claassen A, Yntema HG, Hoefsloot LH, et al. Identification of 51 novel exons of the Usher syndrome type 2A (USH2A) gene that encode multiple conserved functional domains and that are mutated in patients with Usher syndrome type II. Am J Hum Genet. 2004 Apr;74(4):738-44.
9. Dreyer B, Brox V, Tranebjaerg L, Rosenberg T, Sadeghi AM, Moller C, et al. Spectrum of USH2A mutations in Scandinavian patients with Usher syndrome type II. Hum Mutat. 2008 Mar;29(3):451.
10. Baux D, Larrieu L, Blanchet C, Hamel C, Ben Salah S, Vielle A, et al. Molecular and in silico analyses of the full-length isoform of usherin identify new pathogenic alleles in Usher type II patients. Hum Mutat. 2007 Aug;28(8):781-9.
11. Adato A, Lefèvre G, Delprat B, Michel V, Michalski N, Chardenoux S, et al. Usherin, the defective protein in Usher syndrome type IIA, is likely to be a component of interstereocilia ankle links in the inner ear sensory cells. Hum Mol Genet. 2005 Dec;14(24):3921-32.