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Case Report

A De Novo Mutation in *SOX10* in a Chinese Boy with Waardenburg Syndrome Type 2

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Waardenburg syndrome is an autosomal dominant inherited syndromic hereditary hearing loss characterized by varying combinations of sensorineural hearing loss and abnormal pigmentation of the hair, skin, and inner ear. The aim of this study was to analyze the clinical phenotypes and genetic variants of a Chinese boy with Waardenburg syndrome type 2 and to explore the possible molecular pathogenesis of Waardenburg syndrome type 2. Clinical, audiological, and ophthalmologic evaluations were performed on the proband. Clinical data from the principal members in the proband's family were collected through questionnaires. Genetic analysis was conducted, including targeted next-generation sequencing of 144 known deafness genes, Sanger sequencing, and bioinformatic analysis. Waardenburg syndrome type 2was diagnosed in a 4-year-old boy according to the Waardenburg Syndrome Consortium Criteria. The novel missense mutation c.426G>T (p.Trp142Cys) was identified in *SOX10* in the proband but was absent in his parents and the controls. A de novo missense mutation in *SOX10* was the genetic cause of Waardenburg syndrome type 2 in the proband, which was useful for the molecular diagnosis of Waardenburg syndrome type 2.

KEYWORDS: Waardenburg syndrome, *SOX10*, c.426G>T

INTRODUCTION

Waardenburg syndrome is a syndromic hereditary hearing loss characterized by varying combinations of sensorineural hearing loss and abnormal pigmentation of the hair, skin, and inner ear.[1] Waardenburg syndrome is classified into 4 subtypes (WS1-WS4) based on additional symptoms. Waardenburg syndrome type 2 (WS2) is characterized by the absence of dystopia canthorum in patients compared with WS1 Here, we report a boy with WS2 associated with the de novo missense mutation c.426G>T (p.Trp142Cys) in the *SOX10* gene.

CASE PRESENTATION

Subjects and Clinical Features

The proband was a 4-year-old boy suffering from congenital complete sensorineural hearing loss who planned to undergo fitting for a cochlear implant. All genetic diagnoses and prenatal genetic diagnoses were performed with the consent of his parents. Ethical committee approval was received from the Ethics Committee of the 1st Affiliated Hospital of Kunming Medical University (Approval No: 2019L60, Date: 2019.9.2). Clinical data from the principal members in the proband's family were collected through questionnaires. The proband underwent thorough examinations in the areas of intelligence, audiology, ophthalmology, hair, skin, limb joints, and digestive system. He was delivered full-term with a bilateral blue iris and normal inner canthi. An inspection and interview of this boy and his family did not reveal clinical manifestations of any intestinal disorder, such as severe constipation and blockage of the intestine, or abnormalities or limited mobility in the limbs. There were no abnormal manifestations of skin and hair in this family.

Otoacoustic emission, acoustic immittance, auditory brain stem response (ABR), auditory steady-state responses (ASSR), temporal computed tomography (CT), and cranial magnetic resonance imaging (MRI) were performed on the proband. For the assessment of hearing loss in adults, we used pure tone audiometry thresholds, and for children, we used ABR thresholds. Clinical audiological

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examination showed failed bilateral otoacoustic emission. Auditory brain stem response and ASSR showed absent responses to 100 dB nHL stimulus intensity in bilateral ears. Temporal CT and cranial MRI were normal. There were no other family members with congenital hearing loss or blue iris. Clinical features and pedigree analysis are shown in Figure 1. The severity of hearing loss was classified according to the grades of hearing loss and related hearing experience of World Report on Hearing in 2021 (https://www.who.int/publications/i/item/world-report-on-hearing): normal, <20 dB; mild, 20-34 dB; moderate, 35-49 dB; moderately severe, 50-64; severe, 65-79 dB; profound, 80-94 dB; and complete or total hearing loss/deafness, >95 dB.

DNA Preparation and Next-Generation Sequencing

Genomic DNA was extracted from peripheral blood using the QIAGEN DNA Blood Mini Kit (Cat#51106, Qiagen Co., Ltd, Germany.) according to the manufacturer's instructions. Targeted deafness gene capture and next-generation sequencing were conducted by JinYu, Inc. (Guangzhou, China). Sequencing was performed using the TrusightOne sequencing panel on the Illumina NextSeq 500 platform. This panel can analyze 144-inherited deafness-related genes. The sequencing data were analyzed using ANNOVAR software, and several databases, including 1000 Genomes, ESP6500, dbSNP, and HGMD (Human Gene Mutation Database), were used for the screening and annotation of the variants following the The American College of Medical Genetics and Genomics (ACMG) guidelines. The sequencing reads were mapped to the hg19 reference using BWA, and variant calling was performed using SAMtools (0.1.19), Picard (1.123), and GATK (3.3-0-g37228af). All the SNVs and small indels were annotated using ANNOVAR software, through which some important information, such as the gene loci, variant type, frequency of 1000G and ESP6500, and computational prediction results, such as SIFT/PolyPhen, were added to each of the variants. One hundred healthy individuals were enrolled as healthy controls.

Sanger Sequencing

To confirm the mutation type, we used Sanger sequencing to determine whether the *SOX10* mutation co-segregated with the disease phenotype in this family. Based on the results of next-generation sequencing, primers for suspicious pathologic mutations were designed using the online software PRIMER3 (forward primer: 5′-CCAGGGTGGTTGGTGGTAAG-3′, reverse primer: 5′-AAGTGGGCGCTCTTGTAGT-3′). Polymerase chain reaction was performed according to the following procedure: initial denaturation at 95°C for 5 minutes, 30 cycles of denaturation at 95°C for 30 seconds, renaturation at 60°C for 30 seconds, and extension at 72°C for 30 seconds, a final extension at 72°C for 4 minutes. The PCR products were analyzed through gel electrophoresis and then purified. Capillary electrophoresis sequencing was performed using the ABI PRISM 3730 Genetic Analyzer, and the mutations were analyzed.

Validation of Mutations

Several databases were used for annotation, including dbSNP, HGMD, ClinVar, 1000G, and ESP6500, to determine whether the mutation had been previously reported. The functions of proteins with novel mutations were predicted with Mutation Taster (http://www.mutationtaster.org/). We sequenced all the coding exons plus ~100 bp of the flanking intronic sequence of 144 deafness genes in the proband through target gene capture and massively parallel sequencing. One mutation, c.426G>T in exon 2, which leads to a missense mutation, was detected in *SOX10* in the proband. We confirmed the mutation by Sanger sequencing, but it was absent from his parents and 100 unrelated Chinese controls (Figure 2). The mutation was predicted to be damaging by Mutation Taster. As shown in Figure 3, the mutation led to a change from tryptophan to cysteine at position 142 and resulted in a change in protein function. According to multiple databases, including HGMD, ClinVar, 1000G, and ESP6500, c.426G>T (p.Trp142Cys) is a novel mutation in *SOX10* that has not been previously reported.

DISCUSSION

Waardenburg syndrome is an auditory-pigmentary disorder with an incidence of 1 in 40 000 that manifests with sensorineural deafness and abnormal pigmentation of the hair, skin, and iris.[1] Waardenburg syndrome is classified into 4 subtypes, WS1-WS4, depending on the presence of additional symptoms. Waardenburg syndrome is subdivided into 2 types (1 and 2) on the basis of dystopia canthorum. The combination of WS type 1 characteristics with upper limb abnormalities has been called Klein-Waardenburg syndrome or WS type 2. The combination of recessively inherited WS type 2 characteristics with Hirschsprung disease has been called Waardenburg-Shah syndrome or WS type 4. Waardenburg syndrome is genetically heterogeneous, with 6 genes already identified, including *PAX3, MITF, SNAI2*, *SOX10*, *EDNRB*, and *EDN3*.[2]

SOX10 mutations are associated with WS2, WS4, and PCWH (peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, WS, and Hirschsprung disease) and account for approximately 15% of WS2.[3] *SOX10* is extensively expressed in the early development of the inner ear.[4] These results are consistent with animal studies in which heterozygous mutant *Sox10*+/mut mice show an HL phenotype due to the absence of inner and outer hair cells and supporting cells (Deiters' cells) in the cochlea.[3] *SOX10* encodes a member of the SOX (SRY-related HMG-box) family of transcription factors involved in the regulation of embryonic development and in the determination of cell fate. It is composed of 4/5 exons located on chromosome 22q13.1.[2] Three exons code for a 466 amino acid protein containing 3 main functional domains: a highly conserved HMG (high mobility group) domain (102-181 amino acids) that is believed to bind the minor groove of DNA, a SOX Group E domain, and the carboxyterminal (C-terminal) transactivation (TA) domain (354-466 amino acids), through which *SOX10* interacts with other proteins.[3] MITF, one of the most important nuclear transcription factors, is the master regulator of melanogenesis and a target gene of *SOX10*. MITF regulates melanogenesis by activating tyrosinase, TRP1, and TRP2 transcription through the E-box (CANNTG) of their promoters. MITF itself is insufficient to regulate the expression of TYR and DCT without *SOX10*. *SOX10* mutants reduce melanogenesis by attenuating the transactivity of the MITF promoter and the TYR promoter, leading to WS.[3]

In our case, the proband suffered from bilateral complete hearing loss, bilateral blue iris, and normal inner canthi, which are in accordance with the diagnosis of WS2. Hearing loss was found in 71.0% of WS patients and was predominantly bilateral and sensorineural. The prevalence of HL among the different clinical types significantly differed (WS1: 52.3%, WS2: 91.6%, WS3: 57.1%, WS4: 83.5%). Mutations in *SOX10* (96.5%), MITF (89.6%), and SNAI2 (100%) are

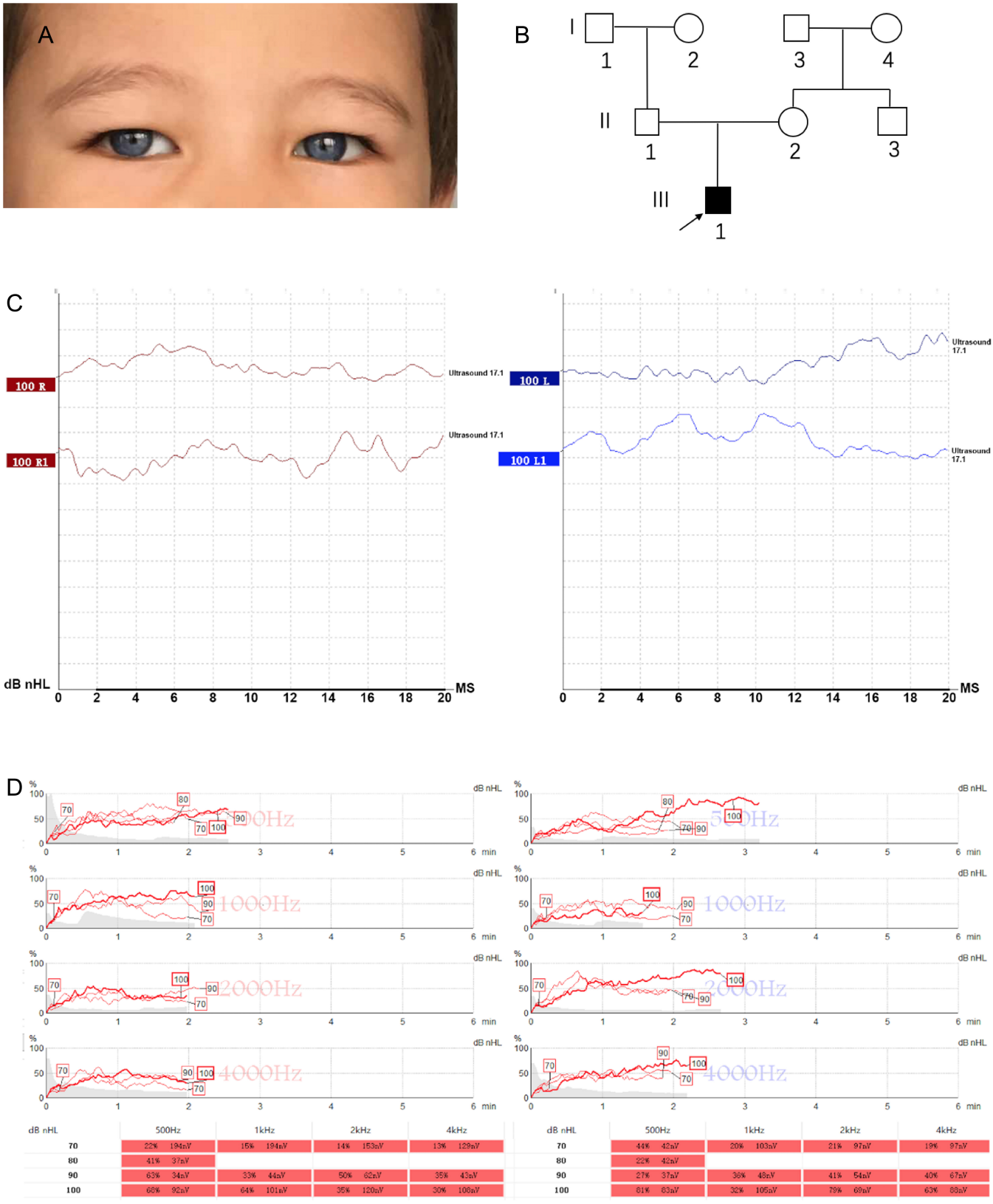

Figure 1. Clinical features and pedigree of the proband. (A) Clinical features of the proband included blue iris in both eyes; (B) family pedigree of the proband; and (C) and (D) auditory brain stem response (ABR) and auditory steady-state responses (ASSR) showed absent responses to 100 dB nHL stimulus intensity in bilateral ears. The patient's father gave consent to use patient's photos in academic journals.

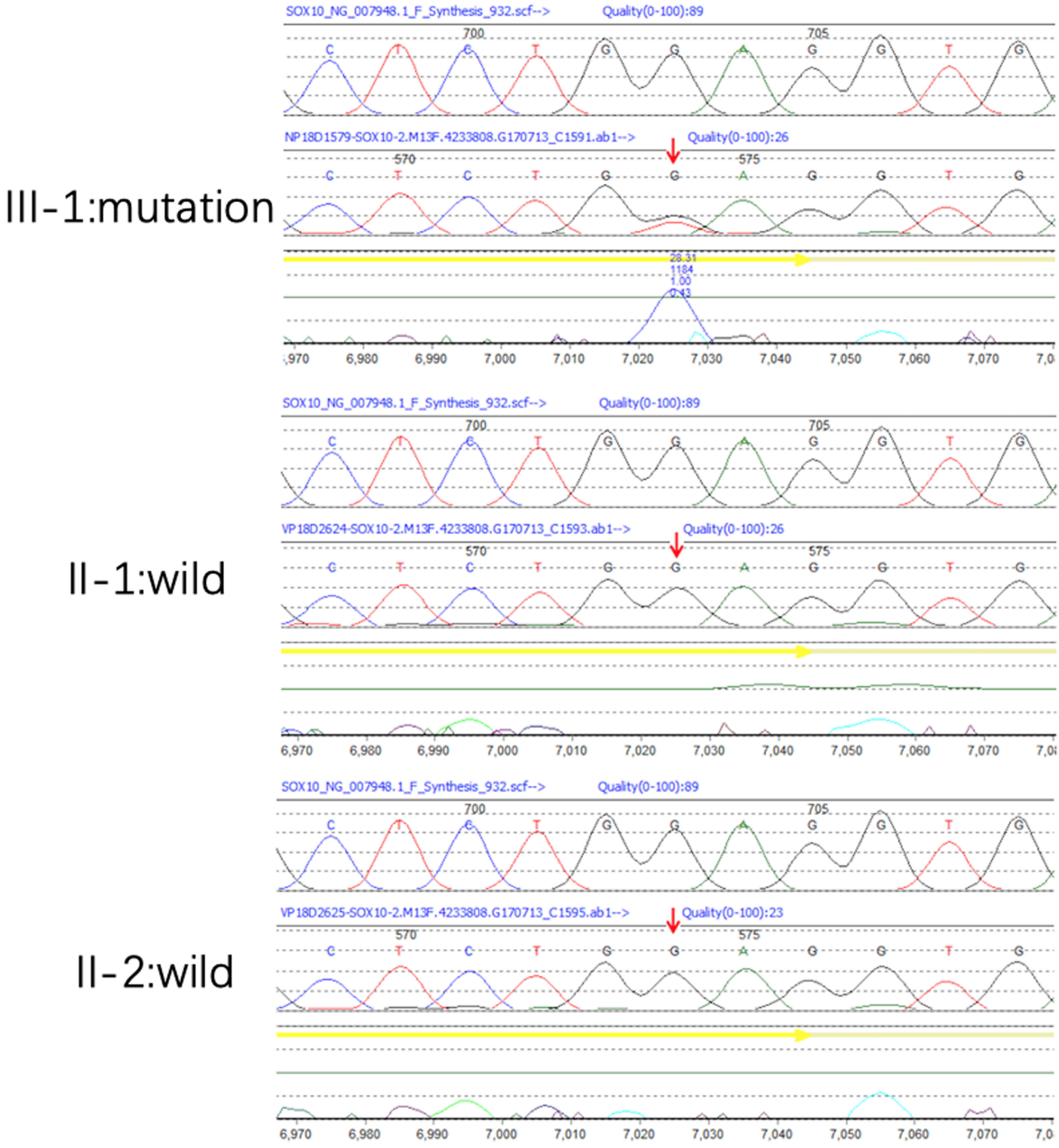

Figure 2. Sanger sequencing map for the family with Waardenburg syndrome. The red arrow indicates the site of the base mutation.

more frequently associated with hearing impairment than other mutations.[5] Most WS-associated *SOX10* mutants are frameshift and nonsense mutations that are unable to transactivate the MITF promoter effectively. The heterozygous c.426G>T *SOX10* mutation we reported is a missense mutation that is located in the HMG domain of *SOX10*. This mutation may cause a change from tryptophan to cysteine at position 142 of the protein (Figure 3). It may damage the DNA-binding capacity of *SOX10*, and it cannot bind the MITF promoter to activate its expression. This mutation was predicted to be damaged by Polyphen-2 and Mutation Taster. It has been reported in ClinVar that c.426G>C and c.426G>A are both pathogenic mutations in WS2. The novel heterozygous c.426G>T *SOX10* mutation we reported was not found in unaffected family members or in the controls.

Figure 3. The mutation led to a change from tryptophan to cysteine at position 142 and resulted in a change in protein function.

CONCLUSION

In summary, we reported the clinical and genetic characteristics of a Chinese family with WS2. We identified a de novo missense mutation in *SOX10* through next-generation sequencing of the deafness gene, Sanger sequencing, and bioinformatic analysis. The more detailed relationship between phenotype and genotype in WS patients has yet to be determined in future research.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of the 1st Affiliated Hospital of Kunming Medical University (Approval No: 2019L60, Date: 2019.9.2).

Informed Consent: Written informed consent was obtained from the patient's parents who agreed to take part in the study.

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Declaration of Interests: The authors have no conflict of interest to declare.

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