A de novo deletion mutation in SOX10 in a Chinese family with Waardenburg syndrome type 4

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Waardenburg syndrome type 4 (WS4) or Waardenburg-Shah syndrome is a rare genetic disorder with a prevalence of <1/1,000,000 and characterized by the association of congenital sensorineural hearing loss, pigmented abnormalities, and intestinal aganglionosis. There are three types of WS4 (WS4A–C) caused by mutations in endothelin receptor type B, endothelin 3, and SRY-box 10 (SOX10), respectively. This study investigated a genetic mutation in a Chinese family with one WS4 patient in order to improve genetic counselling. Genomic DNA was extracted, and mutation analysis of the three WS4 related genes was performed using Sanger sequencing. We detected a de novo heterozygous deletion mutation [c.1333delT (p.Ser445Glnfs*57)] in SOX10 in the patient; however, this mutation was absent in the unaffected parents and 40 ethnicity matched healthy controls. Subsequent phylogenetic analysis and three-dimensional modelling of the SOX10 protein confirmed that the c.1333delT heterozygous mutation was pathogenic, indicating that this mutation might constitute a candidate disease-causing mutation.

Methods
Subjects and clinical evaluation. The patient, his unaffected parents, and 40 unrelated healthy controls were included in this study, and ophthalmic and audiologic examinations were performed. Written informed consent was obtained from all participants, and this study was formally approved by the Ethics Committee of

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Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. All procedures were performed in accordance with the approved guidelines.

**Mutation screening.** Peripheral blood was collected, and genomic DNA was extracted using a DNeasy blood and tissue kit from Qiagen (Hilden, Germany). Polymerase chain reaction (PCR) was performed to amplify all coding exons and intron/exon boundaries of the EDNRB, EDN3, SOX10, PAX3, MITF, and SNAI2 genes. Some of the primers used in the study were referenced from a master’s thesis (title here, Dong Siqi; Chinese PLA General Hospital, Beijing, China), and other primers were designed using Primer 5. Primers are shown in Table 1. PCR of the SOX10 exons was performed in a total volume of 50 μL containing 60 ng of genomic DNA, 400 nM each of the forward and reverse primers, 40 mM dNTPs, and 2.5 U LA Taq DNA polymerase with GC buffer I from TAKARA (Tokyo, Japan). The amplification consisted of an initial denaturation stage at 94 °C for 3 min, followed by 35 cycles consisting of denaturation at 94 °C for 30 s, annealing for 30 s at 60 °C, and extension at 72 °C for 50 s, with an extension step performed at 72 °C for 3 min. Amplification of exons for the remaining genes was performed using 2× PCR master mix under similar conditions, except for annealing at 57 °C. PCR products were purified and sequenced using an ABI 3500 Dx genetic analyser with a BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA, USA), and the sequences were analysed using NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

**Paternity testing and haplotype analysis.** Five short tandem-repeat markers (STRs; D22S283, D22S1177, D22S1045, D22S272, and D22S423) ranging from chr22:36750705 to chr22:40382524 and five single nucleotide polymorphisms (SNPs; rs139873, rs139885, rs4821733, rs3952, and rs5756908) were selected from the UCSC Genome Browser (http://genome.ucsc.edu/), and linkage-disequilibrium analysis was performed based on LD TAG SNP selection (TagSNP; http://snpinfo.niehs.nih.gov/snpinfo/snptag.php). STR and SNP primers are shown in Table 1.

**Protein structure prediction.** Both the wild-type and mutated SOX10 protein sequences were used to perform protein structure prediction using I-TASSER (http://zhanglab.ccmb.med.umich.edu/ITASSER/) as previously reported16–19. In I-TASSER, the B-factor, which indicates the extent of the inherent thermal mobility of residues/atoms in proteins, is calculated from threading template proteins from the Protein Data Bank along with sequence profiles derived from sequence databases. The normalized B-factor of the target protein was defined by $B = (B' - \bar{u})/s$, where $B'$ represents the raw B-factor value, and $\bar{u}$ and $s$ represent the mean and standard deviation of the raw B-factors along the sequence, respectively.
| Primer name | Sequence |
|-------------|----------|
| SOX10 E1F (765 bp) | AGATGGGTTTAGCTGGAGCA |
| SOX10 E1R (765 bp) | ACCTGGTCTTCCAGCCCTAT |
| SOX10 E2F (866 bp) | GATATTCTTGGGCTCTACA |
| SOX10 E2R (866 bp) | CTTGGCCAGTATGACTACG |
| SOX10 E3A F (686 bp) | GCTGCCAAATGTGAAACTTA |
| SOX10 E3A R (686 bp) | GATGTGGCAATAAGGGCTCC |
| SOX10 E3BF (561 bp) | AGCCCGAGTGAAAGACAGA |
| SOX10 E3BR (561 bp) | TCTGTCCAGCCGCTTCTCCT |
| EDN3 E1 F (407 bp) | CAGAAGCCAGAAAGGCGA |
| EDN3 E1 R (407 bp) | CCAGGGCAAGAGGGGAGG |
| EDN3 E2 F (597 bp) | TTTGCAGACATTCTGCTTG |
| EDN3 E2 R (597 bp) | CTCGACCTGCAAGAGAGC |
| EDN3 E3 F (480 bp) | GGTGCACAGTTCACTCCAGA |
| EDN3 E3 R (480 bp) | CCCACAGGGACAGTAGGT |
| EDN3 E4 F (607 bp) | CGTCTGTGAAACCCAGTGT |
| EDN3 E4 R (607 bp) | CATCACTGCCAGACGTCA |
| EDN3 E5 F (424 bp) | GATCTGGCAATTGGCGTGAAG |
| EDN3 E5 R (424 bp) | TCTTGGGTTGGGTGTCCTG |
| EDN3 E6 F (748 bp) | CTTTTGAGCGTGGATACTGG |
| EDN3 E6 R (748 bp) | GGGAGCTAAAGGGAAGCTC |
| EDN3 E7 F (498 bp) | AACACACTTTCCCTGTCACATAC |
| EDN3 E7 R (498 bp) | TTCTACTTGCTGTCATTTTGG |
| EDNRB E1 F (555 bp) | CTGTGAGGACAGTCAGTGA |
| EDNRB E1 R (555 bp) | AGCTTGAGTCTTATTGACCA |
| EDNRB E2 F (666 bp) | CAGAAGAACAGATAGCTCTG |
| EDNRB E2 R (666 bp) | CACCTGCGTTCCACTTCACA |
| EDNRB E3 F (466 bp) | CTTCCCTGTCCTCCTCAACA |
| EDNRB E3 R (466 bp) | GCCCGAGAAGGGAGGAGT |
| EDNRB E4 F (383 bp) | CACACATTTTGCTGGCCTGA |
| EDNRB E4 R (383 bp) | GAGGGGGAACACAGCACAGA |
| EDNRB E5 F (493 bp) | GCAGTAGGAGTCAGTCCTG |
| EDNRB E5 R (493 bp) | GCCAGGAACTTGCTGGCCTG |
| EDNRB E6 F (466 bp) | AAGAGGGAAATATAAAAGAGC |
| EDNRB E6 R (466 bp) | TTCTTCCATGGCCGTAACAA |
| PAX3 E1F (620 bp) | GAACATTTGCCCAGACTCGT |
| PAX3 E1R (620 bp) | TCCAAAACAACAGGGGACG |
| PAX3-2F (503 bp) | CGATGCTGGCGAGTCCAG |
| PAX3-2R (503 bp) | CAGCACCCTCACAACACTCAG |
| PAX3-3F (420 bp) | TGGGATGTGTCTGTGTCTG |
| PAX3-3R (420 bp) | TCTTCTACTGCTGTCTTTG |
| PAX3-4F (432 bp) | CAGAAGAACAGATAGCTCTG |
| PAX3-4R (432 bp) | CTGTGAGGACAGTCAGTGA |
| PAX3-5F (508 bp) | ATATGCTGTCACAGAG |
| PAX3-5R (508 bp) | TACGGATTTGTTAGACCTGT |
| PAX3-6F (445 bp) | CTGGAGAATGAGGAGT |
| PAX3-6R (445 bp) | GAGGTTGACCTCGGTG |
| PAX3-7F (445 bp) | TCTGCTGGATGGAGT |
| PAX3-7R (586 bp) | TTTGATGGAAGCCAGTAGA |
| PAX3-8F (543 bp) | CAGCATTGCTGCCTGAG |
| PAX3-8R (543 bp) | GTCTCAACAAATTAACCGC |
| MITF E1F (630 bp) | GAGGTCCAGACTGCGGTGTC |
| MITF E1R (630 bp) | GCCATCTCCGAGCTTCCA |
| MITF E2F (628 bp) | GCCCTGATAAAATGGCTTTG |
| MITF E2R (628 bp) | AGCCAGTGAAAGAATTAGG |
| MITF E3F (564 bp) | GACAGTGCTGCCTGTACATA |
| MITF E3R (564 bp) | TGCTCTACACCAATACCCC |
| MITF E4 F (310 bp) | TCATCTTTTGGTGCAGATTCCAC |

Continued
Clinical findings. A 1-year-old male patient was referred to our hospital with the chief complaint of Hirschsprung disease accompanied by heterochromia iridis and congenital hearing loss. Based on these clinical features, he was first suspected to be a WS4 patient. Neither parent of the patient exhibited similar symptoms (Fig. 1).

Identification of a novel SOX10 heterozygous deletion mutation. A heterozygous deletion mutation (c.1333delT) in SOX10 was identified in the patient, resulting in replacement of the 445th Ser with Gln and a shift in the reading frame to produce a longer protein consisting of 501 amino acids (p.Ser445Glnfs*57) as compared with the wild-type SOX10 protein (467 amino acids; Fig. 2, Table 2). We subsequently verified that this mutation did not exist in any of the widely used genomic databases, confirming that c.1333delT constitutes a novel deletion mutation. Moreover, this mutation was not found in the unaffected parents or in 40 unrelated healthy control subjects. However, a heterozygous missense mutation (c.1363C>A) in MITF was found in both the patient and his father, but not in his mother (Fig. 2). This mutation was found in the dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases (rs78962087) and is reportedly benign. Furthermore, no mutation was found in the EDN3, EDNRB, PAX3, or SANI2 genes.

Table 1. Primers used in this study.

| Primer name       | Sequence                      |
|-------------------|-------------------------------|
| MITF-4 R (310 bp) | TGCTTAAGTTTTTCAGGAAGGTG       |
| MITF-5 F (343 bp) | GACCATTATGCTTTTGGAATAA       |
| MITF-5R (343 bp)  | TGTGATCCCTGAGAATATCTCCATT    |
| MITF-6F (425 bp)  | TGGAGGATCCTGATCCCTCTCT       |
| MITF-6R (425 bp)  | AAAAGTTACGTTCCATGAGTTGG      |
| MITF-7F (350 bp)  | GCTTTTGAAAACATGCAAGC         |
| MITF-7R (350 bp)  | GCTGTAGAATCAACTCTCCTCT       |
| MITF-8 F (527 bp) | AAAGGCTCTTGGAATAATGTGGA      |
| MITF-8 R (527 bp) | AGAAAGCCACCTCCCTCACA         |
| MITF-9F (425 bp)  | CTATATCATGAGAACACCAGCA       |
| MITF-9R (425 bp)  | CACACACAGAATCCAAACCAA        |
| MITF-10F (466 bp) | CTATAGGCAGCCACCTCACA         |
| MITF-10R (466 bp) | TCTCTGGCTATTTGATAAAAAGC      |
| SNAI2 E1 F (388 bp) | CGGCTCTGAGTCGTAATAGGA        |
| SNAI2 E1 R (388 bp) | GCTCTCTTTCAGGACACTGTTA      |
| SNAI2 E2 AF (534 bp) | GCCCTCTCAAATGAGCTCTATC    |
| SNAI2 E2 AR (534 bp) | TTTTCTGAGACTGGGCAATGC       |
| SNAI2 E2 BF (565 bp) | GCCCCATTAGTGGTAGAAAG       |
| SNAI2 E2 BR (565 bp) | GATCTTTGAGACCAAAACCTC      |
| SNAI2 E3 F (556 bp) | GCTTTTGCGCTTCCTCTTATAT      |
| SNAI2 E3 R (556 bp) | TCTCTCAATCCTAGCAGCATCAGC     |
| D22S283 F (217 bp) | FAM-ACAAACTAGCTCTAGTCCCTGG  |
| D22S283 R (217 bp) | TGAAGCCAGGAGATTTTCCT       |
| D22S1177 F (186 bp) | GCGCCTCCTGAGGCACCACAT      |
| D22S1177 R (186 bp) | AGCCTGACAGGACAGGCAAG       |
| D22S1045 F (153 bp) | FAM-GCTATAGTTCCTCCCATGAT    |
| D22S1045 R (153 bp) | ATGTAAGTCGCTCTCAAGATGCC     |
| D22S223 F (132 bp) | FAM-GAAGTTTTTGTGGCTGGCAC   |
| D22S223 R (132 bp) | AATGACAGCACCACATTAAG       |
| D22S423 F (123 bp) | FAM-CACACTCTGTACACACATAACA |
| D22S423 R (123 bp) | AAAACCAACTGACTGTTTAA        |
| rs139885 F (625 bp) | CACCCATGCCTACTCTCTTTC     |
| rs139885 R (625 bp) | GAGACCTGGAGACACATAACA      |
| rs3952 F (263 bp)  | CTTCTGTCGAGCTTGGGAATA      |
| rs3952 R (263 bp)  | GTTAGAGGGAGGTGCGGAGA        |
| rs5756908 F (366 bp) | AGTTCCTCCCAAAGATCTGTCCC    |
| rs5756908 R (366 bp) | CAGTGTAGCTCCCTCTCCCAA     |
| rs4821733 F (434 bp) | GAGGCGATGGCAGATACC          |
| rs4821733 R (434 bp) | AATATGCTGTAATGGCGGAG       |
| rs139873 F (374 bp) | AAAAGACTCTCGTGTCTCCA        |
| rs139873 R (374 bp) | CCCACAGTGTCGATTTTC         |

Results

Clinical findings. A 1-year-old male patient was referred to our hospital with the chief complaint of Hirschsprung disease accompanied by heterochromia iridis and congenital hearing loss. Based on these clinical features, he was first suspected to be a WS4 patient. Neither parent of the patient exhibited similar symptoms (Fig. 1).
These results suggested that the heterozygous deletion mutation (c.1333delT) in \textit{SOX10} might be associated with the WS4 phenotype of the patient.

**Paternity testing and haplotype analysis.** \textit{SOX10} c.1333delT is located in chr22:38369570. To confirm the paternity of the father, five STRs (D22S283, D22S1177, D22S1045, D22S272, and D22S423) ranging from chr22:36750705 to chr:40382524 and five SNPs (rs139873, rs139885, rs4821733, rs3952, and rs5756908) ranging from chr22:38359666 to chr:38476579 were selected from the UCSC Genome Browser (http://genome.ucsc.edu/) based on their proximity to the mutation site. Paternity testing by haplotype analysis confirmed that these were the biological parents of the patient with WS4 (Figs 3 and 4).

**Protein structure prediction.** The wild-type \textit{SOX10} protein consists of 467 amino acids and contains three helices, whereas the \textit{SOX10} deletion mutation (c.1333delT) results in a protein consisting of 501 amino acids with four helices (Fig. 5). The wild-type and mutant variants shared identical sequences in the first 444 amino acids, with differences occurring after this point.

**Discussion**

WS is classified into four primary phenotypes. WS1 is caused by mutations in \textit{PAX3} and distinguished by the presence of dystopia canthorum (lateral displacement of the inner canthi). WS2 is caused by mutations in \textit{MITF}, \textit{SOX10}, or \textit{SNAI2} and distinguished from type 1 by the absence of dystopia canthorum. WS3 is caused by mutations in \textit{PAX3}, with patients presenting both dystopia canthorum and upper limb abnormalities. WS4 is caused by mutations in \textit{EDNRB}, \textit{EDN3}, or \textit{SOX10}, with patients presenting with phenotypes associated with Hirschsprung disease\textsuperscript{1,20–23}. Here, we described a Chinese patient with clinical features of WS4 and identified a novel heterozygous deletion mutation [c.1333delT (p.Ser445Glnfs*57)] in \textit{SOX10} that was absent in his unaffected parents and 40 ethnicity matched healthy controls. To the best of our knowledge, this constitutes the first report of this mutation, suggesting it as a candidate disease-causing mutation.
**SOX10** is located on chromosome 22 and encodes an essential DNA-binding nuclear transcription factor consisting of 467 amino acids and belonging to the SOX family involved in modulating embryonic development and determining cell fate. **SOX10** may act as a transcriptional activator upon forming a complex with other proteins and/or as a nucleocytoplasmic shuttle protein critical for neural crest and peripheral nervous system development. Mutations in this gene are associated with WS4 and are present in ~50% of WS4 patients.

**SOX10** contains a highly conserved high mobility group (HMG) DNA-binding domain and a C-terminal transactivation (TA) domain that is enriched in serine, proline, and acidic residues. Additionally, **SOX10** contains two separate TA domains, with one localized in the C-terminal region and the other in the central region of the structure. The C-terminal TA domain is frequently involved in various interactions, whereas the TA domain located in the centre of the structure is only involved in TA-related activity in certain cell types and under certain developmental conditions. **SOX10** binds to the promoters of its target genes via the HMG domain, with several studies reporting the importance of the TA domain for inducing transcriptional activation of its target genes. Wang et al. identified a c.1063C>T (p.Q355*) mutation in **SOX10** in a family with WS4 and reported that the mutated **SOX10** variant retained nuclear localization and DNA-binding capabilities comparable to those observed in wild-type **SOX10**; however, the mutated **SOX10** variant was unable to activate transcription of *MITF* via its promoter and acted as a dominant-negative repressor as compared with activity associated with wild-type **SOX10**. In this study, we detected a c.1333delT (p.Ser445Glnfs*57) mutation in **SOX10** in a family with WS4, with the mutated **SOX10** variant sharing sequence homology with only the N-terminal 444 amino acids of the wild-type protein. Furthermore, we identified an additional helix in the C-terminal region of the mutated **SOX10** variant (Fig. 4), which may affect its normal biological function.

**Table 2. Genetic variants found in this family with WS4.**

| Gene | Variant | Protein level | Type        | Father | Mother | Report |
|------|---------|---------------|-------------|--------|--------|--------|
| Sox10 | c.1333delT | p.Ser445Glnfs*57 | heterozygous | Normal | Normal | No     |
| MITF | c.1363C>A | p.Leu455Ile | heterozygous | heterozygous | Normal | Yes    |

Figure 3. Paternity testing and haplotype analysis. 601, patient; 602, father; 603, mother.
In conclusion, here, we described a de novo heterozygous deletion mutation \( [\text{c.1333delT (p.Ser445Glnfs*57)}] \) in \( \text{SOX10} \) identified in a Chinese family with WS4. Our analyses indicated that this mutation might constitute a candidate disease-causing mutation associated with WS4.

Figure 4. SNP analysis of the Chinese family with WS4. Five SNPs (rs139873, rs139885, rs4821733, rs3952, and rs5756908) were selected.

Figure 5. Protein structure prediction. (a) Wild-type \( \text{SOX10} \) protein structure. (b) The mutated \( \text{SOX10} \) protein structure.
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Author Contributions

H.L. and Y.L. designed this work, X.W. and Y.Z. performed sequencing and analysis, N.S., C.W., and J.P. prepared figures, and X.W. wrote the manuscript.

Additional Information

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