Two novel mutations of PAX3 and SOX10 were characterized as genetic causes of Waardenburg Syndrome

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Abstract

Background: The objective of this study was to investigate the genetic causes of two probands diagnosed as Waardenburg syndrome (WS type I and IV) from two unrelated Chinese families.

Methods: PAX3 and SOX10 were the main pathogenic genes for WS type I (WS I) and IV (WS IV), respectively; all coding exons of these genes were sequenced on the two probands and their family members. Luciferase reporter assay and co-immunoprecipitation (CO-IP) were conducted to verify potential functional outcomes of the novel mutations.

Results: The first proband is a 9 years old girl diagnosed with WS I. A novel PAX3 heterozygous mutation of c.372-373delGA (p.N125fs) was identified, which results in a frameshift and truncation of PAX3 protein. In family II, a 2 years old girl was diagnosed with WS IV, and Sanger sequencing revealed a de novo SOX10 mutation of c.1114insTGGGGCCCCACACTACCCGAC (p.Q372fs), a frameshift mutation that extends the amino acid chain of SOX10 protein. Functional studies indicated that the novel mutation of SOX10 had no effects on the interaction of SOX10 and PAX3, but reduced transactivate capacity of melanocyte inducing transcription factor (MITF) promoter. Both PAX3 and SOX10 mutation-induced defects of MITF transcription might contribute to the WS pathogenesis.

Conclusion: We revealed a novel mutation in PAX3 and a de novo mutation in SOX10, which might account for the underlying pathogenesis of WS. This study expands the database of both PAX10 and PAX3 mutations and improves our understanding of the causes of WS.

KEYWORDS
hearing loss, PAX3, SOX10, Waardenburg syndrome
1 | INTRODUCTION

Waardenburg syndrome (WS) is a rare autosomal dominantly inherited disease, which is characterized by hearing loss and pigment alteration in hair, skin, and iris (Read & Newton, 1997). The incidence of WS is estimated 1/40,000; however, it accounts for 2% to 5% of all congenital hearing loss (Dourmishev, Dourmishev, Schwartz, & Janniger, 1999; Song et al., 2016; Zaman, Capper, & Baddoo, 2015), which is the most common clinical feature of WS. WS is divided into four subtypes (WS I-IV) depending on the presence or absence of additional symptoms such as dystopia canthorum, giant colon, and upper limb abnormalities. WS I (OMIM #193500) and WS II (OMIM #193510) are the most common types, while WS III (OMIM #148820) and WS IV (OMIM #277580) are rare (Read & Newton, 1997). WS is genetically different and six genes have been identified: paired box 3 (PAX3), SRY-box transcription factor 10 (SOX10), SRY-box transcription factor 2 (SNAI2) (Otreba, Milinski, Buszman, Wrzesniok, & Beberok, 2013), PAX3 is associated with WS I and WS II, MITF and SNAI2 are found to be involved in the WS II. SOX10, EDNRB, and EDN3 are responsible for WS II and WS IV (Pingault et al., 2010). All these genes play crucial roles in the formation and development of melanocytes.

WS with dystopia canthorum is diagnosed as WS I and PAX3 is the most pathogenic gene. PAX3 encodes the paired box 3 transcription factor and contributes to the development of the central nervous system, skeletal muscle, and melanocytes (Wildhardt et al., 2013). For WS IV, it is characterized by additional feature of megacolon syndrome. In humans, 45%-55% of WS IV cases are involved in the mutation of SOX10, which works in the development and differentiation of melanocytes. In the present study, PAX3 and SOX10 were individually screened in two Chinese probands with WS I and WS IV, as well as their family members. A novel mutation in PAX3 [c.372-373delGA (p.N125fs)] and a de novo mutation in SOX10 [c.1114insTGGGGCCCCACACTACCCGAC (p.Q372fs)] were identified by Sanger sequencing. The mutation of N125fs resulted in truncated PAX3 while Q372fs caused structural extension of SOX10. To understand functional consequences of Q372fs-induced SOX10 extension, transcriptional activity of MITF was detected by dual-luciferase reporter assay. The effects of mutant SOX10 on the interaction between SOX10 and PAX3 were studied using co-immunoprecipitation assay.

2 | MATERIALS AND METHODS

2.1 | Patients

Two Chinese girls with severe-profound hearing loss and their family members were recruited. Photos and blood were collected before informed consent was signed. Ethics Committees of Beijing Children’s Hospital approved our study. After medical history was described by their parents, the two girls got both physical and radiological examination. Auditory evaluations were conducted by play audiometry (PA), auditory steady-state response (ASSR), auditory brainstem response (ABR), and distortion product otoacoustic emission (DPOAE).

2.2 | Gene mutation analysis

Blood DNA was extracted from the probands and their family members using Blood DNA Kit (CW BIO, China). Polymerase chain reaction (PCR) was used to amplify fragments covering coding exons of PAX3 (NM_181457.3) and SOX10 (NM_006941.4) with specific primers (Table S1). PCR experiment was done as previously described (Yu et al., 2019). All PCR products were evaluated by 1% agarose gel electrophoresis and sequenced by Sanger sequencing (Applied Biosystems, USA). Gene mutation was identified by Sequencher software.
2.3 | Plasmids construction

To generate plasmids of pGV230-PAX3-HA and pGV362-SOX10-Flag, full-length cDNA of PAX3 and SOX10 were PCR amplified and subcloned into their corresponding vectors. For pGV362-SOX10-Q372fs-Flag construction, frameshift mutation (c.1114ins TGGGGCCCCACACTACACCGAC) was synthesized and subcloned into the vector. To construct pGV238-MITF luciferase reporter, MITF promoter region (~1500 bp to +50 bp from transcription start site) was synthesized and subcloned into the luciferase vector. All constructs were verified by direct Sanger sequencing.

2.4 | Dual-luciferase reporter assay

Human melanoma A375 cell line was cultured in DMEM with 10% fetal bovine serum. Cells were seeded in 24-well plates for 24 hr and then transfected by MITF luciferase reporter together with SOX10-wt-Flag or SOX10-Q372fs-Flag using Lipofectamine 2000 (Life Technologies). Renilla luciferase reporter plasmid was co-transfected to normalize transfection efficiencies. Luciferase activity was finally measured (CLARIOstar, BMG labtech) using Dual-Luciferase Reporter Assay system (Promega).

2.5 | Co-immunoprecipitation (CO-IP) and western blotting

SOX10 interacts with PAX3 to regulate MITF expression. To study the interaction between SOX10 and PAX3, Co-IP was performed. Briefly, PAX3-HA plasmid was co-transfected with SOX10-wt-Flag or SOX10-Q372fs-Flag in A375 cells for 48 hr. Total proteins were extracted and concentration was determined. Equal amounts of proteins were incubated with anti-HA antibody (Abcam) overnight at 4°C. Commercial protein A agarose beads (Roche, Switzerland) were added and incubated for 4 hr at room temperature. The beads were washed, collected, and resolved repeatedly in chilled GUO HEPES buffer for six times. Finally, the beads were boiled for western blotting detection, according to standard protocol previously described (Yu et al., 2018). Protein bands were obtained by Odyssey CLx imaging system (LI-COR).

3 | RESULTS

3.1 | Clinical features and evaluation

These two girls were from two unrelated Chinese families. Both of them delayed in speech development and diagnosed...
as bilateral hearing loss. The first proband was 9 years old and diagnosed with WS I. As shown in Figure 1a, physical examination found iris heterochromia in eyes, a pinch of white hair on forehead, and dystopia canthorum. Symptoms of iris heterochromia in eyes and unilateral severe hearing loss were also found in her mother. For one of her younger brothers, iris heterochromia was in left eye with normal hearing. The other younger brother was normal. Temporal bone CT scan showed that the structure of the inner ear of the girl was normal (Figure 1b). However, she suffered from severe sensorineural hearing loss in both ears (Figure 1c). The second proband was 2 years old diagnosed with WS IV. Both her parents were normal. Physical examination found iris heterochromia in her right eye and her hair was gray (Figure 2a). She also suffered from Hirschsprung disease and got surgery when she was 2 months old. Both CT and MRI scan showed that the structure of the inner ear and cochlear nerve was normal (Figure 2b). Based on audiometry (PA) detection, her both ears got severe hearing loss (Figure 2c). Both probands have received unilateral cochlear implantation (CI) and were in our close follow-up.

3.2 Identification of novel mutations in PAX3 and SOX10

The pedigree chart of family I was described in Figure 3a. As shown in Figure 3b, novel heterozygous mutations in both intron and exon of PAX3 were detected. Based on the fact that genotype was in accordance with clinical appearance, c.372-373delGA (p.N125fs) in exon 3 might be the pathogenic mutation. This mutation caused a stop codon at position of 143 amino acid and truncated PAX3 (Figure 3c). The protein truncation retains most of the paired domain (PD) but deletes other domains (Figure 3d), which might be crucial for PAX3 function.

In family II, a fragment of 23bp was inserted in exon 4 of SOX10 at site of 1114bp (Figure 4a,b). It is a de novo mutation because c.1114insTGGGGCCACACTACACCGAC (p.Q372fs) only occurred in the proband but not inherited from her parents. It caused a frameshift mutation of SOX10 from position of 372 to 508 amino acid, which is 41 amino acids longer than wide-type SOX10 (Figure 4c). Functionally, the structural extension might affect its binding with PAX3. The schematic image of SOX10 protein and its mutant is described in Figure 4d.

FIGURE 2 Clinical features of the proband diagnosed with WS IV. (a) Iris heterochromia in right eye and premature graying of the hair. (b) CT and MRI showed that the structure of the inner ear and cochlear nerve is normal. (c) Bilateral play audiometry (PA) detection
3.3 | The effect of mutant SOX10 on MITF transcriptional activity

The role of SOX10 in melanocyte is to regulate MITF expression together with PAX3. To identify whether Q372fs affects SOX10 protein function, luciferase assay was performed. As shown in Figure 5a, wild-type SOX10 induced MITF promoter activity by approximately fourfold than mutant SOX10 in A375 cells. As a result, mutant SOX10 loses its ability to activate MITF promoter.

3.4 | The effect of mutant SOX10 on the interaction between SOX10 and PAX3

We have demonstrated that mutant SOX10 cannot activate MITF expression, but the molecular mechanism is not clear. Since SOX10 interacts with PAX3 to regulate MITF expression (Dai et al., 2019), the interaction changes might help in answering this issue. To investigate whether SOX10 Q372fs affects the interaction between SOX10 and PAX3, Co-IP assay was performed. As shown in Figure 5b, both wild-type SOX10 and mutant SOX10 could be co-immunoprecipitated when co-expressed with PAX3 in A375 cells. This result suggested that Q372fs-induced SOX10 protein extension did not affect its interaction with PAX3.

4 | DISCUSSION

Two patients from two unrelated families were diagnosed as WS I and WS IV, which is genetically associated with mutations in PAX3 and SOX10, respectively. To investigate potential pathogenic causes, a genetic and functional analysis was performed. We identified a novel mutation of PAX3 [c.372-373delGA (p.N125fs)] in proband I, which truncated PAX3 and resulted in protein structure disruption. In proband II, the de novo mutation of SOX10 [c.1114insTGGGGCCCACTACACCCGAC (p.Q372fs)] caused structural extension of SOX10. Further in vitro results demonstrated that the mutant SOX10 inhibited transcriptional activity of MITF but not affected the interaction between SOX10 and PAX3.

The first proband in family I was diagnosed as WS I, based on additional symptoms of dystopia canthorum and phenotypes...
in family members. Genetically, *PAX3* is the main causative gene of WS I, and 90% of WS I patients carry *PAX3* variants. Recently, many novel *PAX3* mutations were reported, such as c.91-95delACTCC, c.808C>G, and c.117C>A (Choi, Choi, & Lee, 2018; Li et al., 2019; Ma, Lin, et al., 2019). To verify the clinical diagnosis in proband I, genetic screening of *PAX3* was performed and c.372-373delGA (p.N125fs) was identified. This frameshift mutation changed *PAX3* structure and may result in loss of gene function. Up to March 2019, a total of 164 variants in *PAX3* have been reported in the Human Gene Mutation Database (HGMD), among which point mutations and deletions comprised more than 90% (Stenson et al., 2017). As no report of c.372-373delGA was found in the HGMD, we consider it is a novel mutation. This mutation detected in the proband and his brother came from their mother and was inherited autosomal dominantly.

*PAX3* locates on chromosome 2q35, encoding a 479 amino acids protein, which is a transcriptional factor from the paired box (PAX) family (Boudjadi, Chatterjee, Sun, Vemu, & Barr, 2018). *PAX3* contributes to the migration and differentiation of melanocytes, which originate from the embryonic neural crest. In melanoblast, *PAX3* is associated with the expression of markers for melanocyte development, including *MITF* (Boudjadi et al., 2018; Dye, Medic, Ziman, & Coombe, 2013). Due to the fact that color change is a typical characteristic for WS, *PAX3* function in pigmentation defects of the hair, skin, and eye might account for the pathogenesis of WS I (Bocangel et al., 2018). Previous functional study has demonstrated that *PAX3* R270G mutation failed to activate *MITF* promoter but retained abilities of nuclear distribution and DNA-binding (Niu et al., 2018). However, another study reported that *PAX3* H80D can retain partial activity (Zhang et al., 2012).

According to the guidelines from ACMG (American College of Medical Genetics and Genomics), one missense variant is known to be pathogenic in most cases. However, frameshift mutation was a null variant, which may disrupt gene function (Richards et al., 2015). Therefore, the new reported frameshift mutation of c.372-373delGA in *PAX3* might be the major molecular pathogenesis of WS I in this family.

With regard to the second proband in family II, a de novo mutation of c.1114insTGGGGCCCCCACACTACCCGAC (p.Q372fs) was found, which induced structural extension of *SOX10*. In humans, half of WS IV cases are associated with *SOX10* mutation and more than 160 variants have been reported in HGMD till March 2019 (Stenson et al., 2017). *SOX10* encodes a transcription factor and acts as a transcriptional activator to regulate *MITF* expression by forming a protein complex with *PAX3* (Kamachi, Cheah, & Kondoh, 1999; Seberg, Van Otterloo, & Cornell, 2017). Consistent with previous studies (Dai et al., 2019; Wang et al., 2017), our results demonstrated that Q372fs *SOX10* lost the ability to activate *MITF* expression. Generally, *SOX10* is localized in the nucleus and promotes target DNA transcription (Seberg et al., 2017).

**FIGURE 4** Pedigree map and *SOX10* (NM_006941.4) sequence in family II. (a) Pedigree map. Squares and circles denote males and females, respectively. (b) Sequence electropherograms showed that c.1114insTGGGGCCCCCACACTACCGAC (p.Q372fs) was a de novo mutation, which was not inherited from parents. (c) Q372fs caused a frameshift mutation from position of 372 to 508 amino acid, which is 41 amino acids longer than wide-type *SOX10*. (d) The putative schematic representation of *SOX10* protein and the extended mutants.
Functional study reported that SOX10 mutation of p.L141P affected DNA or protein-binding capacity, and inhibited MITF expression by inducing aberrant cytoplasmic and nuclear localization (Wang et al., 2017). However, mutant SOX10 was also reported to reduce MITF transcription but not affect nuclear localization and DNA-binding capacity (Dai et al., 2019). Therefore, SOX10 function is not localization-dependent and Q372fs SOX10-induced suppression of MITF might be the genetic cause of WS IV.

Although we have demonstrated that mutant SOX10 affected MITF expression, the molecular mechanism is not clear. Functionally, SOX10 was reported to interact with PAX3 to regulate MITF expression (Dai et al., 2019). To determine whether the reduced MITF transcription was caused by defects in the interaction between PAX3 and SOX10, Co-IP was performed but the results showed that Q372fs SOX10 did not affect the ability to interact with PAX3. Although the structure of SOX10 is not fully characterized (Pingault et al., 1998), it structurally contains a DNA-binding HMG (high mobility group) domain, a dimerization region right upstream to the HMG, a conserved domain in the center, and a trans-activation (TA) domain at the extreme C-terminus (Pingault et al., 2010). Herein, the frameshift mutation of Q372fs made SOX10 protein extended in structure, destroying the TA domain but not affecting other domains. This might be the reason why Q372fs SOX10 failed to transactivate MITF, but still can interact with PAX3.

In summary, we identified a novel mutation in PAX3 and a de novo mutation in SOX10 in two unrelated probands diagnosed with WS I and WS IV, respectively. In family I, the mutation of N125fs in PAX3 leads to a frameshift mutation and truncates PAX3 protein. In family II, the de novo mutation of Q372fs caused structural extension of SOX10. Further in vitro results demonstrated that the Q372fs SOX10 inhibited transcriptional activity of MITF, but not affected the interaction between SOX10 and PAX3. Our finding is expected to expand the mutation spectra of PAX3 and SOX10, which might be the genetic causes of WS pathogenesis.

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CONFLICT OF INTEREST
The authors declare they have no actual or potential competing financial interests.

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REFERENCES
Bocángel, M. A. P., Melo, U. S., Alves, L. U., Pardono, E., Lourenço, N. C. V., Marcolino, H. V. C., … Mingroni-Netto, R. C. (2018). Waardenburg syndrome: Novel mutations in a large Brazilian sample. European Journal of Medical Genetics, 61(6), 348–354. https://doi.org/10.1016/j.ejmg.2018.01.012
Bondurand, N., Dastot-Le Moal, F., Stanchina, L., Collot, N., Baral, V., Marin, S., … Pingault, V. (2007). Deletions at the SOX10 gene locus cause Waardenburg syndrome types 2 and 4. American Journal of Human Genetics, 81(6), 1169–1185. https://doi.org/10.1086/522090
Boudjadi, S., Chatterjee, B., Sun, W., Vemu, P., & Barr, F. G. (2018). The expression and function of PAX3 in development and disease. Gene, 666, 145–157. https://doi.org/10.1016/j.gene.2018.04.087
Choi, E. Y., Choi, W., & Lee, C. S. (2018). A novel PAX3 mutation in a Korean patient with Waardenburg syndrome type 1 and unilateral branch retinal vein and artery occlusion: A case report. BMC Ophthalmol, 18(1), 266. https://doi.org/10.1186/s12886-018-0933-9
Dai, W., Wu, J., Zhao, Y., Jiang, F., Zheng, R., Chen, D.-N., … Li, J.-D. (2019). Functional analysis of SOX10 mutations identified in Chinese patients with Kallmann syndrome. Gene, 702, 99–106. https://doi.org/10.1016/j.gene.2019.03.039
Dourmishev, A. L., Dourmishev, L. A., Schwartz, R. A., MPH, & Ianniger, C. K. (1999). Waardenburg syndrome. *International Journal of Dermatology*, 38(9), 656–663. https://doi.org/10.1046/j.1365-4632.1999.00750.x

Dye, D. E., Medici, S., Ziman, M., & Coombe, D. R. (2013). Melanoma biomolecules: Independently identified but functionally intertwined. *Front Oncol*, 3, 252. https://doi.org/10.3389/fonc.2013.00252

Hogan, A. R., Rao, K. A., Thorson, W. L., Neville, H. L., Sola, J. E., & Perez, E. A. (2019). Waardenburg syndrome type IV De Novo SOX10 variant causing chronic intestinal pseudo-obstruction. *Pediatr Gastroenterol Hepatol Nutr*, 22(5), 487–492. https://doi.org/10.10523/pghn.2019.22.5.487

Kamachi, Y., Cheah, K. S., & Kondoh, H. (1999). Mechanism of regulatory target selection by the SOX high-mobility-group domain proteins as revealed by comparison of SOX1/2/3 and SOX9. *Molecular and Cellular Biology*, 19(1), 107–120. https://doi.org/10.1128/mcb.19.1.107

Li, W. U., Mei, L., Chen, H., Cai, X., Liu, Y., Men, M., … Feng, Y. (2019). New genotypes and phenotypes in patients with 3 subtypes of waardenburg syndrome identified by diagnostic next-generation sequencing. *Neural Plasticity*, 2019, 7143458. https://doi.org/10.1155/2019/7143458

Ma, J., Lin, K., Jiang, H. C., Yang, Y., Zhang, Y., Yang, G., … Ruan, B. (2019). A novel mutation of the PAX3 gene in a Chinese family with Waardenburg syndrome type I. *Mol Genet Genomic Med*, 7(7), e00798. https://doi.org/10.1002/mgg3.798

Ma, J., Zhang, T.-S., Lin, K., Sun, H., Jiang, H.-C., Yang, Y.-L., … Ruan, B. (2016). Waardenburg syndrome type II in a Chinese patient caused by a novel nonsense mutation in the SOX10 gene. *International Journal of Pediatric Otorhinolaryngology*, 85, 56–61. https://doi.org/10.1016/j.ijpilo.2016.03.043

Ma, J., Zhang, Z., Jiang, H. C., Sun, H., Ming, C., Zhao, L. P., … Ruan, B. (2019). A novel dominant mutation in the SOX10 gene in a Chinese family with Waardenburg syndrome type II. *Mol Med Rep*, 19(3), 1775–1780. https://doi.org/10.3892/mmr.2019.9815

Niu, Z., Li, J., Tang, F., Sun, J., Wang, X., Jiang, L. U., … He, C. (2018). Identification and functional analysis of a novel mutation in the PAX3 gene associated with Waardenburg syndrome type I. *Gene*, 642, 362–366. https://doi.org/10.1016/j.gene.2017.11.035

Otreba, M., Milinski, M., Buszman, E., Wrzesniok, D., & Beberok, A. (2013). Hereditary hypomelanocytoses: The role of PAX3, SOX10, MITF, SNAI2, KIT, EDN3 and EDNRB genes. *Postepy Hig Med Dosw*, 67, 1109–1118. https://doi.org/10.5604/17322693.1077722

Pingault, V., Bondurand, N., Kuhlbrodt, K., Goerich, D. E., Préhu, M.-O., Puliti, A., … Goossens, M. (1998). SOX10 mutations in patients with Waardenburg-Hirschsprung disease. *Nature Genetics*, 18(2), 171–173. https://doi.org/10.1038/ng0298-171

Pingault, V., Ente, D., Dastot-Le Moal, F., Goossens, M., Marlin, S., & Bondurand, N. (2010). Review and update of mutations causing Waardenburg syndrome. *Human Mutation*, 31(4), 391–406. https://doi.org/10.1002/humu.21211

Read, A. P., & Newton, V. E. (1997). Waardenburg syndrome. *Journal of Medical Genetics*, 34(8), 656–665. https://doi.org/10.1136/jmg.34.8.656

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., … Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, 17(5), 405–424. https://doi.org/10.1038/gim.2015.30

Seberg, H. E., Van Otterloo, E., & Cornell, R. A. (2017). Beyond MITF: Multiple transcription factors directly regulate the cellular phenotype in melanocytes and melanoma. *Pigment Cell Melanoma Res*, 30(5), 454–466. https://doi.org/10.1111/pcmr.12611

Song, J., Feng, Y., Acke, F. R., Coucke, P., Vleminkx, K., & Dhoooge, I. J. (2016). Hearing loss in Waardenburg syndrome: A systematic review. *Clinical Genetics*, 89(4), 416–425. https://doi.org/10.1111/cge.12631

Stenson, P. D., Mort, M., Ball, E. V., Evans, K., Hayden, M., Heywood, S., … Cooper, D. N. (2017). The human gene mutation database: Towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Human Genetics*, 136(6), 665–677. https://doi.org/10.1007/s00439-017-1779-6

Wang, X.-P., Hao, Z.-Q., Liu, Y.-L., Mei, L.-Y., He, C.-F., Niu, Z.-J., … Feng, Y. (2017). Functional analysis of a SOX10 gene mutation associated with Waardenburg syndrome II. *Biochemical and Biophysical Research Communications*, 493(1), 258–262. https://doi.org/10.1016/j.bbrc.2017.09.034

Wildhardt, G., Zirn, B., Graul-Neumann, L. M., Wechtenbruch, J., Suckfüll, M., Buske, A., … Steinberger, D. (2013). Spectrum of novel mutations found in Waardenburg syndrome types 1 and 2: Implications for molecular genetic diagnostics. *British Medical Journal Open*, 3(3), https://doi.org/10.1136/bmjopen-2012-001917

Yu, Y., Yang, Y., Lu, J., Jin, Y., Yang, Y., Hong, E., … Ni, X. (2019). Two compound heterozygous were identified in SLC26A4 gene in two Chinese families with enlarged vestibular aqueduct. *Clin Exp Otorhinolaryngol*, 12(1), 50–57. https://doi.org/10.21053/coe.2018.00213

Yu, Y., Zhang, J., Jin, Y., Yang, Y., Shi, J., Chen, F., … Ni, X. (2018). MiR-20a-5p suppresses tumor proliferation by targeting autophagy-related gene 7 in neuroblastoma. *Cancer Cell International*, 18, 5. https://doi.org/10.1186/s12935-017-0499-2

Zaman, A., Capper, R., & Baddoo, W. (2015). Waardenburg syndrome: More common than you think!. *Clinical Otolaryngology*, 40(1), 44–48. https://doi.org/10.1111/coa.12312

Zhang, H., Chen, H., Luo, H., An, J., Sun, L., Mei, L., … Feng, Y. (2012). Functional analysis of Waardenburg syndrome-associated PAX3 and SOX10 mutations: Report of a dominant-negative SOX10 mutation in Waardenburg syndrome type II. *Human Genetics*, 131(3), 491–503. https://doi.org/10.1007/s00439-011-1098-2

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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