Prenatal diagnosis and genetic counseling for Waardenburg syndrome type I and II in Chinese families

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Abstract. Waardenburg syndrome (WS) is an auditory-pigmentary disorder with varying combinations of sensorineural hearing loss and abnormal pigmentation. The present study aimed to investigate the underlying molecular pathology and provide a method of prenatal diagnosis of WS in Chinese families. A total of 11 patients with WS from five unrelated Chinese families were enrolled. A thorough clinical examination was performed on all participants. Furthermore, patients with WS underwent screening for mutations in the following genes: Paired box 3 (PAX3), melanogenesis associated transcription factor (MITF), SRY-box 10, snail family transcriptional repressor 2 and endothelin receptor type B using polymerase chain reaction sequencing. Array-based comparative genomic hybridization was used for specific patients whose sequence results were normal. Following identification of the genotype of the probands and their parents, prenatal genetic diagnosis was performed for family 01 and 05. According to the diagnostic criteria for WS, five cases were diagnosed as WS1, while the other six cases were WS2. Genetic analysis revealed three mutations, including a nonsense mutation PAX3 c.583C>T in family 01, a splice-site mutation MITF c.909G>A in family 03 and an in-frame deletion MITF c.649_651delGAA in family 05. To the best of the authors' knowledge the mutations (c.583C>T in PAX3 and c.909G>A in MITF) were reported for the first time in Chinese people. Mutations in the gene of interest were not identified in family 02 and 04. The prenatal genetic testing of the two fetuses was carried out and demonstrated that the two babies were normal. The results of the present study expanded the range of known genetic mutations in China. Identification of genetic mutations in these families provided an efficient way to understand the causes of WS and improved genetic counseling.

Introduction

Hearing loss can be present in >400 genetic syndromes. Waardenburg syndrome (WS) is the most common type of autosomal dominant syndromic hearing loss (1). It accounts for 2% of congenital deafness, is most commonly described in western populations and affects ~1 in 42,000 people. WS is characterized by congenital sensorineural hearing loss, and pigmentation defects of the eyes, hair and skin (for example, heterochromia iridum, white forelock and patchy hypopigmented skin), and dystopia canthorum (2). WS is commonly hypothesized to be caused by the absence of neural crest-derived melanocytes (3-5).

WS has been classified into four subtypes (WS1-4) based on the presence or absence of additional symptoms. WS1 and WS2 are more common than WS3 and WS4. Type 1 [Mendelian inheritance in man (MIM) no. 193500] is characterized by congenital sensorineural hearing loss, heterochromia iridis or vivid blue eyes, and depigmented patches of the skin and hair. Type 2 (MIM no. 193510) is distinguished from WS1 by the absence of dystopia canthorum. The presence of limb abnormalities separates type III WS (Klein-Waardenburg syndrome; MIM no. 148820) from type I. Type IV WS (Shah-Waardenburg syndrome disease; MIM no. 277580) exhibits features of Hirschsprung disease (an aganglionic megacolon) in addition to WS2 (5).

Currently, there are six genes, paired box 3 (PAX3), SRY-box 10 (SOX10), melanogenesis associated transcription factor (MITF), endothelin-2, endothelin receptor type B (EDNRB) and snail family transcriptional repressor 2 (SNAI2), reported to be associated with WS (6). PAX3 on chromosome 2q35 is a member of the mammalian PAX gene family and encodes a DNA-binding transcription factor expressed in neural crest cells. Human PAX3, which is 98% homologous to the murine PAX3, has four structural motifs, including a paired domain, an octapeptide sequence, a homeodomain and a Pro-Ser-Thr-rich-COOH terminus (7). PAX3 serves an
important role in the migration and differentiation of melanocytes, which originate from the embryonic neural crest. The dysfunction of melanocyte migration and differentiation affects pigmentation of skin, hair and eyes, and hearing function in the cochlea. Almost all patients with WS1 and WS3 present with heterozygous mutations of \( \text{PAX3} \) (8,9). Researchers have proposed that 15% of WS2 patients possess heterozygous mutations of \( \text{MITF} \), which is also a transcription factor with a basic helix-loop-helix leucine zipper (b-HLH-Zip) motif. \( \text{MITF} \) has differentially expressed isoforms. The M-isoform, which is composed of nine exons (2-9) and is routinely studied in the WS field, encodes a protein of 419 amino acids (10). \( \text{MITF} \) is vital to the development and survival of melanocytes, osteoclasts and mast cells (11). It is estimated that the \( \text{SOX10} \) gene accounts for ~15% of WS2 cases and 50% of WS4 cases (12). A homozygous deletion in the \( \text{SNAI2} \) gene was described in two unrelated patients with WS2 (13) and a heterozygous \( \text{EDNRB} \) mutation has been identified in patients with WS2 (14).

Little is known about the genetic background of WS in China. In the present study, detailed analyses of the clinical manifestations and molecular mechanisms of patients with WS1/2 from five Chinese families were conducted. Prenatal diagnosis and counseling for WS was performed, as there is no specific treatment for the disease.

### Materials and methods

**Patients and samples.** All the patients with WS were identified at Henan Provincial People's Hospital (Zhengzhou, China; March 2014 to December 2015). There were 11 patients with WS (from five families), aged 33 months-44 years old, and unaffected 10 family members who agreed to take part in the study following audiological and general physical examinations. Among the five families, family 05 was a sporadic case and the remaining families had multiple affected patients (Fig. 1). Patient characteristics are presented in Table I. The present study was approved by the ethics committee of Henan Provincial People's Hospital. Written informed consent was obtained from all adult subjects and guardians on behalf of the children, prior to clinical evaluation and blood sample collection.

EDTA-K$_2$ peripheral venous blood was taken from the 11 proposituses and the 10 unaffected family members (Table II). The chorionic villi sampling with ultrasonic guidance was performed for the high-risk women at 11 weeks of pregnancy. Genomic DNA was extracted using the DNA extraction kit (Tiangen Biotech Co., Ltd., Beijing, China), according to the manufacturer's protocols. The PowerPlex\textsuperscript{®} 16 HS System kit (Promega Corporation, Madison, WI, USA) was used to exclude genetic contamination from the mother. The results were analyzed using ABI3130xl genetic analyzer and GeneMapper version 3.2 (both from Thermo Fisher Scientific, Inc., Waltham, MA, USA) software.

**Clinical evaluation.** Patients with WS were diagnosed according to the criteria proposed by the WS consortium (15). The diagnosis of WS can be made when ≥two major, or one major and two minor, phenotypic criteria are met. A comprehensive clinical history was taken, and audiological, neurological, ophthalmological and dermatological examinations were performed on all the subjects. The audiological and neurological examination included otoscopy, pure-tone audiometry and auditory steady-state response, immittance testing, and auditory brainstem response. Attention was paid to the color of skin, hair and iris, as well as developmental defects including dystopia canthorum and limb abnormalities. The degree of hearing loss (HL) was defined according to the pure-tone average, which was based on three frequencies (500, 1000 and 2000 Hz) as follows: Normal, <26 dB HL; mild, 26-40 dB HL; moderate, 41-70 dB HL; severe, 71-90 dB HL; and profound, >90 dB HL. The audiological follow-ups in infants were performed using Transient Evoked Otoacoustic Emissions and Automated Auditory Brainstem Response at 0 and 3 months.

**Mutational analysis.** Polymerase chain reaction (PCR) was carried out using the DNA from the patients with specific primers to amplify all exons and intron/exon boundaries of the \( \text{PAX3} \) [National Center for Biotechnology Information reference number (NM)_181458.2], \( \text{MITF-M} \) isoform (NM_000248.2), \( \text{SOX10} \) (NM_006941.3), and \( \text{SNAI2} \) (NM_003068.3) and \( \text{EDNRB} \) (NM_001201397.1) genes. The primers (Table III) were designed using the online program PRIMER3 (biotools.ummassmed.edu/bioapps/primer3 www.cgi). The PCR reactions were carried out in a total volume of 25 µl reaction mixture containing 40 ng genomic DNA, 2 pmol primer, 150 µmol dNTP, 0.125 units HotstartTaqDNA polymerase and 2.5 mmol MgCl$_2$. PCR conditions consisted of an initial denaturation at 94°C for 4 min; 30 cycles of denaturation at 94°C for 30 sec, annealing at various temperatures (Table III) for 45 sec for the different primers and extension at 72°C for 45 sec; followed by a 7 min final extension at 72°C.

PCR fragments were purified and sequenced in each direction on an ABI3130xl DNA sequencer, with a BigDye\textsuperscript{TM} Terminator Cycle Sequencing kit (Thermo Fisher Scientific, Inc.), with the same primers used for PCR. The raw sequence data were aligned with the wild-type sequence using the GeneTool program version 2.0 (Syngene Europe, Cambridge, UK). Following the identification of mutations of the WS genes in the propositus, samples from the related family members and normal controls were further screened for the mutations.

**Array-based comparative genomic hybridization (aCGH).** a-CGH was performed on patients whose sequence results were normal, a-CGH was carried out by using the SurePrint G3 Human CGH Microarray 8x60K kit (Agilent Technologies, Inc., Santa Clara, CA, USA). For each sample, 500 ng genomic DNA was used for each a-CGH experiment. DNA was labeled by direct incorporation of Cy5-deoxyuridine triphosphate (dUTP) and Cy3-dUTP, according to the Agilent protocol. Labeled DNA was purified and then hybridized to the arrays. Following performance of the hybridization step and the recommended washes, the arrays were scanned with a SureScan Dx Microarray scanner and analyzed using CytoGenomics 2.9 (both from Agilent Technologies, Inc., Santa Clara, CA, USA).

**Results**

**Clinical findings.** According to the diagnostic criteria for WS, five cases (IV:5, IV:7 and V:7 in family 01, I:1 and II:1 in
family 02) were diagnosed as WS1, while the other six cases (III:3, III:9 and IV:8 in family 03, I:1 and II:1 in family 04, II:1 in family 05) were WS2. Among these patients, deafness and heterochromia iridum were the most frequent features, and nine patients exhibited sensorineural hearing impairment varying from moderate to profound. Additionally, the

| Pedigree | Gender | Age | Iris | Skin | W   | Left ear | Right ear | Gene  | Variant     |
|----------|--------|-----|------|------|-----|----------|----------|-------|-------------|
| 01-IV:5  | M      | 44  | -    | -    | 1.85| Moderate | Severe   | PAX3  | c.583C>T    |
| 01-IV:7  | F      | 39  | -    | -    | 1.85| Normal   | Normal   | PAX3  | c.583C>T    |
| 01-V:7   | M      | 10  | +    | -    | 1.85| Profound | Moderate | PAX3  | -           |
| 02-I:1   | F      | 32  | +    | -    | 1.83| Profound | Moderate | PAX3  | -           |
| 02-II:1  | F      | 5   | +    | -    | 1.86| Profound | Profound | PAX3  | -           |
| 03-III:3 | M      | 48  | -    | +    | 1.75| Severe   | Severe   | MITF  | c.909G>A    |
| 03-III:9 | F      | 41  | -    | +    | 1.73| Normal   | Normal   | MITF  | c.909G>A    |
| 03-IV:8  | M      | 18  | -    | +    | 1.71| Profound | Profound | MITF  | c.1060C>A   |
| 04-I:1   | F      | 28  | +    | -    | 1.75| Profound | Profound | MITF  | c.1060C>A   |
| 04-II:1  | M      | 3   | -    | +    | 1.42| Severe   | Profound | MITF  | c.649_651del|

Iris, heterochromia iridis; skin, numerous brown freckles on the face; W, W index (2); HL, hearing loss; M, male; F, female; PAX3, paired box 3; MITF, melanogenesis associated transcription factor.

Figure 1. Pedigrees of the Waardenburg syndrome families. Pedigrees of families (A) 01, (B) 02, (C) 03, (D) 04 and (E) 05. The shading is as follows: Lower right, dystopia canthorum (in A and B) or freckles on face (in C-E); upper right, sensorineural hearing loss; lower left, premature graying; upper left, heterochromia iridis (3,5).
characteristic brilliant blue irides (unilateral or bilateral heterochromia irides), were observed in 6 patients. Each of the WS1 cases exhibited dystopia canthorum and left eye heterochromia iridis. Three heterozygous mutations in \textit{PAX3} exon 4 were identified in 3 affected members of family 01 exhibited dystopia canthorum, and left eye heterochromia iridis and synophrys. (C) The proband IV:7 of family 03 presented with brown freckles on the face. The probands of family (D) 04 (II:1) and (E) 05 (II:1) presented with eyes heterochromia iridis. (F) II:1 of family 05 presented with skin pigmentation disorder on both hands.

**Discussion**

WS may not be a rare disease in China as reported previously, up to now, >50 cases had been reported (17-19). More patients may be identified in special schools for children with hearing loss, although a large-scale, population-based study is required to confirm this. The syndrome involves a number of highly variable clinical manifestations. A previous study suggested that sensorineural deafness is the most frequent feature of WS (60% in WS1 and 90% in WS2) (20). In the present study, the majority of the patients (81.81%; 9/11) exhibited bilateral sensorineural hearing loss, ranging from moderate deafness to a progressive profound hearing loss. The syndrome exhibits a highly variable phenotype within families and incomplete penetrance. The affected individuals may exhibit multiple symptoms; however, certain patients exhibit few symptoms of WS, including IV:7 and V:7 in family 01. In addition, the propositus's father in family 05 was a healthy subject, despite the fact that he carried the same mutation as propositus. The father exhibited only a skin pigmentary disorder on both hands and is not a patient with WS according to the diagnostic criteria. It was speculated that the symptom may be associated with the genetic abnormalities. The skin pigmentation disorder is a rare clinical feature in WS families in China. This supported the results of the study by Liu et al (21) that skin pigmentation disorders are less frequent. Conversely, features including deafness or facial freckles are relatively common, which makes the clinical diagnosis difficult, particularly for WS2 (IV:8 from family 03). Therefore, molecular genetic analyses are required to confirm this. The syndrome involves a number of highly variable clinical manifestations. A previous study suggested that sensorineural deafness is the most frequent feature of WS (60% in WS1 and 90% in WS2) (20). In the present study, the majority of the patients (81.81%; 9/11) exhibited bilateral sensorineural hearing loss, ranging from moderate deafness to a progressive profound hearing loss. The syndrome exhibits a highly variable phenotype within families and incomplete penetrance. The affected individuals may exhibit multiple symptoms; however, certain patients exhibit few symptoms of WS, including IV:7 and V:7 in family 01. In addition, the propositus's father in family 05 was a healthy subject, despite the fact that he carried the same mutation as propositus. The father exhibited only a skin pigmentary disorder on both hands and is not a patient with WS according to the diagnostic criteria. It was speculated that the symptom may be associated with the genetic abnormalities. The skin pigmentation disorder is a rare clinical feature in WS families in China. This supported the results of the study by Liu et al (21) that skin pigmentation disorders are less frequent. Conversely, features including deafness or facial freckles are relatively common, which makes the clinical diagnosis difficult, particularly for WS2 (IV:8 from family 03). Therefore, molecular genetic analyses of WS genes are important diagnostic steps to explain the molecular cause of WS, and facilitate genetic counseling of affected patients and their families.

Currently six genes have been identified to be associated with WS. To date, >280 mutations in these genes have been reported in patients with WS (grenada.lumc.nl/LOVD2/WS). \textit{PAX3} mutations account for the majority of WS1 and WS3; however, few of them are recurrent. Missense and nonsense mutations are less frequent, which makes the clinical diagnosis difficult, particularly for WS2 (IV:8 from family 03). Therefore, molecular genetic analyses of WS genes are important diagnostic steps to explain the molecular cause of WS, and facilitate genetic counseling of affected patients and their families.
mutations, frameshifts, small in-frame insertions or deletions, and splice alterations of the \( PAX3 \) gene have been reported in patients with WS1. Altogether, ~50% of the mutations are missense and 50% are truncating variations. The majority of the novel mutations in \( PAX3 \) are localized in exons 2-6 and therefore influence functionally relevant domains. In the present study, the \( PAX3 \) mutation c.583C>T in exon 4 was detected within family 01 cases, which led to premature termination at the 195th amino acid and was void of functional domains. This mutation has been previously reported by Read et al (2). No mutation was identified in family 02 by \( PAX3 \) exon sequencing which cannot reliably detect whole exon or whole gene copy number alterations. Drozniakiewska et al (22) reported a patient with a de novo 2q36.1 deletion of 862 kb, including the \( PAX3 \) gene, which indicates that deletion or duplication screening is indispensable for the molecular genetic diagnosis of WS. Neither deletion nor duplication was observed in family 02 by a-CGH. It’s possible that the mutation remains to be identified as:

i) Mutations deep in introns or promoter regions were not sequenced; and

ii) mutations in other genes may be involved in pathogenesis (23,24).

Currently, ~30% of WS2 cases can be explained at the molecular level. It is estimated that \( MITF \) mutations occur in approximately 50% of the patients with WS. The remaining cases are likely due to mutations in other genes.

Table III. Amplification and sequencing primers.

| Gene   | Exon | Forward primer sequence (5’-3’) | Reverse primer sequence (5’-3’) | Product size (bp) | Annealing temperature (˚C) |
|--------|------|---------------------------------|---------------------------------|-------------------|---------------------------|
| PAX3   | 1    | TCACCACAGGAGGAGACTCA            | GAGGCCCTCCCTTTACCTTC            | 472               | 57                        |
|        | 2    | TACGTGCTGCTGTTCTTCTG            | TTACGCACCTCACAACACTC            | 442               | 58                        |
|        | 3    | TCTGGTCTGCTCTTTCTCAA           | ATTTGAGGTGATTACGTCGG            | 388               | 57                        |
|        | 4    | GCTGGAGAAGATGGAGATGAGT         | CTACAAGTGACCCCAAGCAGT           | 351               | 57                        |
|        | 5    | TGTCTTCGACTGCGAGAG            | GGTGACCTGCTGTTGCTG             | 492               | 58                        |
|        | 6    | AATTCGCCCACAAACACACA        | CAGAGAAATTCGCCCTTAAA           | 359               | 58                        |
|        | 7    | TGGGAGTAAACATTTGGA            | GGTTGGAGAAGAAAGAACC             | 451               | 58                        |
|        | 8    | TCGTCGAGGTGATGTAATA          | AGGAGAAATTCGCCCTTAAA           | 359               | 58                        |
|        | 9    | GAATGGTCCACGATGACGCT        | TGCTGCGAGGTCTCTCCTTCTC         | 311               | 60                        |
|        | 10a  | ACTGGCCCTGTTCTCTGCTTCT       | TGGCAAAACATCTGACTCTG           | 943               | 58                        |
|        | 10b  | CCAGTTTACATTATTGTA            | TCTCATGAAAGGGCTTCC             | 887               | 58                        |
| MITF   | 1    | TGTTGCTGCTGCGGAGACTTGG        | TGGCATCACAATATAAACAGCA         | 304               | 57                        |
|        | 2    | CGTTAGCACAAGTGCCTGTGTA        | GTGGCACAAGGCACAACTA            | 482               | 57                        |
|        | 3    | CATCTTGTGCTCTGTGCCATC        | AAGGTGATCCACCCAAACA            | 253               | 56                        |
|        | 4    | GACCATATTCTGCTTGGGTTAAAA       | TGTGATCCTGAGATAATTGTCCCATT     | 343               | 57                        |
|        | 5    | TGAGGAGTACCTGCTACTCTCTT       | AAAATTTACTGCTAGTGCTGGA         | 425               | 58                        |
|        | 6    | GCCTTTGAAACACATGCAACG        | GCTGTGAAATCAACTCCTTCCTC        | 350               | 58                        |
|        | 7    | CATGACCTGGAGAGATTATAATGTC    | AGTGTCACAATACCTCTTCGC          | 398               | 56                        |
|        | 8    | CACCTGTTCACACATACAAA         | GTCCTACCCCTTATGCGGC            | 372               | 58                        |
|        | 9    | CTAATGACGCGCCATCTACCA        | TCAAGAAACCCCTTTAGGT            | 594               | 57                        |
| SOX10  | 1    | GAGTTTAGCAGTGAGGAGGAGGAGGAGGA | CTTGAGATTTTCCACCTTTT           | 500               | 58                        |
|        | 2    | AGATGGTGGTTAGCTGGAGAC         | ACCCTGCTCTTCACCGCTAT           | 765               | 58                        |
|        | 3    | GTATTTCCTCTGCGCCTACA         | CTTTGCCAGACTAGTACGC           | 686               | 58                        |
|        | 4-a  | CATGTCCACACGGATGCTGA         | ATAGGGTCCTAGGCGTGT             | 678               | 58                        |
|        | 4-b  | AGGCCAGGTTGGAAGAGAGAGAG    | TCTGTGACGTCCTGTCTTCCTT         | 561               | 57                        |
| EDNRB  | 1    | CATTTCCTGGTCTCTGCAGT         | ACCCAAACCAAAGTGGCAAT           | 361               | 58                        |
|        | 2    | CTTTTAGAGCTGTAGATCTGG        | AGGGAGCTAAAGGGAGCT            | 748               | 58                        |
|        | 3/4  | CCAACACACCTCTGCCTGCC        | TTCTTGGCAGCTGAGTATTCG         | 816               | 57                        |
|        | 5    | TGTTCATGTAATGTGGCTGCTGA      | CAAGAAGAAAGGAAATGCTGTGG        | 432               | 58                        |
|        | 6    | CACTTTGCGTTTACCTCTCA         | CTTCCTGCTTCCTCCTCA             | 466               | 58                        |
|        | 7    | GAGGGGGACACAGCAAGAGAGA       | GCAGTAGGGAGTGGTGACT            | 493               | 57                        |
|        | 8    | AAGAGGGAAAATAAAGAGGACACTG    | TTCTTTCCATGCCGTAACA           | 466               | 58                        |
| SNAI2  | 1    | GCTGTGATTGATTTCTTTCTTGC      | GTGAAGCTCTCCTTTCCAGGACAC       | 449               | 58                        |
|        | 2    | GTGTTGATATCGCGGCTGTTG        | CTCTATGCAAAATCCCAACAGC         | 700               | 58                        |
|        | 3    | ATTTCTGTATATGGTGAGCAGC       | GCTTGGAGTGGAAGAAATGC          | 471               | 56                        |

PAX3, paired box 3; MITF, melanogenesis associated transcription factor; SOX10, SRY-box 10; EDNRB, endothelin receptor type B; SNAI2, snail family transcriptional repressor 2; bp, base pairs.
in ~15% of WS2. Through binding to DNA sequences, MITF regulates melanocyte differentiation and the transcription of several melanocyte-specific genes. Mutant MITF proteins are thought to possess defects in homo- or heterodimerization, and DNA binding through their basic regions. Point mutations are not evenly scattered along the gene, the majority of them are located in exons 7 and 8 that correspond to the basic HLH-Zip motifs. Interruption of the basic HLH-Zip domain decreases the ability of the mutant MITF protein to bind to the CATGTG core DNA sequence in the human tyrosinase promoter (25).

Wildhardt et al (26) reported a patient with WS2 carrying two MITF mutations (missense mutation and small deletion) within the same copy of the gene. In the present study, three MITF mutations, c.909G>A (p.Thr303Thr), c.649_651delGAA and c.1060C>A (p.L354I) were identified. The synonymous mutation c.909G>A was demonstrated to create a novel splice acceptor site that removes the 52 bps from the mRNA and results in the introduction of seven novel amino acids with premature termination and loss of the terminal 133 amino acids (16). The in-frame deletion c.649_651delGAA, del(R217) mutation in exon 7 was identified in family WS05, which removes one of four arginines in the basic domain. Conservation analyses revealed that the Arg residues at 217 in MITF are conserved across humans, mice, chickens, cows, and dogs. The mutation had been previously reported in a Caucasian family (8). In the family, the mother and son exhibited severe congenital sensorineural hearing loss and pigmentary disorder. The phenotype resembles the albinism-deafness syndrome of Tietz (MIM no. 103500) to an increased extent compared with classical Waardenburg syndrome, which is different from the patients in the present study. It can be speculated that there is interplay between genetic modifiers, environmental factors and stochastic events, in addition to the mutation itself. The mutation c.649_651delGAA in the MITF gene may be a hot spot for 7 cases reported in the mutation database.

Variant c.1060C>A (L354I) was identified in WS04. The mutation p.L354I fell outside of the important MITF domain, and was predicted to exhibit a 'benign' or 'tolerated' effect by the PolyPhen-2 (http://tiddlyspace.com/bags/icgc_public/tiddlers/PolyPhen2), and SIFT (http://sift.jcvi.org) software, respectively.

Simultaneously, the child inherited it from his unaffected father, while his affected mother did not carry the same mutation. Other variants of MITF have been reported, including: c.20A>G(p.Tyr7Cys), c.332C>T(p.Ala111Val), c.483A>T(p.Gln161His), c.608G>A (p.Arg203 Lys) and c.892T>C (p.Ser298Pro), all of which are thought to be likely neutral variants or the Single Nucleotide Polymorphism database (5,8,19).

The above mutations (c.583C>T in PAX3 and c.909G>A in MITF) were first identified in the Chinese population. These mutations in the WS genes enable prenatal diagnosis to be performed for the high-risk fetus. If one of the parents carries a dominant altered WS gene, there is a 50% chance that the children will inherit the disease. Preimplantation genetic diagnosis (PGD) is an alternative to prenatal diagnosis and is an almost safe, harmless, non-invasive and ethically acceptable procedure (27). However, PGD could not be easily obtained by the families for economic reasons. Invasive prenatal diagnosis may be another effective means of preventing recurrence of genetic disease. Therefore, prenatal diagnosis for the two families (01 and 05) was performed. The results demonstrated that the two fetuses were normal, which was confirmed by audiological follow-ups. However, a positive test result may be derived from a patient exhibiting a normal phenotype despite the presence of mutations as there may be reduced penetrance. For this reason, it is important to discuss these issues during pregnancy.
genetic counseling. In the future, non-invasive prenatal diagnosis of WS by examination of cell-free fetal DNA in maternal blood will be performed.

In conclusion, the results of the present study expand the database of known mutations in Chinese patients with WS. Certain cases remain unexplained at the molecular level, which require further study. The exact description of the mutations responsible for the WS type is of importance in genetic counseling of patients with WS and their families.

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