

# 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 neuralcrest-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 I [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

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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 *PAX3* (8,9). Researchers have proposed that 15% of WS2 patients possess heterozygous mutations of *MITF*, which is also a transcription factor with a basic helix-loop-helix leucine zipper (b-HLH-Zip) motif. *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). *MITF* is vital to the development and survival of melanocytes, osteoclasts and mast cells (11). It is estimated that the *SOX10* gene accounts for ~15% of WS2 cases and 50% of WS4 cases (12). A homozygous deletion in the *SNAI2* gene was described in two unrelated patients with WS2 (13) and a heterozygous *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<sub>2</sub> peripheral venous blood was taken from the 11 probands 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® 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, 1,000 and 2,000 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 *PAX3* [National Center for Biotechnology Information reference number (NM)\_181458.2], *MITF-M* isoform (NM\_000248.2), *SOX10* (NM\_006941.3), and *SNAI2* (NM\_003068.3) and *EDNRB* (NM\_001201397.1) genes. The primers (Table III) were designed using the online program PRIMER3 (biotools.umassmed.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 HotstarTaqDNA polymerase and 2.5 mmol MgCl<sub>2</sub>. 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™ 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 proband, 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

Table I. Clinical characteristics and gene variants of Waardenburg syndrome families.

Pedigree	Gender	Age	Iris	Skin	W	Severity of HL		Gene	Variant
						Left ear	Right ear		
01-IV:5	M	44	-	-	1.85	Moderate	Severe	<i>PAX3</i>	c.583C>T
01-IV:7	F	39	-	-	1.87	Normal	Normal	<i>PAX3</i>	c.583C>T
01-V:7	M	10	+	-	1.85	Profound	Moderate	<i>PAX3</i>	c.583C>T
02-I:1	F	32	+	-	1.83	Profound	Moderate	<i>PAX3</i>	-
02-II:1	F	5	+	-	1.86	Profound	Profound	<i>PAX3</i>	-
03-III:3	M	48	-	+	1.75	Severe	Severe	<i>MITF</i>	c.909G>A
03-III:9	F	41	-	+	1.73	Normal	Normal	<i>MITF</i>	c.909G>A
03-IV:8	M	18	-	+	1.71	Profound	Profound	<i>MITF</i>	c.909G>A
04-I:1	F	28	+	-	1.75	Profound	Profound	<i>MITF</i>	c.1060C>A
04-II:1	M	3	+	-	1.42	Severe	Profound	<i>MITF</i>	c.1060C>A
05-II:1	M	2	+	-	1.41	Profound	Profound	<i>MITF</i>	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.

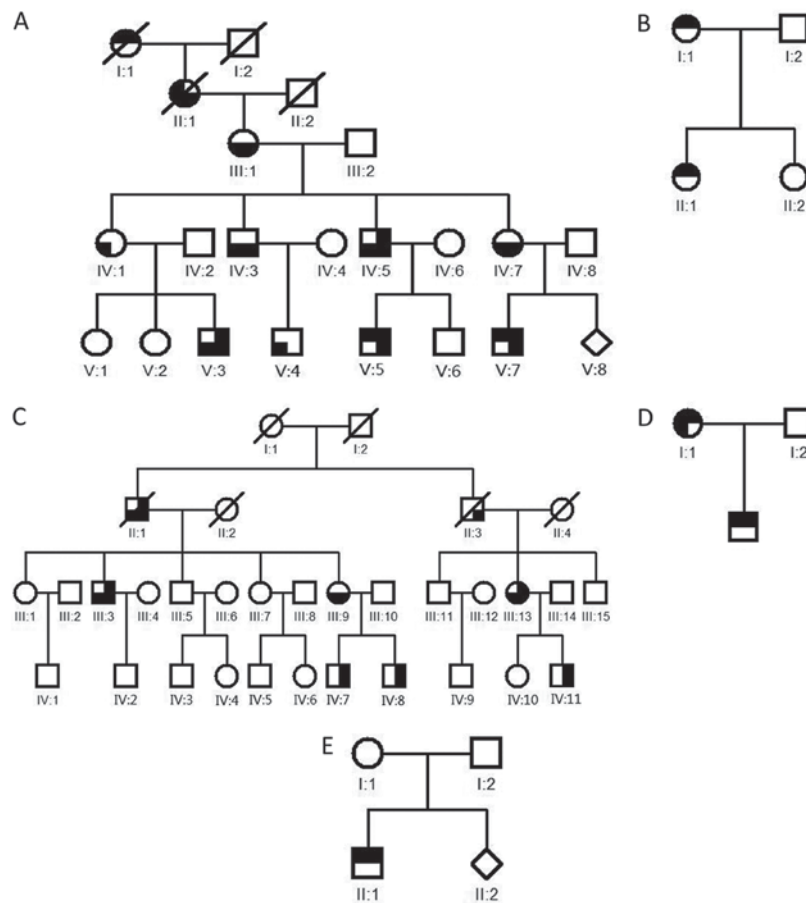


Figure 1. Pedigrees of the Waardenburg syndrome families. Pedigrees of families (A) 01, (B) 02, (C) 03, (D) 04 and (E) 05. The shading is as follows: Lower right, dystopia canthorum (in A and B) or freckles on face (in C-E); upper right, sensorineural hearing loss; lower left, premature graying; upper left, heterochromia iridis (3,5).

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

Table II. Characteristics of the normal controls.

Pedigree	Gender	Age
01-IV8	M	40
01-V6	M	13
02-I2	M	31
02-II2	F	3
03-III5	M	46
03-III7	F	43
03-III10	M	43
04-I2	M	30
05-I1	F	27
05-I2	M	29

M, male; F, female.

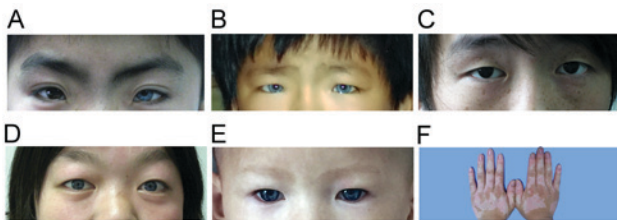


Figure 2. Images of specific affected individuals. (A) The proband V:7 of family 01 presented with dystopia canthorum and left eye heterochromia iridis. (B) The proband II:1 of family 02 presented with eyes heterochromia iridis and synophrys. (C) The proband IV:7 of family 03 presented with brown freckles on the face. The probands of family (D) 04 (II:1) and (E) 05 (II:1) presented with eyes heterochromia iridis. (F) I:1 of family 05 presented with skin pigmentation disorder on both hands.

characteristic brilliant blue irides (unilateral or bilateral heterochromia irides), were observed in 6 patients. Each of the WS1 cases exhibited dystopia canthorum, and three of them (01-V7, IV7 and 02-III1) also presented with broad nasal roots. A total of 3 patients in family 03 had numerous brown freckles on the faces. Patchy skin depigmentation was only observed in family 05. Other clinical features, a white forelock and hypopsia for example, were not observed in the present study (Table I). Representative clinical findings and the typical characteristics of these WS cases were illustrated in Fig. 2.

**Identification of mutations.** Six variants were identified in these proband cases. A heterozygous nonsense mutation c.583C>T in *PAX3* exon 4 was identified in 3 affected members of family 01, resulting in a premature termination codon at the 195th nucleotide within the octapeptide domain of the *PAX3* protein (Fig. 3A). The sequencing and a-CGH assays failed to detect the mutation in family 02.

Three heterozygous mutations in *MITF* were identified in these WS2 patients, including splice-site, missense and deletion mutations. The splice-site mutation c.909G>A was identified in family 03 (Fig. 3B), which has been reported by Brenner *et al* (16). Unlike the previous splicing variants reported, the mutation did not destroy the acceptor or donor splicing sites, but created a new splice acceptor site. The variant

c.1060C>A (p.L354I) in exon 9 was found in family 04, which led to a substitution from Leu to Ile at the 354th amino acid (Fig. 3C). An in-frame deletion c.649\_651delGAA, del (R217) mutation was identified in family WS05, which removed one of a run of four arginines in the basic domain (Fig. 3D). The mutations (c.583C>T in *PAX3*, c.909G>A and c.649\_651delGAA in *MITF*) identified in these patients were not observed in any other unaffected family members. Other synonymous substitutions were, c.927T>C in *SOX10* (family 03) and c.831A>G in *EDNRB* (family 04).

**Prenatal diagnosis and follow-up.** Following identification of the genotype of the probands and their parents, prenatal genetic diagnosis was performed for the two fetuses of family 01 and family 05, as a carrier, the couples have a 50% chance of affected offspring. DNA was extracted from chorionic villi samples and mutations of the *PAX3/MITF* gene were detected. The prenatal genetic diagnosis revealed the two babies were normal for the genes examined (data not shown). Furthermore, the audiological follow-ups between 0 and 6 months revealed hearing was within normal thresholds.

## 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 proband's father in family 05 was a healthy subject, despite the fact that he carried the same mutation as proband. 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). *PAX3* mutations account for the majority of WS1 and WS3; however, few of them are recurrent. Missense and nonsense

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	GAGGCCCTCCCTTACCTTC	472	57
	2	TACGTGCTGCTGTTCTTTGC	TTACGCACCTTCACAAACCTC	442	58
	3	TCTGGTCTGCCCTTTCTAA	ATTGGGGTGATTACGTCTGG	388	58
	4	GCTGGAGAAGGATGAGGATG	CTCCAAGTGACCCAGCAAGT	351	57
	5	TGTCTTGCAGTCGGAGAGAG	GGTGGACTTCTGTGTGTCGT	492	58
	6	AATTCGCCCAAACAACACA	CAGAGAAATCGCCTGGAAGT	368	58
	7	TGGCGATGAAC TTTGCAC	GGGTGGAGAGAAAGGAAACC	451	58
	8	TCGTCGGGCATGATGTAATA	AGGAGAAATTGCCCCCTAAA	359	58
	9	GAATTGTCCCAGCATGACCT	TGCTCCAGGTCTTCCTCTTC	311	60
	10a	ACTGGCCCTGTTTCTGGTCT	TGGCAAACATCACTGCACTC	943	58
10b	CCAGTTCACATTTATTTGG	CTCATAGAAAGGGTCCAC	887	58	
MITF	1	TGGTGTCTCGGGATACCTTG	TGGCATCAAATAATAAACAGCA	304	57
	2	CGTTAGCACAGTGCCTGGTA	GTGGCCACAAGGACAAACTA	482	57
	3	CATCTTGTTGCTCTGTGCCATC	AAGGTGATCCACCACAAA	253	56
	4	GACCATTATTGCTTTGGGTAAAA	TGTGATCCTGAGATAATTCTCCATT	343	57
	5	TGAGGAGATCCTGTACCTCTCTT	AAAAGTTACGTCCATGAGTTGGA	425	57
	6	GCTTTTGAAAACATGCAAGC	GCTGTAGGAATCAACTCTCCTCT	350	58
	7	CATGACCTGGAGAAGTTAATATGC	AGTGTCCAACAATCCTTTTGC	398	56
	8	CACCTGTTCCCAAAAATA	GTCAACTCCCCTATGGCTCA	372	58
	9	CTAATGACGCGCATCTACCA	TCAAGAAAACCCCTTAGGT	594	57
SOX10	1	GAGTGTGGGGATGAAGGAA	CCTGGAATTTCCACCTTTT	500	58
	2	AGATGGGTTTAGCTGGAGCA	ACCTGGTCTTCCAGCCCTAT	765	58
	3	GTTATTCTTGGGCCTCACA	CTTTGCCAGTAGGATCAGC	686	57
	4-a	CATGCTGCCAAAATGTGAAA	ATAGGGTCCTGAGGGCTGAT	678	56
	4-b	AGCCAGGTGAAGACAGAGA	TCTGTCCAGCCTGTTCTCCT	561	57
EDNRB	1	CATTTCTGTTCCCTGACT	ACCAAACCAAAGTGCCAAT	361	58
	2	CTTTTGAGCGTGGATACTGG	AGGGAGCTAAAGGGAAGCTC	748	58
	3/4	CCCAACACACTTTCCTGTCC	TTCTTGACAGCTTGAGTCATTG	816	57
	5	TGTTTCAGTAAGTGTGGCCTGA	CAAGAAAAGGAAATATGCTCTGG	432	56
	6	CACTTCGGTTCCTTCCACTCACA	CTTCCCTGTCCCTCTCAACA	466	58
	7	GAGGGGGACACAGACAGAGA	GCAGTAGGGAGTGGCTGACT	493	57
	8	AAGAGGGAAAATAAAAGAGCACTG	TTCTTTCCATGCCGTAAACA	466	57
	SNAI2	1	GCTGTGATTGGATCTTTCTTGC	TGTAAGCTCCCTTTCAGGACAC	449
2		TGTGTGTATACTTGCCTGTGG	CTTCATGCAAATCCAACAGC	700	58
3		ATTTCTGTATGATTGGCAGCAG	GCTTCGGAGTGAAGAAATGC	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.

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. Drozniewska *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

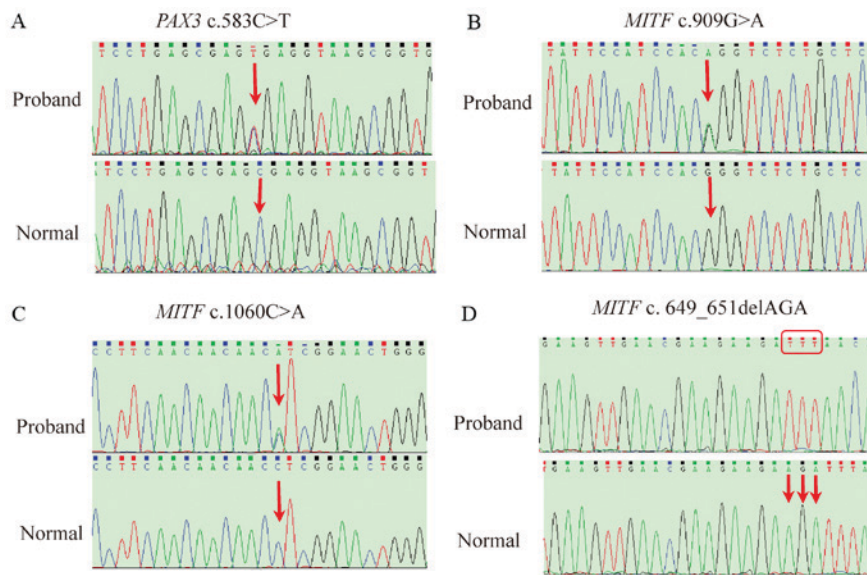


Figure 3. Mutation analyses of Chinese Waardenburg syndrome families 01, 03, 04 and 05. (A) DNA sequence chromatograms presenting heterozygous missense mutation c.583C>T in *PAX3* identified in family 01, compared with wild-type control. (B) DNA sequence chromatograms presenting heterozygous missense mutation c.909G>A in *MITF* identified in family 03, compared with wild-type control. (C) DNA sequence chromatograms presenting heterozygous mutation c.1060C>A in *MITF* identified in family 04, compared with wild-type control. (D) DNA sequence chromatograms presenting heterozygous mutation c.649\_651delGAA in *MITF* identified in family 05, compared with wild-type control. *PAX3*, paired box 3; *MITF*, melanogenesis associated transcription factor.

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 b-HLH-Zip motifs. Interruption of the b-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](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

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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### References

- Zaman A, Capper R and Baddoo W: Waardenburg syndrome: More common than you think! *Clin Otolaryngol* 40: 44-48, 2015.
- Read AP and Newton VE: Waardenburg syndrome. *J Med Genet* 34: 656-665, 1997.
- Baxter LL, Hou L, Loftus SK and Pavan WJ: Spotlight on spotted mice: A review of white spotting mouse mutants and associated human pigmentation disorders. *Pigment Cell Res* 17: 215-224, 2004.
- Mollaaghababa R and Pavan WJ: The importance of having your SOX on: Role of SOX10 in the development of neural crest-derived melanocytes and glia. *Oncogene* 22: 3024-3034, 2003.
- Pingault V, Ente D, Dastot-Le Moal F, Goossens M, Marlin S and Bondurand N: Review and update of mutations causing Waardenburg syndrome. *Hum Mutat* 31: 391-406, 2010.
- Song J, Feng Y, Acke FR, Coucke P, Vlemminckx K and Dhooge IJ: Hearing loss in Waardenburg syndrome: A systematic review. *Clin Genet*, Jun 22, 2016 (Epub ahead of print).
- DeStefano AL, Cupples LA, Arnos KS, Asher JH Jr, Baldwin CT, Blanton S, Carey ML, da Silva EO, Friedman TB, Greenberg J, *et al*: Correlation on between Waardenburg syndrome phenotype and genotype in a population of individuals with identified PAX3 mutations. *Hum Genet* 102: 499-506, 1998.
- Tassabehji M, Newton VE, Liu XZ, Brady A, Donnai DK, ajewska-Walasek M, Murday V, Norman A, Obersztyn E, Reardon W, *et al*: The mutational spectrum in Waardenburg syndrome. *Hum Mol Genet* 4: 2131-2137, 1995.
- Wollnik B, Tükel T, Uyguner O, Ghanbari A, Kayserili H, Emiroglu M and Yuksel-Apak M: Homozygous and heterozygous inheritance of PAX3 mutations causes different types of Waardenburg syndrome. *Am J Med Genet A* 122A: 42-45, 2003.
- Shibahara S, Takeda K, Yasumoto K, Udono T, Watanabe K, Saito H and Takahashi K: Microphthalmia-associated transcription factor (MITF): Multiplicity in structure, function, and regulation. *J Invest Derm Symp Proc* 6: 99-104, 2001.
- Grill C, Bergsteinsdottir K, Ogmundsdóttir MH, Pogenberg V, Schepsky A, Wilmanns M, Pingault V and Steingrimsdóttir E: MITF mutations associated with pigment deficiency syndromes and melanoma have different effects on protein function. *Hum Mol Genet* 22: 357-4367, 2013.
- Tassabehji M, Newton VE and Read AP: Waardenburg syndrome type 2 caused by mutations in the human microphthalmia (MITF) gene. *Nat Genet* 8: 251-255, 1994.
- Sánchez-Martín M, Rodríguez-García A, Pérez-Losada J, Sagraera A, Read AP and Sánchez-García I: SLUG (SNAI2) deletions in patients with Waardenburg disease. *Hum Mol Genet* 11: 3231-3236, 2002.
- Jabeen R, Babar ME, Ahmad J and Awan AR: Novel mutations of endothelin-B receptor gene in Pakistani patients with Waardenburg syndrome. *Mol Biol Rep* 39: 785-788, 2012.
- Farrer LA, Grundfast KM, Amos J, Arnos KS, Asher JH Jr, Beighton P, Diehl SR, Fex J, Foy C, Friedman TB, *et al*: Waardenburg syndrome (Ws) type-I is caused by defects at multiple Loci, one of which is near alpp on chromosome-2-1st report of the Ws consortium. *Am J Hum Genet* 50: 902-913, 1992.
- Brenner L, Burke K, Leduc CA, Guha S, Guo J and Chung WK: Novel splice mutation in microphthalmia-associated transcription factor in Waardenburg Syndrome. *Genet Test Mol Biomarkers* 15: 525-529, 2011.
- Chen H, Jiang L, Xie Z, Mei L, He C, Hu Z, Xia K and Feng Y: Novel mutations of PAX3, MITF, and SOX10 genes in Chinese patients with type I or type II Waardenburg syndrome. *Biochem Biophys Res Commun* 397: 70-74, 2010.
- Jiang L, Chen H, Jiang W, Hu Z, Mei L, Xue J, He C, Liu Y, Xia K and Feng Y: Novel mutations in the SOX10 gene in the first two Chinese cases of type IV Waardenburg syndrome. *Biochem Biophys Res Commun* 408: 620-624, 2011.
- Yang S, Dai P, Liu X, Kang D, Zhang X, Yang W, Zhou C, Yang S and Yuan H: Genetic and phenotypic heterogeneity in Chinese patients with Waardenburg syndrome type II. *PLoS One* 8: e77149, 2013.
- Nayak CS and Isaacson G: Worldwide distribution of Waardenburg syndrome. *Ann Oto Rhinol Laryn* 112: 817-820, 2003.
- Liu X, Newton V and Read A: Hearing loss and pigmentary disturbances in Waardenburg syndrome with reference to WS type II. *J Laryngol Otol* 109: 96-100, 1995.
- Drozniewska M and Haus O: PAX3 gene deletion detected by microarray analysis in a girl with hearing loss. *Mol Cytogenet* 7: 30, 2014.
- Berlin I, Denat L, Steunou AL, Puig I, Champeval D, Colombo S, Roberts K, Bonvin E, Bourgeois Y, Davidson I, *et al*: Phosphorylation of BRN2 modulates its interaction with the Pax3 promoter to control melanocyte migration and proliferation. *Mol Cell Biol* 32: 1237-1247, 2012.
- Degenhardt KR, Milewski RC, Padmanabhan A, Miller M, Singh MK, Lang D, Engleka KA, Wu ML, Li J, Zhou D, *et al*: Distinct enhancers at the Pax3 locus can function redundantly to regulate neural tube and neural crest expressions. *Dev Biol* 339: 519-527, 2010.
- Takeda K, Takemoto C, Kobayashi I, Watanabe A, Nobukuni Y, Fisher DE and Tachibana M: Ser298 of MITF, a mutation site in Waardenburg syndrome type 2, is a phosphorylation site with functional significance. *Hum Mol Genet* 9: 125-132, 2000.
- Wildhardt G, Zirn B, Graul-Neumann LM, Wechtenbruch J, Suckfüll M, Buske A, Bohring A, Kubisch C, Vogt S, Strobl-Wildemann G, *et al*: Spectrum of novel mutations found in Waardenburg syndrome types 1 and 2: Implications for molecular genetic diagnostics. *Bmj Open* 3: pii: e001917, 2013.
- Millan JM, Aller E, Jaijo T, Grau E, Beneyto M and Najera C: Genetic counselling in visual and auditory disorders. *Arch Soc Esp Ophthalmol* 83: 689-702, 2008 (In Spanish).



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