PAX3 mutations and clinical characteristics in Chinese patients with Waardenburg syndrome type 1

Juan Wang, Shiqiang Li, Xueshan Xiao, Panfeng Wang, Xiangming Guo, Qingjiong Zhang

State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, P.R. China

Purpose: To detect paired box gene 3 (PAX3) mutations and associated phenotypes in Chinese patients with Waardenburg syndrome type 1 (WS1).

Methods: Five unrelated families with suspected WS1 were selected from our Genomic DNA Repository for Hereditary Eye Diseases. The coding and adjacent intronic regions of PAX3 were amplified by polymerase chain reaction and the amplicons were then analyzed by cycle sequencing. Variations detected were further evaluated in available family members as well as one hundred controls with heteroduplex-single strand conformational polymorphism (heteroduplex-SSCP) analysis and/or clone sequencing.

Results: Three novel and two known mutations in PAX3 were detected in five patients, respectively: c.567_586+17del (p.Asp189_Gln505delinsGluGlyGly), c.456_459dupTTCC (p.Ile154PhefsX162), c.795_800delCTGGTT (p.Trp266_Phe267del), c.799T>A (p.Phe267Ile), and c.667C>T (p.Arg223X). Two novel mutations proved to be de novo as their parents did not carry the mutations. All five patients with PAX3 mutations had dystopia canthorum and different iris color and fundi between their two eyes. However, none had white forelock, skin hypopigmentation, and deafness.

Conclusions: Our findings expand the frequency and spectrum of PAX3 mutations and ethnic-related phenotypes in Chinese patients with WS1. De novo mutations in PAX3 have not been reported before.

Waardenburg syndrome (WS) is an inherited disorder characterized by varying degrees of hearing loss and pigmentary anomalies affecting the eye, hair, and skin [1-6]. WS is clinically heterogeneous and has been classified into four major types and 10 subtypes as listed in Table 1 [5, 7-17]. WS type 1 (WS1, OMIM 193500) and type 2 (WS2) are more common than type 3 (WS3) and type 4 (WS4). Overall, the syndrome affects perhaps 1 in 42,000 people [6].

Except for auditory-pigmentary disorder, dystopia canthorum is the typical phenotype of WS1 (Table 1). Mutations in the paired box gene 3 (PAX3, OMIM 606597) have been identified to be responsible for WS1 [18,19]. PAX3 encodes a member of the mammalian PAX family of transcription factors, which contains two highly conserved domains for DNA binding, paired box domain and paired-type homeodomain [20]. Alternative splicing of PAX3 results in several different-length transcripts, of which the longest transcript contains 10 exons, and consequent proteins with distinct carboxyl termini [21]. PAX3 plays a regulatory role in the early embryonic development of the pigment system [22] and is required to expand a pool of committed melanoblasts or restricted progenitor cells early in development [23]. Heterozygous mutations in PAX3 have been reported in familial and sporadic WS1, while heterozygous or homozygous mutations have been detected in patients with WS3 [8,13,24,25]. Although many mutations have been identified in Caucasians, several cases have been determined in the Chinese population [26,27]. Fundus changes for WS1 patients with PAX3 mutations have not been reported.

In the present study, five mutations in PAX3, including three novel ones and two known ones, were identified in five unrelated Chinese families with WS1. All patients with the 5 mutations presented dystopia canthorum and different colors of the irises and fundi but none of those showed visible pigmentary changes on their hair and skin, indicating an ethnic specific phenotypes.

METHODS

Patients: Five unrelated patients were recruited from our Pediatric and Genetic Eye Clinic, Zhongshan Ophthalmic Center, Guangzhou, P.R. China. Diagnosis of WS1 was based on criteria previously described [4,28]. Informed consent conforming to the tenets of the Declaration of Helsinki and following the Guidance of Sample Collection of Human Genetic Diseases (National 863-Plan) by the Ministry of Public Health of China was obtained from participating individuals before the study. All participants received detailed ophthalmological examinations performed by ophthalmologists (Q.Z. or X.G.). Unrelated controls (100) were collected from normal volunteers. This study was approved by the Institutional Review Board of Zhongshan Ophthalmic Center.

Correspondence to: Qingjiong Zhang, M.D., Ph.D., State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, 54 Xianlie Road, 510060, China; Phone: (+86)-20-87330422; FAX: (+86)-20-87333271; email: qingjiongzhang@yahoo.com
Variation analysis: Genomic DNA was isolated from venous leukocytes. Genomic fragments encompassing coding regions and adjacent intronic regions of PAX3 were amplified by polymerase chain reaction (PCR), using eleven primer pairs (PAX3: NCBI human genome build 36.1, NC_000002.11, NM_181459.2, NP_852124.1), including previously reported primers for exons 1–9 [26] and two new primer pairs for exon 10 (Table 2). The amplicons from individual exon were purified and analyzed by cycle sequencing with ABI BigDye Terminator Cycle Sequencing Kit v3.1 (ABI Applied Biosystems, Foster City, CA) on an automatic DNA sequencer (ABI 3100 Genetic Analyzer, Applied Biosystems). Sequencing results from patients as well as the consensus sequences from the NCBI Human Genome Database were imported into the SeqManII program of the Lasergene package (DNASTar Inc., Madison, WI) and aligned to identify variations. Each variation was confirmed by bidirectional sequencing. Variations were named following the nomenclature recommended by the Human Genomic Variation Society (HGVS).

Any variation detected by sequence analysis was further evaluated in 100 controls by heteroduplex-SSCP analysis. In addition, one multiple-nucleotide deletion was further analyzed by clone sequencing, using the method we described previously [29]. NNSPLICE version 0.9 was used to predict splice sites.

RESULTS

Clinical phenotype: The most significant sign in all five unrelated patients is different colors between two eyes, which resulted from heterochromia iridis (Figure 1, Table 3). All patients had dystopia canthorum (Figure 1). Ocular fundus examination revealed different colors between two fundi (Figure 2 and Table 3). In all 5 patients the eye with generalized iris hypopigmentation also had mild retinal hypopigmentation. In the eyes with pigmentary changes, however, the fundus vessel distribution, macular architectural and visual acuity seemed to be normal (Figure 2 and Table 3). None of the 5 patients had pigmentary changes on their skin, hair, eyebrows, and eyelashes, which are the common signs in Caucasian patients. Deafness was not
observed in three patients while the hearing function could not be measured in the other two babies. Anomalies on limb development were not observed in all 5 patients.

**Variation detection:** In the 5 patients, five heterozygous mutations in *PAX3* were detected, including c.567_586+17del (p.Asp189_Gln505delinsGluGlyAlaLeuAlaGly), c.456_459dupTTCC (p.Ile154PhefsX162), c.795_800 delCTGGTT (p.Trp266_Phe267del), c.799T>A (p.Phe267Ile), and c.667C>T (p.Arg223X; Table 3, Figure 3). The first three mutations were novel and, therefore, were further confirmed by heteroduplex-SSCP analysis (Figure 3). The other two mutations were known mutations. All five mutations were absent in 100 normal controls based on heteroduplex-SSCP analysis (data not shown).

The c.567_586+17del mutation was identified in a baby from Family A (A-II:1). Direct sequencing revealed a heterozygous variation involving multiple nucleotides in exon 4 region. Cloning sequencing revealed a 37 bp deletion affecting both exon 4 and intron 4 (Figure 3A). A new splice site is predicted to be created downstream by NNSPLICE. The encoded protein would be truncated.

The c.456_459dupTTCC and c.795_800delCTGGTT mutations were only present in the probands (Figure 3, B-II:

---

**Figure 1.** Photographs of eyes from WS1 patients and controls. **A:** Baby with normal iris pigmentation and normal facial characteristics. **B:** 15-month-old girl (II:1 from family A in Figure 3) with dystopia canthorum, heterochromia iridis, broad nasal root, and a horizontal distance between the inner canthi of 28 mm. **C:** Normal adult (I:2 from family A). **D, E:** Adult II:1 from family E showed dystopia canthorum, heterochromia iridis (left iris).
| ID   | Sex | Age (yrs) | OD | OS | Mutation             | Effect              | Differently colored eyes | Fundus hypopigmentation | Dystopia canthorum | Deafness | Family history |
|------|-----|-----------|----|----|----------------------|---------------------|-------------------------|-------------------------|---------------------|----------|----------------|
| A-II:1 | F   | 1         | NA | NA | c.567_586+17del      | p.Asp189_Gln505del  | OS                      | OS                      |         |          |               |
| B-II:1 | M   | 0.6       | NA | NA | c.456_459dupTTCC     | p.Ile154_PhefsX162  | OS                      | OS                      | Yes     | NA        | No          |
| C-II:1 | M   | 7         | 1.00 | 0.90 | c.795_800delCTGGTT  | p.Trp266_Phe267del  | OS                      | OS                      | Yes     | No        | No          |
| D-IV:1 | M   | 6         | 0.90 | 1.00 | c.799T>A             | p.Phe267le         | OD                      | OD                      | Yes     | No        | Yes         |
| E-II:1 | F   | 23        | 1.50 | 1.50 | c.667C>T             | p.Arg223X           | OS                      | OS                      | Yes     | No        | No          |

Note: NA: Not available because they are too young. None of the 5 probands had white forelock and skin hypopigmentation.
but not in their parents, demonstrating de novo mutations that have been rarely reported in PAX3.

DISCUSSION

In this study, three novel and two known mutations in PAX3 were identified in five unrelated Chinese patients. The three novel mutations would result in frameshift or inframe deletion if transcribed and translated, suggesting putative disease-causing. Unilateral sapphire iris with pink pupil and retinal depigmentation as well as dystopia canthorum without other abnormalities suggested a diagnosis of WS1.

Of the five PAX3 mutations, three were novel (c.567_586+17del, c.456_459dupTTCC and c.795_800delCTGGTT) and the other two were previously reported (c.799T>A and c.667C>T) [30,31]. Two novel mutations, c.456_459dupTTCC and c.795_800delCTGGTT, were proved to be de novo as their parents did not carry the mutations, suggesting that natural occurring new mutations in PAX3 of the Chinese population is not uncommon. Based on available information, de novo mutations in PAX3 have rarely been mentioned before. Three of the five mutations, c.567_586+17del (p.Asp189_Gln505delinsGluGlyGlyAlaLeuAlaGly), c.456_459dupTTCC (p.Ile154PhefsX162) and c.667C>T (p.Arg223X), are predicted to encode premature truncated proteins affecting the paired-type homeodomain [20]. The other two mutations, c.795_800delCTGGTT (p.Trp266_Phe267del) and c.799T>A (p.Phe267Ile), would also affect the paired-type homeodomain, if translated.

Clinical manifestation of the 5 Chinese patients with PAX3 mutations is consistent with the phenotypes of WS1. However, pigmentary changes on skin, hair, eyebrows, and eyelashes are absent in these Chinese patients, indicating an ethnic specific variations in clinical expression. Fundus hypopigmentation in WS1 patients have been demonstrated in the Chinese patients. Although fundus hypopigmentation
was recorded in WS in previous reports, it has not been described in WS1 patients with PAX3 mutations before. Understanding the typical and atypical phenotypes of Chinese WS1 patients is of clinical importance as such patients may be misdiagnosed as unilateral ocular albinism, especially since mild dystopia canthorum is not uncommon in Southern Chinese population.

ACKNOWLEDGMENTS

The authors thank all patients and family members for their participation. This study was supported by the National Science Fund for Distinguished Young Scholars (30725044 to Q.Z.).
REFERENCES

1. Hageman MJ, Delleman JW. Heterogeneity in Waardenburg syndrome. Am J Hum Genet 1977; 29:468-85. [PMID: 331943]

2. Markova TG, Megrelishvilli SM, Shevtsov SP, Shvarts EI. Clinical and molecular genetic investigation of Waardenburg syndrome type 1. Vestn Otorinolaryngol 2003; 1:17-9. [PMID: 12666593]

3. Kondoh T, Matsumoto T. Waardenburg syndrome. Ryoikibetsu Shokogun Shirizu 2000; 30:255-7. [PMID: 11057219]

4. Read AP, Newton VE. Waardenburg syndrome. J Med Genet 1997; 34:656-65. [PMID: 9279758]

5. Newton VE. Clinical features of the Waardenburg syndrome. Adv Otorhinolaryngol 2002; 61:201-8. [PMID: 12408085]

6. Waardenburg PJ. A new syndrome combining developmental anomalies of the eyelids, eyebrows and nose root with pigmentary defects of the iris and head hair and with congenital deafness. Am J Hum Genet 1951; 3:195-253. [PMID: 14902764]

7. Winship I, Beighton P. Phenotypic discriminants in the Waardenburg syndrome. Clin Genet 1992; 41:181-8. [PMID: 1576755]

8. Farrer LA, Arnos KS, Asher JH Jr, Baldwin CT, Diehl SR, Friedman TB, Greenberg J, Grundfast KM, Hoth C, Lalwani AK, Landa B, Leverton K, Milunsky A, Morell R, Nance WE, Newton V, Ramesar R, Rao VS, Reynolds JE, San Agustin TB, Wilcox ER, Winship I, Read AP. Locus heterogeneity for Waardenburg syndrome is predictive of clinical subtypes. Am J Hum Genet 1994; 55:728-37. [PMID: 7942851]

9. Tassabehji M, Newton VE, Read AP. Waardenburg syndrome type 2 caused by mutations in the human microphthalmia (MITF) gene. Nat Genet 1994; 8:251-5. [PMID: 7874167]

10. Selicorni A, Guermeri S, Ratti A, Pizzuti A. Cytogenetic mapping of a novel locus for type II Waardenburg syndrome. Hum Genet 2002; 110:64-7. [PMID: 11810298]

11. Sanchez-Martin M, Rodriguez-Garcia A, Perez-Losada J, Sagrera A, Read AP, Sanchez-Garcia I. SLUG (SNAI2) deletions in patients with Waardenburg disease. Hum Mol Genet 2002; 11:2321-6. [PMID: 12444107]

12. Bondurand N. Review and update of mutations causing Waardenburg syndrome. Mol Vis 2006; 12:1169-85. [PMID: 17999358]

13. Pasteris NG, Trask BJ, Sheldon S, Gorski JL. Discordant phenotype of two overlapping deletions involving the PAX3 gene in chromosome 2q35. Hum Mol Genet 1993; 2:953-9. [PMID: 8103404]

14. Puffenberger EG, Hosoda K, Washington SS, Nakao K, deWit D, Yanagisawa M, Chakravart A. A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. Cell 1994; 79:1257-66. [PMID: 8001158]

15. Edery P, Attie T, Amiel J, Pelet A, Eng C, Hofstra RM, Martelli H, Bidaud C, Munnich A, Lyonnet S. Mutation of the endothelin-3 gene in the Waardenburg-Hirschsprung disease (Shah-Waardenburg syndrome). Nat Genet 1996; 12:442-4. [PMID: 8630502]

16. Pingault V, Bondurand N, Kuhlbrodt K, Goerich DE, Prehu MO, Puliti A, Herbarth B, Hermans-Borgmeyer I, Legius E, Matthijs G, Amiel J, Lyonnnet S, Ceccherini I, Romeo G, Smith JC, Read AP, Wegner M, Goossens M. SOX10 mutations in patients with Waardenburg-Hirschsprung disease. Nat Genet 1998; 18:171-3. [PMID: 9462749]

17. Lalwani AK, Baldwin CT, Morell R, Friedman TB, San Agustin TB, Milunsky A, Adair R, Asher JH, Wilcox ER, Farrer LA. A locus for Waardenburg syndrome type II maps to chromosome 1p13.3-2.1. Am J Hum Genet 1994; 55:1A4.

18. Epstein DJ, Vekemans M, Gros P. Splotch (Sp2H), a mutation affecting development of the mouse neural tube, shows a deletion within the paired homeodomain of Pax-3. Cell 1991; 67:767-74. [PMID: 1682057]

19. Tassabehji M, Newton VE, Leverton K, Turnbull K, Seemanova E, Kunje Z, Sperling K, Strachan T, Read AP. PAX3 gene structure and mutations: close analogies between Waardenburg syndrome and the Splotch mouse. Hum Mol Genet 1994; 3:1069-74. [PMID: 7981674]

20. Apuzzo S, Gros P. Cooperative interactions between the two DNA binding domains of Pax3: helix 2 of the paired domain is in the proximity of the N-terminus of the homeodomain. Biochemistry 2007; 46:2984-93. [PMID: 17323927]

21. Apuzzo S, Abdelhakim A, Fortin AS, Gros P. Cross-talk between the paired domain and the homeodomain of Pax3: DNA binding by each domain causes a structural change in the other domain, supporting interdependence for DNA binding. J Biol Chem 2004; 279:33601-12. [PMID: 15148315]

22. Boissy RE, Nordlund JJ. Molecular basis of congenital hypopigmentary disorders in humans: a review. Pigment Cell Res 1997; 10:12-24. [PMID: 9170158]

23. Hornyak TJ, Hayes DJ, Chiu LY, Ziff EB. Transcription factors in melanocyte development: distinct roles for Pax-3 and Mitf. Mech Dev 2001; 101:47-59. [PMID: 11231058]

24. Tassabehji M, Read AP, Newton VE, Patton M, Gruss P, Harris R, Strachan T. Mutations in the PAX3 gene causing Waardenburg syndrome type 1 and type 2. Nat Genet 1993; 3:26-30. [PMID: 8490648]

25. Pingault V, Ente D, Dastot-Le Moal F, Goossens M, Marlin S, Bondurand N. Review and update of mutations causing Waardenburg syndrome. Hum Mutat 2010; 31:391-406. [PMID: 20127975]

26. Qin W, Shu A, Qian X, Gao J, Xing Q, Zhang J, Zheng Y, Li X, Li S, Feng G, He L. A novel mutation of PAX3 in a Chinese family with Waardenburg syndrome. Mol Vis 2006; 12:1001-8. [PMID: 16971891]

27. Yang SZ, Cao JY, Zhang RN, Liu LX, Liu X, Zhang X, Kang Y, Li S, Feng G, He L. A novel mutation of PAX3 in a Chinese family with Waardenburg syndrome. Mol Vis 2006; 12:1001-8. [PMID: 16971891]

28. Farrer LA, Grundfast KM, Amos J, Arnos KS, Asher JH Jr, Beighton P, Diehl SR, Fex J, Foy C, Friedman TB, Greenberg J, Herbarth B, Hermans-Borgmeyer I, Legius E, Matthijs G, Amiel J, Lyonnnet S, Ceccherini I, Romeo G, Smith JC, Read AP, Wegner M, Goossens M. SOX10 mutations in patients with Waardenburg-Hirschsprung disease. Nat Genet 1998; 18:171-3. [PMID: 9462749]
first report of the WS consortium. Am J Hum Genet 1992; 50:902-3. [PMID: 13491988]

29. Wang J, Liu J, Zhang Q. FOXL2 mutations in Chinese patients with blepharophimosis-ptosis-epicanthus inversus syndrome. Mol Vis 2007; 13:108-13. [PMID: 17277738]

30. Baldwin CT, Lipsky NR, Hoth CF, Cohen T, Mamuya W, Milunsky A. Mutations in PAX3 associated with Waardenburg syndrome type I. Hum Mutat 1994; 3:205-11. [PMID: 8019556]

31. Nakamura M, Ishikawa O, Tokura Y. A novel missense mutation in the PAX3 gene in a case of Waardenburg syndrome type I. J Eur Acad Dermatol Venereol 2009; 23:708-9. [PMID: 18761541]