Biochemical and Biophysical Research Communications 397 (2010) 70–74

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Novel mutations of PAX3, MITF, and SOX10 genes in Chinese patients with type I or type II Waardenburg syndrome

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ARTICLE INFO

Article history:
Received 6 May 2010
Available online 15 May 2010

Keywords:
Waardenburg syndrome
PAX3
MITF
SNAI2
SOX10

ABSTRACT

Waardenburg syndrome (WS) is a rare disorder characterized by distinctive facial features, pigment disturbances, and sensorineural deafness. There are four WS subtypes. WS1 is mostly caused by PAX3 mutations, while MITF, SNAI2, and SOX10 mutations are associated with WS2. More than 100 different disease-causing mutations have been reported in many ethnic groups, but the data from Chinese patients with WS remains poor. Herein we report 18 patients from 15 Chinese WS families, in which five cases were diagnosed as WS1 and the remaining as WS2. Clinical evaluation revealed intense phenotypic variability in Chinese WS patients. Heterochromia iridis and sensorineural hearing loss were the most frequent features (100% and 88.9%, respectively) of the two subtypes. Many brown freckles on normal skin could be a special subtype of cutaneous pigment disturbances in Chinese WS patients. PAX3, MITF, SNAI2, and SOX10 genes mutations were screened for in all the patients. A total of nine mutations in 11 families were identified and seven of them were novel. The SOX10 mutations in WS2 were first discovered in the Chinese population, with an estimated frequency similar to that of MITF mutations, implying SOX10 is an important pathogenic gene in Chinese WS2 cases and should be considered for first-step analysis in WS2, as well as MITF.

1. Introduction

Waardenburg syndrome (WS) is a rare autosomal dominant inherited disorder characterized by sensorineural hearing loss and pigment disturbances of the hair, skin, and iris. It was first described in Northern Europeans [1], and has been reported in many other ethnic and racial groups [2,3]. Four subtypes of WS have been classified based on the presence or absence of additional symptoms. Type I WS (WS1, MIM ID: 193500) and type II WS (WS2, MIM ID: 193510) are distinguished by the presence or absence of dystopia canthorum, respectively. Type III WS (Klein–Waardenburg syndrome, WS3, MIM ID: 148820) is similar to type I with additional musculoskeletal abnormalities; while type IV WS (Shah–Waardenburg syndrome or Waardenburg–Hirschsprung disease, WS4, MIM ID: 277580) is characterized by the presence of an aganglionic megacolon. WS1 and WS2 are more common clinically.

This syndrome is both clinically and genetically heterogeneous. Dystopia canthorum is the most penetrant feature of WS1, being present in 99% of those affected, but not in WS2. Apart from dystopia canthorum, all features of WS1 and WS2 show marked interfamilial and intra-familial variability. There are mutations in six genes reported to be associated with WS, with PAX3 mutations accounting for the majority of WS1 and WS3. Nearly all WS1 patients are present with heterozygous mutations of PAX3 [4,5]. WS3, an extreme presentation of WS1, is also caused by heterozygous or homozygous mutations of PAX3 [6]. Fifteen percent of WS2 have heterozygous mutations of MITF, but about 85% of WS2 cases are still unexplained at the molecular level, although homozygous deletions of SNAI2 have been found in two patients [5,7]. WS4 is mainly caused by mutations of the EDN3, EDNRB, and SOX10 genes. SOX10, a key transcription factor of neural-crest development, is crucial for the survival and maintenance of pluripotency of migrating neural-crest progenitors. Recently, Bondurand et al. have identified SOX10 deletions in WS2 patients, making SOX10 a new gene related to WS2 [8].

To date, over 100 mutations in PAX3, MITF, SNAI2, and SOX10 genes have been reported in WS patients. However, there are limited reports on WS in the Chinese population. Yang et al. studied 35 Chinese patients with WS1 or WS2 and observed that patients with

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0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.05.066

WS2 displayed many brown freckles on the skin [9], which is obviously different from cutaneous pigment abnormalities of their Western counterparts [10]. In addition, Sham et al. have found two SOX10 mutations in two Chinese WS4 patients [11], without any SOX10 mutation being identified in Chinese WS2 patients. In the present study, we determined the phenotypic gene expression of WS patients in 15 Chinese WS families. More importantly, we identified three novel SOX10 mutations in WS2 for the first time in the Chinese population. Our results may be helpful in determining possible genotype-phenotype correlations for WS patients.

2. Materials and methods

2.1. Patients and families

All the patients with WS were seen in the Otology Clinic at Xiangya Hospital and three training schools for the deaf and mute in Central South China in the past five years. There were 18 WS patients (from 15 families), age of 1–42 years old, and 21 family members who agreed to take part in the study. Among the 15 families, only family WS01 and family WS02 had more than one patient (Fig. 1). Two hundred randomly selected normal individuals were also included in this study. The study was approved by the Xiangya Ethics Committee, and signed informed consent was obtained from each of the subjects.

2.2. Clinical evaluation

A comprehensive clinical history was taken, and neurological, ophthalmologic, and dermatologic examinations were performed on all of the subjects. The audiological and neurological examinations consisted of otoscopy, pure-tone audiometry, immittance, distortion product otoacoustic emission (DPOAE), and auditory brain-stem response (ABR). The ophthalmologic examination included visual acuity measurement, visual field examination, and fundus ophthalmoscope. Special attention was given to the color of skin, hair, and iris, and to other developmental defects, such as dystopia canthorum and limb abnormalities. The degree of hearing loss was defined according to the pure-tone averages (PTA), which were based on the three frequencies (500, 1000, and 2000 Hz), as follows: normal <26 dB (decibel) HL (hearing level), mild 26–40 dBHL, moderate 41–70 dBHL, severe 71–90 dBHL, and profound >90 dBHL.

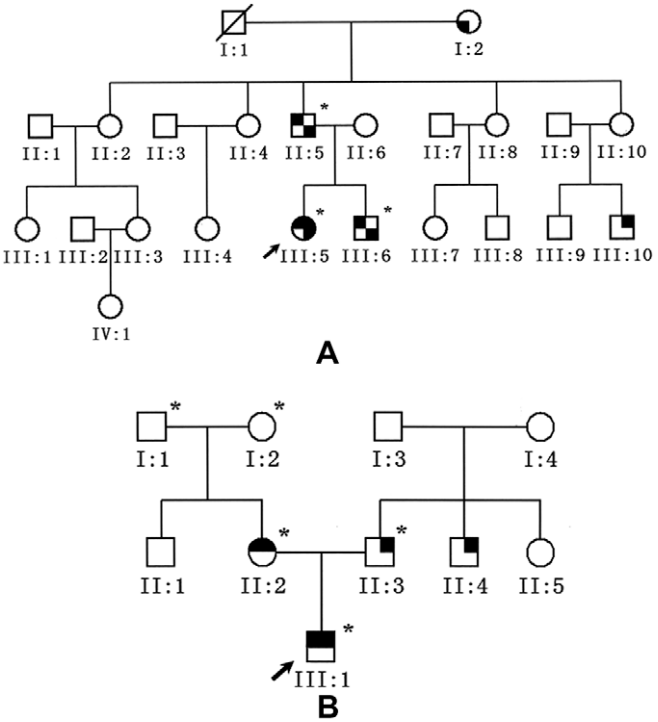

Fig. 1. Pedigrees of the families WS01 (A) and WS02 (B). Circle, female; square, male; filled quadrants indicate phenotype associated with WS, upper left: blue irides; lower left: premature graying; upper right: hearing loss; lower right: dystopia canthorum; arrow, the proband; *, DNA samples available.

2.3. Molecular analysis

The genomic DNA from all the subjects was extracted from peripheral blood cells using the phenol/chloroform method. The primers to amplify all coding region and intron/exon boundaries of PAX3, MITF, SNAI2, and SOX10 were designed using the on-line program PRIMER3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). After mutations of the four genes were identified in these patients, samples from the related family members and the controls were further screened for the mutations identified in the patients. Some PCR products were cloned and sequenced in order to confirm the mutations.

The PCR reaction was carried out in a total volume of 10 ul of reaction mixture containing 30 ng of genomic DNA, 30 ng each of forward and reverse primers, 150 umol dNTP, 0.2 U Hotstar Taq DNA polymerase, 2.5 mmol $MgCl_2$, 10 mmol Tris–HCl, and 50 mmol KCl. The amplification consisted of an initial denaturation at 95 °C for 15 min, followed by 10 cycles consisting of denaturation at 94 °C for 1 min, annealing for 45 s at 62 °C, and extension at 72 °C for 50 s; followed by 25 cycles consisting of denaturation at 94 °C for 1 min, annealing for 45 s at 57 °C, and extension at 72 °C for 50 s, and a final extension at 72 °C for 10 min. The amplified fragments were purified and sequenced in both ends on an ABI_Prism 3100 DNA sequencer®, with a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems®, Foster City, USA). The sequencing results were analyzed using the SeqMan II program of the Lasergene package (DNASTAR Inc., Madison, WI).

3. Results

3.1. Clinical characteristics of WS patients

According to the diagnostic criteria for WS proposed by the Waardenburg Consortium [12], five cases (WS05, WS10, and II:5, III:5, III:6 in the family WS01) were diagnosed as WS1, while the other 13 cases were WS2. All the patients showed unilateral or bilateral heterochromia irides, and fundus examinations demonstrated a generalized decrease in retinal pigment with a focal hypopigmented lesion in all the eyes with blue irides. Each of the WS1 cases displayed dystopia canthorum, and two of them also presented broad nasal roots and synophrys. However, hypopsia and pigmentary abnormalities of hair and skin were only observed in WS2 families, in which five patients and one family member had hyposia and/or much brown freckles on the bodies extensively. Premature graying and ptosis were only found in some of the WS2 cases. Other clinical features, such as white forelock, were not observed in this study. All the patients displayed congenital, bilateral profound sensorineural hearing loss, except two WS1 cases. Representative clinical findings and the typical characteristics of these WS cases are illustrated in Fig. 2.

3.2. Identification of mutations

Nine heterozygous mutations, including seven previously unrecognized, were identified from 11 of the 15 families, including one indel, three missense and five deletion mutations.

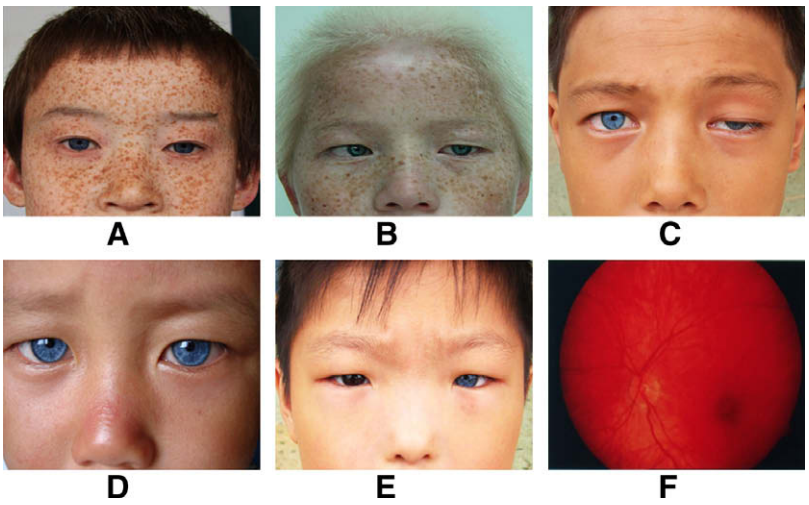

Fig. 2. Photographs of affected individuals. The probands in the family WS12 (A) and WS08 (B) presented with bilateral blue irides and special brown freckles on the faces, and the latter also showed premature graying. Another two probands in the family WS09 (C) and WS13 (D) displayed bilateral brilliant blue irides, and the former accompanied with congenital ptosis. But they all (A–D) had no evidence of dystopia canthorum and were diagnosed as WS2. The proband (E) in the family WS05 displayed blue irides (left), dystopia canthorum, broad nasal root, synophrys and was diagnosed as WS1, and the fundus photograph (F) of the left eye with blue irides demonstrated a generalized decrease in retinal pigment with a focal hypopigmented lesion. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

All the three PAX3 mutations, c.238C > G, c.808C > T and c.556delC, were detected within WS1 families. Three patients of the WS01 family carried the mutation, c.808C > T, which led to a substitution from Arg to Cys at the 270th amino acid, which has been previously reported. The novel mutation, c.238C > G, found in the proband of the WS05 family and his unaffected father, replaced the histidine at codon 80 with aspartic acid. Another novel mutation, c.556delC, identified in the WS10 family, resulted in a truncated protein with a premature termination codon at 192.

Three MITF mutations were found in five WS2 families. The mutation c.650G > T was detected in the family WS08 and c.648_650delAAG in the families of WS06, WS07 and WS12, respectively, which caused an amino acid change (Arg to Ile) and the deletion of arginine at amino acid 217 separately. The mutation c.650G > T had not been reported previously. Another novel mutation, c.575delC, leading to a truncated protein of 210 amino acids, was found in the proband of the WS04 family and her affected father.

Three novel SOX10 mutations, c.113delG, c.110_219del110, and c.126_127delinsTT, were identified in the WS02, WS09, and WS11 families, respectively. These mutations led to truncation of the SOX10 protein at varying distances from the start codon, due to frameshift or introduction of premature termination codon.

All the mutations identified in these patients were not seen in any other unaffected family members and 200 unrelated control subjects. No *SNAI2* mutations were detected in our study.

4. Discussion

The incidence of WS is about 1 in 42,000, and both sexes and all races are equally affected by this disease. But the frequencies of each type of the syndrome in different populations are variable. Pardono E claimed that WS1 is more common than WS2 [13], however, other groups reported more cases of WS2 instead [3,5]. In the present study, WS2 cases were apparently more than WS1. Other research on Chinese WS also displayed that WS2 patients were more common [9], suggesting WS2 may be the most common subtype in the Chinese population; although a large-scale, population-based study is needed to confirm this.

The clinical manifestations of WS vary widely within and between families. In the present study, dystopia canthorum is the most frequent sign in WS1 (100%), followed by heterochromia irides (100%), sensorineural deafness (60%), broad nasal roots (40%), and synophrys (40%). In WS2, sensorineural deafness (100%) and heterochromia irides (100%) are the most common findings, followed by pigment abnormalities of the skin (38.5%), hypopsia (30.8%), ptosis (15.4%), and premature graying (7.8%), suggesting that heterochromia irides and sensorineural deafness are the most frequent features of both WS1 and WS2, which have higher incidences than in Westerners [4]. Unilateral heterochromia irides were present more in WS1, while deafness was more associated with WS2. The special brown freckles on the skin shown in our WS2 patients might be a special subtype of cutaneous pigment disturbances in Chinese WS patients. It is obviously different from cutaneous pigment abnormalities of WS in Westerners, who usually manifest hypopigmented and depigmented patches on the skin, and sometimes hyperpigmented patches, can be seen within the areas of hypomelanosis [10]. Furthermore, the patients with cutaneous pigment disturbances always had hypopsia, and most were seen in WS2 cases in our study.

There were 13 different mutations of PAX3, MITF, and SOX10 described in Chinese patients with WS before (Table 1), including nine missense mutations and four deletion mutations [5,9,10,14–18], and most of these mutations are unique for one kindred. In our study, nine heterozygous mutations were identified, including each three mutations in PAX3, MITF, and SOX10. All the patients with WS1 carried PAX3 mutations, while MITF and SOX10 mutations in WS2 were more common than in Westerners [5], with the detection rates being 38.5% and 30.8%, respectively. Our results greatly expanded the database of known mutations in Chinese WS patients.

PAX3 is a transcription factor expressed during embryonic development and has four structural motifs, including paired domain, octapeptide sequence, homeodomain, and Pro-Ser-Thr-rich COOH terminus [19]. The three mutations in PAX3, c.238C > G, c.808C > T and c.556delC, are located in the highly conserved paired domain, octapeptide motif and homeodomain, respectively. Alternations in these domains may lead to decreasing DNA binding affinity or changing DNA binding specificity.

MITF also is a transcription factor, containing a basic helix-loop-helix zipper motif, which is vital for the development and survival of melanocytes, osteoclasts, and mast cells. The basic domain

Table 1
The reported mutations of PAX3, MITF, and SOX10 in Chinese WS patients.

Gene	Mutation[a]	Effect[b]	Position	References
MITF	C.20A > G[c]	p.Y7C[c]	Exon1	[9]
MITF	c.648_650delAAG	p.R217del	Exon 7	[9,5] this study
MITF	c.639delA[c]	p.E213fs[c]	Exon 7	[18]
MITF	c.328C > T[c]	p.R110X[c]	Exon 3	[9]
MITF	c.763C > T[c]	p.R255X[c]	Exon 8	[9]
MITF	c.649A > G[c]	p.R217G[c]	Exon 7	[9]
MITF	c.332C > T[c]	p.A111V[c]	Exon 3	[9]
PAX3	c.812G > A	p.R271H	Exon 6	[5,9,15]
PAX3	c.626_627delCT[c]	p.S209X[c]	Exon 5	[17]
PAX3	c.667C > T	p.R223X	Exon 5	[14,15,17]
PAX3	c.701T > C[c]	p.L234P[c]	Exon 5	[16]
SOX10	c.168delG[c]	p.E57fs[c]	Exon 3	[10]
SOX10	c.1399T > A[c]	p.X467K[c]	Exon 5	[10]

[a] Description of the mutations is based on cDNA sequence, +1 corresponding to the A of the ATG translation initiation codon of the reference sequence. GenBank reference sequences: PAX3 (Genebank ID: NM_181458.2); MITF (Genebank ID: NM_000248.2); SNAI2 (Genebank ID: NM_003068.3); SOX10 (Genebank ID: NM_006941.3).

[b] Amino acid numbering is based on GenBank reference sequences: PAX3 (Genebank ID: NP_852123); MITF (Genebank ID: NP_000239); SNAI2 (Genebank ID: NP_003059); SOX10 (Genebank ID: NP_008872). Amino acids were numbered from the first methionine.

[c] The mutation which has been only described in Chinese population.

makes specific DNA contacts while the helix-loop-helix leucine zipper mediates homodimerization and heterodimerization with related transcription factors [20]. The two MITF mutations, c.650G > T and c.648_650delAAG, likely affect the dimerization by disrupting the helix-loop-helix or zipper motifs, and result in disease through the mechanism of haploinsufficiency [20]. The mutation c.648_650delAAG may be a frequent mutation in Chinese patients with WS2 as three families carried the same mutation in our cases. While the mutation c.575delC results in a premature termination codon, ten amino acids after the start of the HLH leucine zipper motif, the mutant protein is void of functional domains.

SOX10, a member of the group E SOX genes, contains a central high mobility group (HMG) domain and a C-terminal transactivation domain [21]. More than 20 mutations causing different neurocristopathies have been reported in the SOX10 gene, and most of them generate premature stop codons and result in WS4 or the more severe PCWH (peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease) [22,23]. In this study, we identified SOX10 mutations to be associated with WS2 in the Chinese population for the first time. Thirty point eight percent of WS2 cases carried SOX10 mutations with an estimated frequency similar to that of MITF (38.5%), suggesting that SOX10 is an important pathogenic gene in Chinese WS2 and should be considered for first-step analysis in WS2. However, the SOX10 mutations characterized so far are mostly truncating mutations [2], and no SOX10 missense mutation has been found in WS2 [8,24]. It may be possible that screening larger numbers of patients with WS2 would result in the identification of SOX10 missense mutations. The SOX10 mutations identified in our families were two deletions and one indel mutations, resulting in the truncation of the proteins which lack part of or the whole HMG DNA binding domain and transactivation domain, and likely to be nonfunctional. A similar mutation (c.126_127delinsCT) in SOX10 that also resulted in a premature termination at the 43rd amino acid (R43X) had been reported in a Caucasian child [22]. The Caucasian child displayed sensorineural hearing loss and Hirschsprung disease, which is obviously different from the patient in our study.

Few conclusions can be drawn with regard to the relationship between a specific mutation or class of mutation and phenotype in Chinese WS. The diversity of mutations seems not to be the cause of the clinical variability of WS. Even within a family, the presentation is very variable, and no correlation between the type of mutation and clinical features has been found to be strong enough as a predictive marker for individuals. In the present study, 83.3% of cases with MITF mutations manifested premature graying and/or much freckles on the skin, while patients with SOX10 mutations only displayed mild pigmentary disturbance of the skin and half of them showed congenital ptosis, which was not seen in patients with MITF mutations. Therefore, the more detailed relationship between phenotype and genotype in Chinese WS patients is still to be determined in future research.

Acknowledgments

We thank the patients and families for their collaboration in this project. We are grateful to Dr. Danling Wang for helpful editing of the manuscript. Contract grant sponsor: Chinese National Programs for High Technology Research and Development; contract grant number: 2007AA02Z445. Contract grant sponsor: National Nature Science Foundation of China; contract grant number: 30470954 and 30671150. Besides, this study has been supported by another National Nature Science Foundation of China, the contract grant number of which is 30971589.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.05.066.

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