Dystrophia canthorum in Waardenburg syndrome with a novel *MITF* mutation

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Abstract

● **AIM:** To reveal a novel *MITF* gene mutation in Waardenburg syndrome (WS), which is an autosomal dominant inherited neurogenic disorder that consists of various degrees of sensorineural deafness and pigmentary abnormalities in the eyes, hair and skin.

● **METHODS:** The genetic analysis of the Chinese family was conducted by whole-exome sequencing, then the results were confirmed by Sanger sequencing.

● **RESULTS:** WS is classified into type I to IV, which are identified by the W index, clinical characteristics and additional features. The *MITF* gene mostly accounts for WS type II. In this study, a *de novo* heterozygous mutation in the *MITF* gene, c.638A>G in exon 7, was identified in the patient diagnosed with WS type I features, as the W index was 2.17 (over 2.10), with dystrophia canthorum, congenital bilateral profound hearing loss, bilateral heterochromia irides, premature greying of the hair, and excessive freckling on the face at birth. She also underwent refractive errors and esotropia, reduced pigmentation of the choroid and visible choroid vessels. The mutation was not found in previous studies or mutation databases.

● **CONCLUSION:** The novel mutation in the *MITF* gene, which altered the protein in amino acids 213 from the glutamic acid to glycine, is the genetic pathological cause for WS features in the patient. Those characteristics of this family revealed a novel genetic heterogeneity of *MITF* in WS, which expanded the database of *MITF* mutations and offered a possible in correcting the W index value of WS in distinct ethnicities. Moreover, ocular symptoms should be emphasized in all types of WS patients.

INTRODUCTION

Waardenburg syndrome (WS), which accounts for 0.9%-2.8% among the congenitally deaf and has an incidence of 1 in 42000 in the general population, is the most common cause of syndromic deafness[1-2]. Sensorineural deafness and various types of pigmentary abnormalities, including the skin, hair, eyes, ears craniofacial region, urogenital tract, gastrointestinal tract and central nervous system, are the major WS characteristics[3]. The significant symptoms of WS result from the abnormal functional melanocytes[4]. Based on the clinical characteristics and additional features, WS has been classified into four types, including WS type I with dystrophia canthorum (WS1, OMIM193500), WS type II without dystrophia canthorum (WS2, OMIM193510), WS type III with dystrophia canthorum and upper limb anomalies (WS3, OMIM148820) and WS type IV only with intestinal aganglionosis (WS4, OMIM277580)[5]. Dystopia canthorum is the most prominent sign for WS type classification, and the W index was introduced to diagnose this symptom[6]. WS1 is diagnosed with a W index value >1.95 in Caucasian[7], and >2.10 in Chinese, which was corrected in a recent study[8].

Except showing few significant symptoms, the clinical phenotypes of WS are highly varied in expression, leading to a significant challenge in clinical diagnosis[9]. In the clinic, genetic examination is often used to improve the accuracy of WS diagnosis. Until now, six genes have been incriminated as causative factors for WS, including *EDN3* (encoding the endothelin-3), *EDNRB* (encoding the endothelin receptor type B), *MITF* (encoding the microphthalmia-associated transcription factor), *PAX3* (encoding the paired box 3 transcription factor),

SNAI2 (encoding the snail homologue 2 transcription factor) and *SOX10* (encoding the sex determining region Y box 10 transcription factor) with different frequencies in different types of WS[5].

Among them, MITF is a member of the basic-helix-loop-helix leucine zipper family of transcription factors, which acts as a regulator of melanocyte development, function and survival by modulating other related genes[10]. Nearly 15% of WS2 cases are caused by the heterozygous mutations in the *MITF* gene[11-12], while a homozygous mutation in the *MITF* gene has been identified in a case of WS4[13]. Moreover, other genes responsible for WS can have a link with the regulation of MITF activity or expression level, such as *PAX3*, which is most related with WS1 and WS3. Meantime, as a regulator gene in the neural crest, PAX3 has been directly associated with modulation of the MITF promoter[14].

To understand the molecular function of *MITF* and its association with WS will hopefully confer possible clinical strategies for this disease. In this study, a patient diagnosed with WS was reported a new pathogenic mutation in *MITF*, which offers a new insight for studying the *MITF*-linked pathogenesis mechanism in WS.

SUBJECTS AND METHODS

Ethical Approval The study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine. All participants had been fully informed of the purpose and methods of this study, and provided informed consent to join in this study.

Patients and Clinical Evaluations The proband diagnosed with WS was recruited through her visit to Eye Center, the Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, China. The W index was calculated as follows: W=(2×ICD−0.2119×OCD−3.909)/OCD+(2×ICD−0.2497×IPD−3.909)/IPD+ICD/IPD, and the ICD, OCD, and IPD are inner canthal distance, outer canthal distance and interpupillary distance (millimeter), respectively[6,15]. The proband was completely examined in audiology, hair, skin, ophthalmology, limb joints, digestive system and intelligence. Additionally, a temporal CT and cranial MRI were also conducted. She reported WS major criteria: the congenital sensorineural deafness, characteristic hair depigmentation, dystopia canthorum, patchy depigmentation of the skin, pigmentary anomalies of the iris and excessive freckle[3], and some ocular manifestations, including choroidal hypopigmentation[16], convergent strabismus and refractive error[17].

Next-generation Sequencing and Data Analysis Genomic DNA was extracted from peripheral blood for each member from the family, then it was fragmented to an average

size of 180-280 base pair (bp) and used to create a DNA library following established Illumina paired-end protocols. The Agilent Sure Select Human All ExonV6 Kit (Agilent Technologies, Santa Clara, CA, USA) was used for exome capture. Subsequently, genomic DNA sequencing, which was analyzed by the Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA) generated 150 bp paired-end reads with a minimum coverage of 10× for ~99% of the genome (mean coverage of 100×).

Given to the pedigree of WS, the classification system of the American College of Medical Genetics and Genomics (ACMG), the 100% coverage of the causative related genes, including *END3, EDNRB*, *MITF, PAX3, SOX10* and *SNAI2*, and possible pathogenic effects of the missense mutations were evaluated by computational tools including Polyphen-2, Mutation Taster, SIFT (cut-off score <0.05), PROVEAN (cut-off score <-2.5) and CADD (cut-off score >20).

Sanger Sequencing for the Candidate Variants The potentially mutated base was amplified using polymerase chain reaction (PCR) and sequenced by Sanger sequencing, detected by ABI 3730 Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Then the similarities between the reference sequences and the detected sequences were evaluated by the nucleotide-nucleotide BLAST (blastn) tool (https://blast.ncbi.nlm.nih.gov).

Variation in *MITF* gene was evaluated by those computational tools and classified as likely pathogenic according to ACMG. In addition, a missense *de novo* variation in the *MITF* gene (GenBank accession No. NM_000248.3) was found in the proband, with one bp substitution on exon 7, p.Glu213Gly (c.638A>G). No mutations were identified in other unaffected family members. The detected mutation was searched in the HapMap database, the 1000 Genomes database, the NCBI dbSNP database and the Human Gene Mutation Database (HGMD, http://www.hgmd.org/), and known polymorphic sites were excluded.

RESULTS

Clinical Characteristics of the Patient The proband, a 7-year-old girl, was delivered full-term in this family with unaffected parents (Figure 1A). She was suffered from congenital bilateral profound hearing loss, bilateral heterochromia irides, broadened inner canthi, premature greying of the hair and excessive freckle on the face at birth (Figure 1B). When she was one year old, she failed bilateral transient evoked otoacoustic emissions (TEOAE), all bilateral auditory brainstem response (ABR) thresholds were over 99 dB nHL, and auditory steady-state response (ASSR) 500-4000 Hz thresholds were over 100 dB HL. In her recent ocular inspection, she underwent refractive errors (hyperopia with 2.75 D/3.25 D in atropine dilation optometry) with both
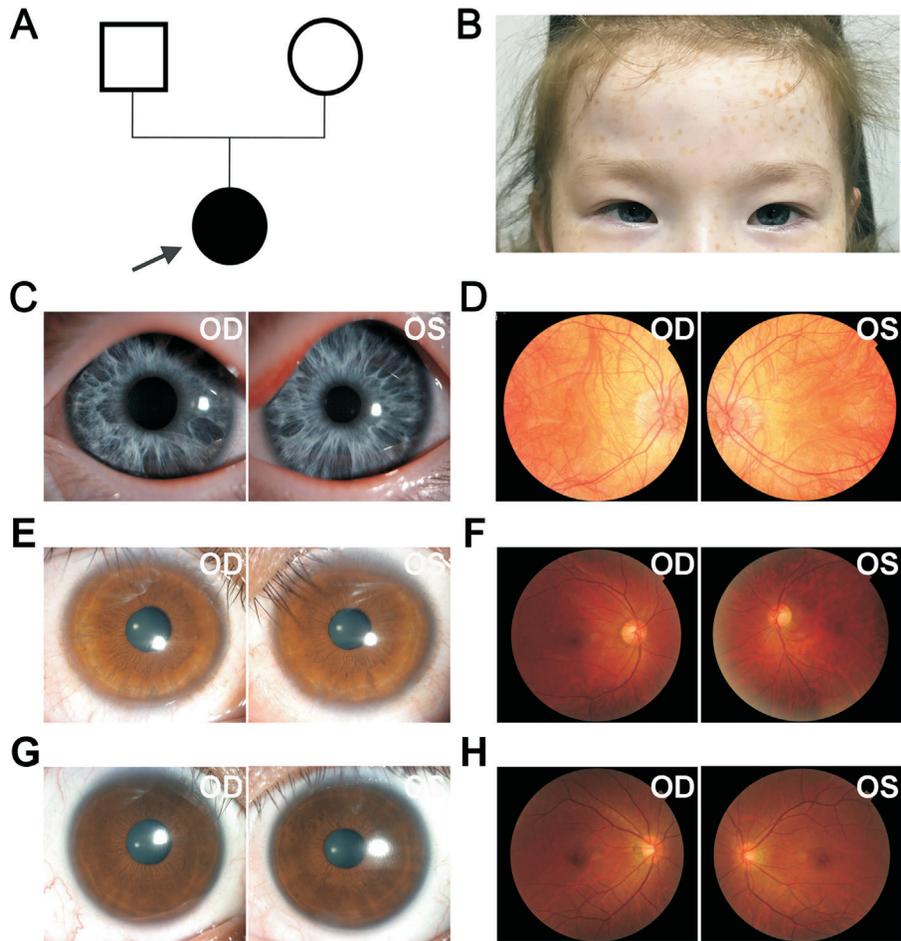
Figure 1 Pedigree, phenotype characterization of this patient A: Pedigree, the proband was pointed by the arrow; B: Clinical features, the patient has bilateral heterochromia irides, dystrophia canthorum, premature greying of the hair, and excessive freckling on the face; C: Bilateral heterochromia irides in both eyes of this patient; D: Reduced pigmentation of the choroid in both eyes of this patient; E-F: Anterior imaging and fundus photography of her father's; G-H: Anterior imaging and fundus photography of her mother's. OD: Oculus Dexter, OS: Oculus Sinister.

corrections only to 16/20, and esotropia with 35 PD, and the external examination was normal with brisk pupillary reaction to light. Extra-ocular muscle movements, ocular tension and fundus were normal. Complete hypoplastic blue irises were detected *via* anterior imaging, as well as reduced pigmentation of choroid and visible choroid vessels were observed *via* fundus photography (Figure 1C-1D), compared with her unaffected parents' irises and choroid (Figure 1E-1H).

However, her temporal CT, cranial MRI and the inner ear imaging showed no obvious abnormalities. No dysfunction was detected in intelligence, and no abnormality was detected in the musculoskeletal system and gastrointestinal tract. The measured ICD was 41 mm, IPD was 64 mm, and OCD was 101 mm, so the W index of this proband was 2.17 (over 2.10[8]), and she was diagnosed with WS1.

Fortunately, the girl received the left cochlear implant surgery at age one and wore a hearing aid in the right ear, because of congenital bilateral sensorineural hearing loss, and the parents were informed of the disease, the necessary follow-up treatment and rehabilitation training. The hearing rehabilitation

was successful, and visual acuity was generally able to be corrected. At her third follow-up visit in ophthalmology, after correction with glasses for 9mo, both eyes had 20/20 vision and esotropia was corrected to 8.0 PD with glasses in a recent examination.

Identification of the Novel *MITF* Gene Mutation The genomic DNA of the proband and family members was extracted, and a coverage of ~99 % of the genome, including the whole exons and the 20 bp of splicing sites of deafness-associated genes and WS-associated genes, was sequenced. A novel *de novo* variation adenine (A) to guanine (G) in position 638 (c.638A>G) was located in exon 7 of the *MITF* gene, leading to a substitution of the 213rd codon (p.E213G): the glutamic acid was replaced by glycine, which was confirmed by Sanger sequencing (Figure 2A). The mutation changed a highly conserved E213 residue (Figure 2B) in the N-terminal α helix of the basic helix-loop-helix (bHLH) motif (Figure 2C). According to the standards and the guidelines of the American College of Medical Genetics and Genomics (ACMG), the mutation in the *MITF* gene is considered to be likely
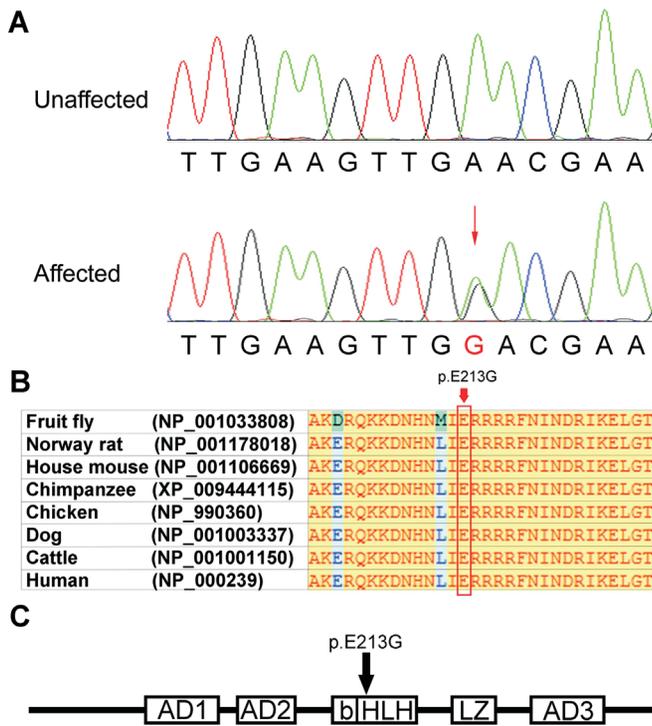
Figure 2 The c.638A>G (p.E213G) mutation identified in the family A: Chromatograms showed the heterozygous missense c.638A>G (p.E213G) mutation (arrow); B: Conservative analysis of *MITF* in different species showed the highly conserved 213rd residue (arrow); C: The position of p.E213G in *MITF* gene.

Table 1 Disease-causing prediction of this *MITF* gene mutation

Gene	*MITF*
Reference transcript	NP_000239.1
Candidate variant	p.E213G (c.638A>G)
1000 genomic databases	Not reported
ExAC	Not reported
Mutation taster	Disease causing
PolyPhen-2	
Prediction	Probably damaging
HumVar score[a]	0.987
PROVEAN	
Prediction	Deleterious
Score[b]	-6.514
SIFT	
Prediction	Damaging
Score[c]	0.000

[a]HumVar score, sensitivity: 0.53; specificity: 0.95. [b]Default threshold is -2.5, which means variants with a score ≤-2.5 are considered deleterious, and variants with a score >-2.5 are considered neutral. [c]Score ranges from 0 (deleterious) to 1 (neutral) with cut-off score set at 0.05.

pathogenic. The mutation was predicted to be disease-causing by different computational tools, such as PolyPhen-2, Mutation Taster, PROVEAN and SIFT (Table 1). In addition, the mutation was not found in the 1000 Genomes database, the ExAC database, the Human Gene Mutation Database (HGMD) and known polymorphic sites.

DISCUSSION

In the family, the patient has typical clinical characteristics of WS: all major diagnostic criteria, including congenital sensorineural deafness, hair depigmentation, pigmentary anomalies of the iris, dystopia canthorum, and minor criteria, including depigmented choroid. The heterozygous mutation found in *MITF* is located in exon 7 and causes missense alteration in the 213rd amino acid (p.E213G), which is a highly conserved E213 residue in vertebrates. Interestingly, both parents of the proband have no clinical characteristics or genetic mutations, implying this case occurred *de novo*[18]. No clinical evidence of upper limb anomalies or intestinal aganglionosis excluded WS3 and WS4. With the characteristic dystopia canthorum and the current value of the W index, the girl was diagnosed with WS1.

WS has been known for its highly varied expression and penetrance. Mutations in a gene can account for different types and characteristics of WS. In animal models, mutations in *MITF* homologues are also responsible for the microphthalmia

phenotypes of the *mibA* rat mutant, the anophthalmic white and *Wh*[V203] hamster mutants and the *nacre* and *nac*[W2] zebrafish mutants[19-21]. However, in humans, the majority number of mutations in *MITF* cause more moderate phenotypes with WS2, and most of mutations are expected to be located in exon 7 and 8, which correspond to the b-HLH-Zip motifs[5], including the mutation in this study. The *MITF* gene, containing nine alternative promoters, has differentially expressed isoforms with a complex pattern of splicing[22]. Although differing in the amino terminus, all isoforms contain basic domains, including the DNA binding domain (b), an HLH and a leucine zipper (Zip)[23]. The b-HLH-Zip motif controls the ability to bind to the CATGTG core DNA sequence in the human tyrosinase promoter[24]. Some missense mutations located out of the basic b-HLH-Zip motifs lie in alpha helices of the functional domains, and other exceptions also be found[5].

Although most of the *MITF* mutations were reported to be responsible for WS2[25-27], some *MITF* mutations seemed to be related with other types of WS[13,28]. One recent report showed a new clinical possibility between WS and MITF, as a homozygous *MITF* mutation in two WS4 children was found in a family with heterozygous *MITF*-mutated WS2 patients[13]. In this study, we found a heterozygous *MITF* mutation in a WS1 patient with a current value of the W index. The definition of WS1 and WS2 can be distinguished by dystopia canthorum, which is calculated by the W index. Aria *et al* introduced the W index first in Caucasian[29], and the

threshold was identified to its current value of >1.95. However, in a study of 16 WS Japanese families, the same mutation found in Japanese and Caucasian patients diagnosed with WS1 and WS2, respectively[15,29], which depended on the W index. Another study was conducted in Chinese patients, Sun *et al*[8] diagnosed WS1 with a corrected W index >2.10. Considering that ICD, IPD, and OCD are also changeable in infants, children and adults[6], more data on dystopia canthorum and genetic mutations would be necessary to establish a clinical classification consistent with genetic classification for WS patients with distinct ethnicities.

Certain cases remain still unexplained in the pathogenesis mechanism and genetic variety, but those revealed a dosage effect in *MITF*-linked WS, which is similar to the pattern of *PAX3* and *EDNRB* mutations reported in WS1, WS2 and WS4[30]. More importantly, the clinical characteristics, including ocular features, of WS patients should be emphasized.

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