Gene 642 (2018) 362–366

Contents lists available at ScienceDirect

Gene

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

Research paper

Identification and functional analysis of a novel mutation in the PAX3 gene associated with Waardenburg syndrome type I

Zhijie Niu[a,b], Jiada Li[c], Fen Tang[d], Jie Sun[a,b], Xueping Wang[a,b], Lu Jiang[a,b], Lingyun Mei[a,b], Hongsheng Chen[a,b], Yalan Liu[a,b], Xinzhang Cai[a,b], Yong Feng[a,b,c,*], Chufeng He[a,b,*]

[a] Department of Otolaryngology-Head and Neck Surgery, Xiangya Hospital, Central south University, Changsha 410008, People's Republic of China
[b] Key Laboratory of Otolaryngology Major Disease Research of Hunan Province, Changsha, 410008, People's Republic of China
[c] State Key Laboratory of Medical Genetics, Central South University, Changsha 410078, People's Republic of China
[d] State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-Sen University, Guangzhou 510060, People's Republic of China

ARTICLE INFO

Keywords:
Mutation
PAX3
Waardenburg syndrome
Sensorineural hearing loss
Haploinsufficiency

ABSTRACT

Waardenburg syndrome type 1 (WS1) is a rare autosomal dominant genetic disorder of neural crest cells (NCC) characterized by congenital sensorineural hearing loss, dystopia canthorum, and abnormal iris pigmentation. WS1 is due to loss-of-function mutations in paired box gene 3 (*PAX3*). Here, we identified a novel *PAX3* mutation (c.808C > G, p.R270G) in a three-generation Chinese family with WS1, and then analyzed its in vitro activities. The R270G PAX3 retained nuclear distribution and normal DNA-binding ability; however, it failed to activate *MITF* promoter, suggesting that haploinsufficiency may be the underlying mechanism for the mild WS1 phenotype of the study family.

1. Introduction

Waardenburg syndrome (WS) is a rare auditory-pigmentary disorder characterized by sensorineural deafness and pigmentation abnormalities of the hair, eye and skin (white forelock or premature graying, hypoplastic blue irises and white skin patches) (Baxter et al., 2004; Pingault et al., 2010), which results from defects of neural crest cell (NCC)-derived melanocytes. As a clinically and genetically heterogeneous disorder, WS is classified into four subtypes (WS1–4) based on the presence or absence of additional symptoms (Read and Newton, 1997), including dystopia canthorum, upper limb abnormalities, and Hirschsprung disease. Currently, six genes have been implicated in the various types: *PAX3* (OMIM #606597), *MITF* (OMIM #156845), *SOX10* (OMIM #602229), *SNAI2* (OMIM #602150), *EDN3* (OMIM #131242) and *EDNRB* (OMIM #131244).

Waardenburg syndrome type 1 (WS1; OMIM# 193500) is characterized by additional feature of dystopia canthorum, which is the most penetrant feature of WS1 with an incidence of 99%. Its prevalence is estimated to be 1/42,000 of the general population, and 1–3% of the congenital hearing loss (Read and Newton, 1997). It's well known that *PAX3* is the pathogenic gene responsible for WS1, accounting for approximately all the cases. *PAX3* (NM_181457.3, NP_852122) encodes a 479-amino-acids protein belonging to the nine-member family of paired box-containing transcription factors. PAX3 protein contains two DNA-binding region (a paired domain and a homeodomain), an octapeptide motif, and a Ser/Thr/Pro-rich transactivation domain (TA). It is expressed in neural crest cells during embryogenesis and involved in the proliferation, migration and differentiation of myogenic precursor cells and many neural crest derivatives including the melanocyte (Goulding et al., 1991; Kubic et al., 2008). With current knowledge of the molecular basis of WS, *PAX3* mutations are involved in both WS1 and WS type 3 (WS3, OMIM #148820). These mutations are heterozygous in WS1 cases, while homozygous or compound heterozygous in the allelic disease WS3, with extended musculoskeletal abnormalities (Wollnik et al., 2003), sometimes leading to death in early infancy or in utero. To date, > 100 different WS-associated mutations in the *PAX3* gene have been identified in many ethnic and racial groups (http://www.lovd.nl/3.0/home).

In the present study, we reported a small non-consanguineous Chinese family with WS1 with intra-familial phenotypic heterogeneity, and identified a novel missense mutation (c.808C > G) in the *PAX3* gene by Sanger sequencing. To understand functional consequences of

Abbreviations: WS1, Waardenburg syndrome type 1; *PAX3*, Paired box gene 3; NCC, Neural crest cells; *MITF*, The Microphthalmia-Associated Transcription Factor; WS3, Waardenburg syndrome type 3; HRCT, The high-resolution computed tomography; MRI, Magnetic resonance imaging; DPOAE, Distortion productotoacoustic emission; ABR, Auditory brain-stem response

* Corresponding authors at: Department of Otolaryngology, Head and Neck Surgery, Xiangya Hospital, Central South University, 87 Xiangya Road, Changsha, Hunan, People's Republic of China.
E-mail addresses: fyong@xysm.net (Y. Feng), hechufeng@csu.edu.cn (C. He).

https://doi.org/10.1016/j.gene.2017.11.035
Received 15 June 2017; Received in revised form 7 October 2017; Accepted 13 November 2017
Available online 20 November 2017
0378-1119/

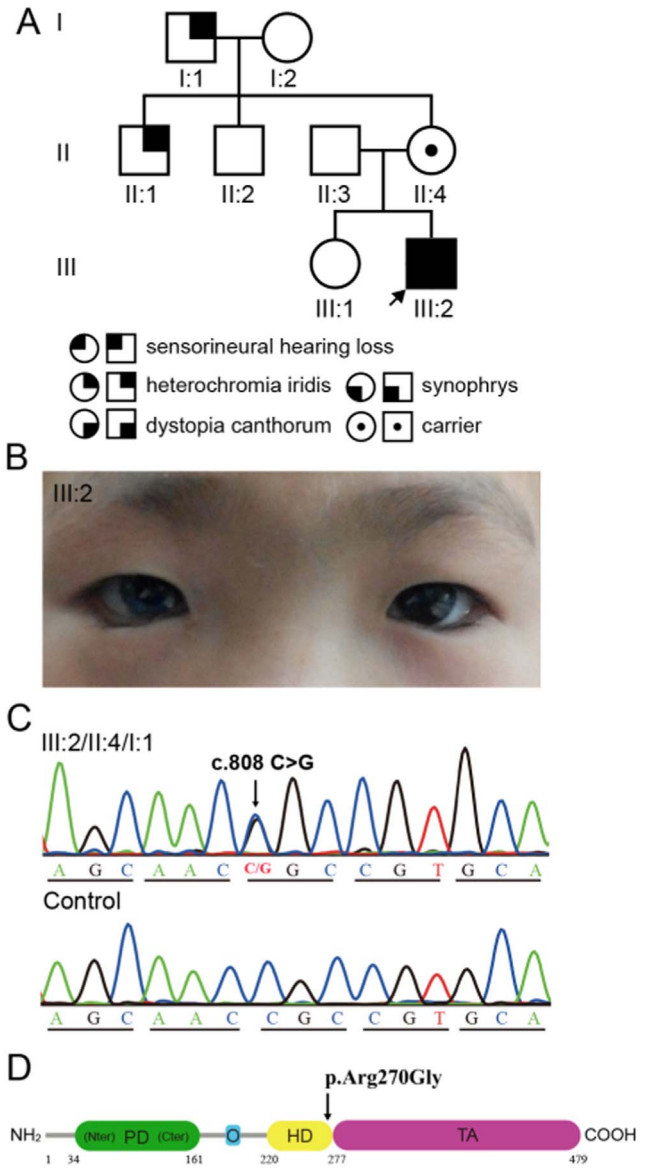

Fig. 1. Clinical features and genetic information of the WS1 family

A: The pedigree of the family is shown. The squares indicate men, and the circles indicate women. Filled quadrants indicate phenotype associated with WS1. The arrow denotes the proband.

B: Photograph of eyes from the proband (III: 2) with Waardenburg syndrome type 1. Dystopia canthorum (W index: 2.34), right-side brilliant blue iris, synophyris and broad nasal root were present in a 4-year old boy.

C: Sequence chromatography. The heterozygous change, c.808C > G, was identified in three family members (III: 2, II: 4 and I: 1). However, unaffected member (II: 3) and 200 healthy controls are wild-type at this position.

D: Schematic representation of PAX3 protein with the paired domain (PD), octapeptide motif (O), homeodomain domain (HD), and the transactivation domain (TA). The arrow highlights the Arg270Gly (R270G) mutation site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the mutation, we analyzed the expression, subcellular distribution, in vitro transcriptional activity and DNA-binding ability of the mutant PAX3 protein.

2. Materials and methods

2.1. Participants and clinical evaluation

The proband was identified in the Otology Clinic at Xiangya Hospital, Central-south University. In total, four subjects (Fig. 1A, I: 1, II: 3, II: 4, and III: 2) of three generations in this family and two hundred normal individuals of Chinese background were enrolled in the present study. The clinical evaluation was carried out on each study participant as previously described (Chen et al., 2010). The high-resolution computed tomography (HRCT) and magnetic resonance imaging (MRI) were performed on proband to exclude complications other than hearing disorders. This study was approved by the Xiangya Hospital Medical Ethics Committee, and signed informed consent was obtained from all subjects or their parents. All procedures were performed in accordance with the approved guidelines and regulations.

2.2. Mutation analysis

Genomic DNA was extracted from peripheral blood of all participants using the standard phenol/chloroform method. Sanger sequencing was then performed to investigate the pathogenic mutation in this family. Genomic fragments including the all coding exons and adjacent intronic regions of *PAX3*, *MITF*, *SOX10*, and *SNAI2* genes were amplified with PCR, using primers described previously (Chen et al., 2010) (Table 1). The novel mutation has been submitted to the Locus Specific Database (LOVD, DB-ID PAX3_00130).

2.3. Biological molecular analysis

Human melanoma UACC903 cell lines and Human embryonic kidney 293T (HEK 293T) cells were used in present study. The luciferase reporter *MITF* promoter (pGL3-MITF-Luc), human pCMV-Flag-SOX10 vector and pcDNA3-HA-PAX3 vector were generated as described earlier (Zhang et al., 2012; Wang et al., 2014; Sun et al., 2017). PAX3 mutant, p.R270G, expression vector was generated by the Nanjing Genescript Biotechnology Company (China). All plasmids were confirmed by direct nucleotide sequencing. As described previously (Zhang et al., 2012), we performed cell culture, transfection, luciferase reporter assays, Western blot analysis, immunofluorescence assays and biotinylated DNA affinity precipitation assays.

3. Results

3.1. Clinical findings

The proband (Fig. 1A, III: 2) is a 4-year-old boy with deafness and dumbness. He was born at 38 weeks gestation after a normal pregnancy and delivery. The boy was the second child of non-consanguineous parents. His elder sister was phenotypically normal. The boy exhibited right-sided pale blue iris (Fig. 1B), dystopia canthorum (W index: 2.34), bilateral congenital hearing loss, mild synophrys and broad nasal root, but lacked white central forelock or depigmented patches of the skin. Musculoskeletal anomalies and Hirschsprung disease were absent. Thus, according to the diagnostic criteria proposed by the International Waardenburg Consortium (Farrer et al., 1992), he was diagnosed with WS1 on the basis of clinical findings. However, his mother did not present any symptoms of WS. His mother's elder brother and father showed clinical characteristics consistent with WS1. HRCT and MRI showed normal middle-ear cavity with regular ossicles, a normal mastoid and inner-ear structures, which made it possible for the proband to receive a cochlear implant. A congenital sensorineural hearing loss was suspected after universal neonatal hearing screening. At 1 month of age, the results of distortion productotoacoustic emission (DPOAE) and auditory brain-stem response (ABR) confirmed the diagnosis of profound bilateral sensorineural hearing loss.

3.2. Identification a novel PAX3 heterozygous mutation

After mutation analysis of four pathogenic genes using sequencing technology, a novel heterozygous mutation, c.808C > G, in exon 6 of *PAX3* was detected in the proband, his mother and grandfather, but