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Germinal mosaicism of *PAX3* mutation caused Waardenburg syndrome type I

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ABSTRACT

Objectives: Waardenburg syndrome mutations are most often recurrent or *de novo*. The rate of familial recurrence is low and families with several affected children are extremely rare. In this study, we aimed to clarify the underlying hereditary cause of Waardenburg syndrome type I in two siblings in a Chinese family, with a mother affected by prelingual mild hearing loss and a father who was negative for clinical symptoms of Waardenburg syndrome and had a normal hearing threshold.

Methods: Complete characteristic features of the family members were recorded and genetic sequencing and parent-child relationship analyses were performed.

Results: The two probands were found to share double mutations in the *PAX3/GJB2* genes that caused concurrent hearing loss in Waardenburg syndrome type I. Their mother carried the *GJB2* c.109G > A homozygous mutation; however, neither the novel *PAX3* c.592delG mutation, nor the Waardenburg syndrome phenotype, was observed in either parent.

Conclusion: These previously unreported digenic mutations in *PAX3/GJB2* resulted in deafness associated with Waardenburg syndrome type I in this family. To our knowledge, this is the first report describing germinal mosaicism in Waardenburg syndrome. This concept is important because it complicates genetic counseling of this family regarding the risk of recurrence of the mutations in subsequent pregnancies.

1. Introduction

Isolated hereditary sensorineural hearing impairment corresponds to the classification of non-syndromic hearing impairment (NSHI), while cases where additional symptoms exist are classified as syndromic hearing impairment (SHI). To date, effective genetic counseling can be offered on the premise that it is possible to diagnose inheritance patterns conclusively with a clear genetic etiology. Nevertheless, genetic heterogeneity in NSHI/SHI is widely accepted and complex affected families are emerging continuously worldwide. One example of this is the co-existence of SHI and NSHI induced by different genes in the same family, as illustrated by Yan et al., who reported the existence of a novel mutation in the *MITF* gene that may be digenic with *GJB2* mutations in a large Chinese family affected by Waardenburg syndrome (WS) type II [1]. Other research groups have identified unrelated families in which two children shared the *GJB2* genotype and features of keratitis-ichthyosis-deafness within unaffected parents [2,3].

WS, which is commonly associated with SHI, is characterized by sensorineural hearing impairment and skin depigmentation, and can be classified into four main phenotypes (WS1–4) according to the physical characteristics of the patients [4]. The prevalence of WS is estimated to be 1/42,000, and is responsible for between 1% and 3% of cases of profound congenital deafness. Several genes, such as *PAX3, MITF,* and *SOX10*, are thought to be responsible for WS. A comprehensive review estimated that most of the WS-related mutations reported are recurrent or have arisen *de novo* [5]. The rate of familial recurrence is low and families with several affected children are extremely rare [5].

Here, we present our study of a rare non-consanguineous Chinese family with symptoms/signs of profound hearing loss and/or WS1. In this family, the combination of both SHI and NSHI genes were involved, implying the complexity of hereditary hearing impairment. Most significantly, the relatively healthy status of the parents is highly suggestive of hidden germinal mosaicism. This unique pattern of inheritance has not previously been defined in WS, and appears to be uncommon in inherited hearing loss.

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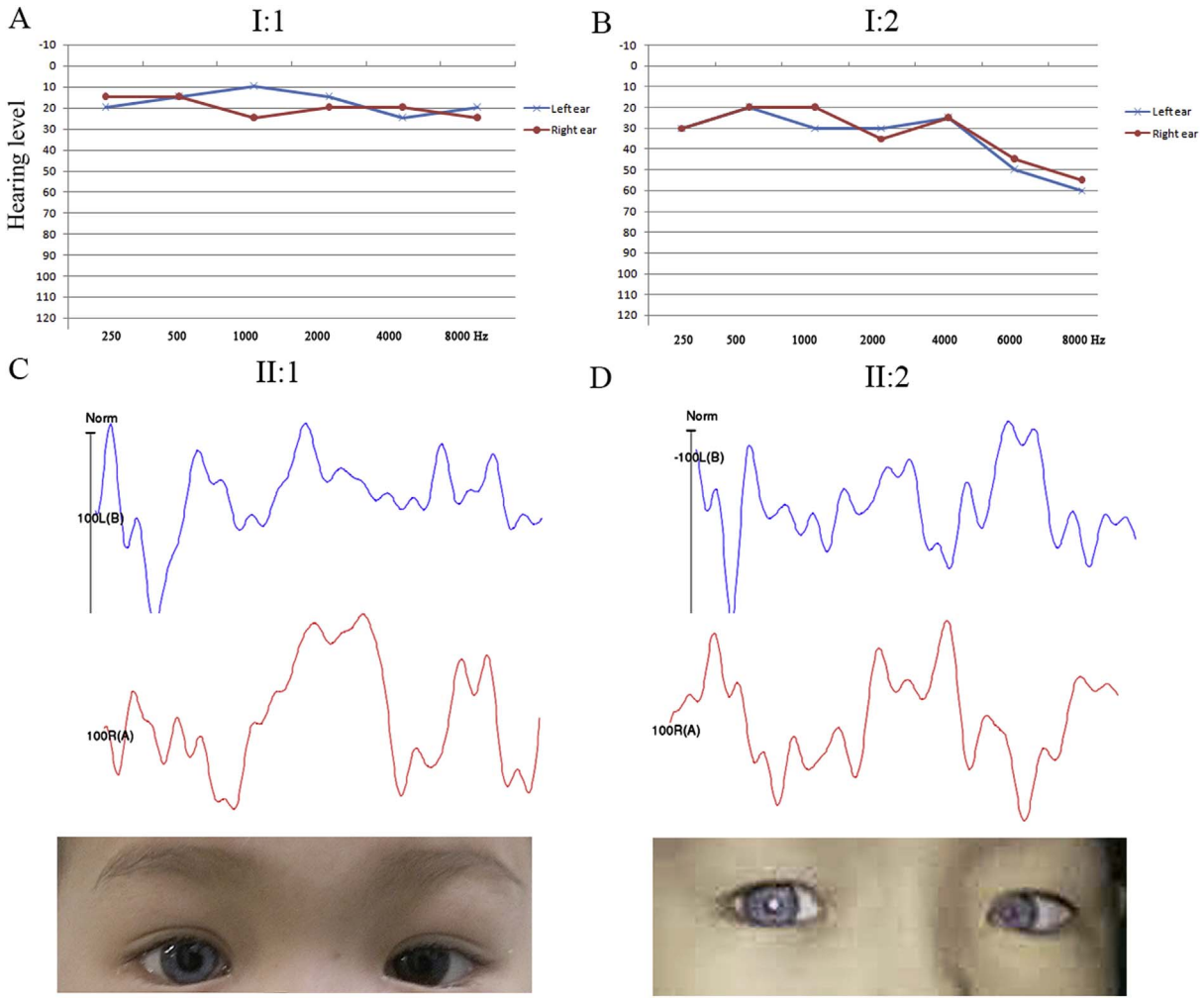

Fig. 1. Diverse phenotypes were seen in this family.

2. Materials and methods

2.1. Study participants and clinical evaluations

This study formed part of the extensive genetic analysis and counseling project [6] for hereditary hearing impairment conducted at the First Affiliated Hospital, Sun Yat-sen University according to a protocol that was approved by the Institutional Review Board. Informed consent was obtained from all the participants or their guardians prior to each study.

The family described here (Fig. 1) was of Han origin from Guangdong Province, China. Complete medical history and physical examinations excluded the possibility of environmental causes of deafness. Despite uneventful pregnancy and delivery, both probands (II-1 and II-2; aged 7 and 6 years, respectively) failed newborn hearing screening at birth and received hearing aids for language rehabilitation. Subsequently, cochlear implantation was arranged for both children at the ages of five and three years, respectively, due to the failure of hearing aids to compensate for the profound deafness, as evaluated by auditory examinations, including auditory brainstem response and auditory steady-state evoked response. Temporal computed tomography findings were normal. Ophthalmologic assessments with procedures such as slit lamp biomicroscopy and optical coherence tomography revealed normal vision, dystopia canthorum and iris heterochromia, which were consistent with features reported for WS1. Neither vestibular dysfunction nor neurological defects (ie. peripheral neuropathy, mental retardation, cerebellar ataxia or spasticity) was identified. Both children gained obtainable hearing function after cochlear implantation, at the time of the recent visit. Also, the mother (I-2) was affected by prelingual mild hearing loss, as measured by pure tone audiometry. Only the father (I-1) was negative for clinical symptoms of WS and had a normal hearing threshold.

2.2. DNA preparation, sequencing and functional analysis

Peripheral blood samples were collected from the four subjects and DNA isolation was performed using the QuikeGene 610L (KURABO, Japan) and matching DNA blood kit (DB-L). All coding exons of *GJB2*, *SLC26A4*, and the mitochondrial genes, *SOX10*, *MITF* and *PAX3* genes were subsequently amplified by polymerase chain reaction (PCR, for *PAX3* primer sequences, see Table S1) as previously described [6,7]. A total of 100ng DNA template, 5pmol of each primer, 12.5 μl 2X PCR buffer mixtures and ddH_2O were added in a final volume of 25μl for PCR. Annealing temperature was 56 °C. The products were purified and sequenced by Sanger sequencing using an ABI 3730 Genetic Sequencer. All sequences were aligned and compared with published sequences from the NCBI database. Amino acid conservation alignments across different *PAX3* gene families (*Bos taurus*: NP_001193747.1; *Gallus gallus*: NP_989600.1; *Mus musculus*: NP_001152992.1; *Rattus norvegicus*: NP_446162.1; *Homo sapiens*: NP_000429.2) were completed with ClustalX2 software. We also used *MutationTaster* to evaluate possible pathogenicity (http://www.mutationtaster.org).