Short Report

Double heterozygous mutations of MITF and PAX3 result in Waardenburg syndrome with increased penetrance in pigmentary defects


Waardenburg syndrome (WS) is characterized by sensorineural hearing loss and pigmentary defects of the hair, skin, and iris. Heterozygous mutations of MITF and its transactivator gene PAX3 are associated with Waardenburg syndrome type II (WS2) and type I (WS1), respectively. Most patients with MITF or PAX3 mutations, however, show variable penetrance of WS-associated phenotypes even within families segregating the same mutation, possibly mediated by genetic background or specific modifiers. In this study, we reported a rare Waardenburg syndrome simplex family in which a pair of WS parents gave birth to a child with double heterozygous mutations of MITF and PAX3. Compared to his parents who carried a single mutation in either MITF or PAX3, this child showed increased penetrance of pigmentary defects including white forelock, white eyebrows and eyelashes, and patchy facial depigmentation. This observation suggested that the expression level of MITF is closely correlated to the penetrance of WS, and variants in transcription regulator genes of MITF may modify the relevant clinical phenotypes.

Conflict of interest

The authors declare no conflict of interest.

Waardenburg syndrome (WS) is a hereditary disorder characterized by sensorineural hearing loss (SNHL) and pigmentary defects of the hair, skin, and iris. This autosomal dominant disorder results from abnormal survival, proliferation, migration or differentiation of neural crest-derived melanocytes. Depending on additional symptoms, WS is further classified into four types: type I with dystrophia canthorum (WS1, MIM193500), type II without dystrophia canthorum (WS2, MIM 193510), type III with dystrophia canthorum and upper limb anomalies (WS3, MIM148820), and type IV with aganglionic megacolon (WS4, MIM277580).

Heterozygous mutations of MITF and PAX3 are associated with WS2 and WS1, two most common forms of WS, respectively (1–3). MITF encodes microphthalmia-associated transcription factor, a basic helix–loop–helix protein critical in neural crest-derived melanocyte development (4). Consistent with its pathogenic role in WS2, MITF regulates transcription of several key melanocytic genes encoding tyrosinase (TYR), tyrosinase-related protein 1 (TRP1) and 2 (DCT/TRP2) (5, 6). The MITF gene itself is transactivated by two other transcription factor genes associated with WS: PAX3 (WS1/WS3) and SOX10 (WS2/WS4). PAX3 encodes the paired box 3 transcription factor and is involved in the development of central nervous system, somites, skeletal muscle and several neural crest-derived lineages including melanocytes (7). In synergy...
Double heterozygous mutations of \textit{MITF} and \textit{PAX3}

\begin{figure}
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\includegraphics[width=\textwidth]{Fig_1.png}
\caption{Pedigree (a) and clinical features (b) of the Waardenburg syndrome family showing dystopia canthorum and hypoplastic irides of I:1 and II:1, minor hair hypopigmentation (premature graying) in I:2, and severe hair and skin hypopigmentation (white forelock, white eyebrows, white eyelashes and patchy facial depigmentation) in II:1.}
\end{figure}

with SOX10, PAX3 binds to the \textit{MITF} promoter to directly regulate its expression. Mutant PAX3 fails to activate \textit{MITF} gene expression, providing a molecular basis for SNHL and pigmented defects associated with WS1 and WS3 (8–10).

A striking feature of WS is its highly variable penetrance. Marked intrafamilial and interfamilial variation in SNHL and pigmentary abnormalities of WS patients suggests possible interplay between genetic and environmental factors. Epistatic involvement of genetic modifiers has been proposed based on studies in several WS mouse models (11–14). This modifier effect, however, has yet to be confirmed in human. In this study, we reported a rare WS simplex family segregating with both \textit{MITF} and \textit{PAX3} mutations. Phenotypic comparison of the family members suggested a link between the expression level of \textit{MITF} and the penetrance of WS-associated pigmentary defects, and led to an interesting hypothesis that variants in transcription regulators of \textit{MITF} may serve as genetic modifiers to influence the penetrance of WS phenotypes.

Materials and methods

Family description

A simplex family associated with Waardenburg syndrome was ascertained through the Department of Otolaryngology – Head and Neck Surgery, Xinhua Hospital, Shanghai, China (Fig. 1a). According to the criteria proposed by the WS consortium (15), the proband II:1, a 5-year-old child, and his mother I:1 were diagnosed as WS1, and the proband’s father I:2 was diagnosed as WS2. All three individuals have profound SNHL and various pigmentary defects (see Results for details). Dystopia canthorum was only present in II:1 and I:1. No musculoskeletal anomaly or intestinal aganglionosis were present in any of the family members. High resolution computed tomography of the temporal bones revealed no inner ear abnormalities in proband II:1. He received cochlear implantation at the age of 5 years.

Clinical evaluation

Comprehensive auditory, ophthalmologic, dermatologic and neurological examinations and clinical history inquiry were performed on all three family members. Auditory evaluations included otoscopy, distortion product otoacoustic emission, and pure-tone audiometry (PTA). Additional auditory steady-state response (ASSR) test was performed for individual II:1 who did not respond to PTA test well because of his young age. Special attention was given to the pigmentary changes of hair, iris and skin, and other developmental defects associated with WS such as dystopia canthorum, limb abnormalities and intestinal aganglionosis. W-index, the biometric index of dystopia canthorum...
Fig. 2. Auditory steady-state response audiograms of II:1 (a) and pure-tone audiometry audiograms of I:1 (b) and I:2 (c), showing the bilateral profound sensorineural hearing loss in the family members.

was measured and compared in family members as previously described (15).

Mutation analysis
Genomic DNA was extracted from peripheral blood samples following the standard procedure. Mutation screening of WS1 and WS2 genes PAX3, MITF, SOX10 and SNAI2 was performed by polymerase chain reaction amplification and bidirectional sequencing of all exons and flanking splicing sites. Informed written consents were obtained from the family members to participating in this study following guidelines by the ethics committee of Xinhua Hospital, Shanghai Jiao Tong University School of Medicine.

Discussion
We presented a rare WS family in which a child carrying double heterozygous mutations of PAX3 and MITF showed increased penetrance of WS-associated pigmenitary defects. It is worth noting that many hypopigmentary phenotypes present only in the child but not in his parents, including white forelock, white eyebrows and eyelashes, and patchy depigmentation of skin, were more frequently observed in Caucasian WS patients but were extremely rare in Chinese WS patients. Based on our literature search, white eyebrows or white eyelashes were not reported in any of the 68 Chinese WS cases from 27 families, while white forelock and patchy depigmentation of skin were present in cases from one large Chinese WS1 family (10, 17–20). These data supported that despite the high variability of WS phenotypes in Caucasian patients, the additional pigmenitary defects observed in the child is highly probable because of the increased penetrance.

The identified c.452-2A>G splice mutation in PAX3 was predicted to produce a transcript lacking the in-frame exon 4 and to encode a protein with internal deletion of amino acids 151–195 lacking the 3' end of the paired box and the highly conserved octapeptide.
Double heterozygous mutations of MITF and PAX3

(a) Chromatograms of I:1, I:2 and II:1 showing the c.452-2A>G mutation in PAX3 and the p.R255X mutation in MITF. (b) Schematic illustration of PAX3 and MITF gene structure showing the position of the mutations and the predicted structural alteration of the genes. Gene structures with dashed lines indicate the predicted deletions resulting from the mutations. b, basic domain; HD, homeodomain; HLH, helix-loop-helix domain; LZ, leucine zipper domain; o, octapeptide; PD, paired domain; TA or AD1-3, transactivation domains.

The other identified p.R255X nonsense mutation in MITF was predicted to lead to a premature stopped protein product lacking the 3’ end of the helix-loop-helix domain, the whole leucine zipper domain, and the third transactivation domain (Fig. 3b). Both mutations will result in a functionally disrupted or inactive allele. As mutations in PAX3 fail to transactivate the expression of MITF and lead to auditory–pigmentary symptoms similar to those caused by the MITF mutations (8, 10), we predicted that double heterozygous mutations of PAX3 and MITF decrease the expression level of MITF further than single heterozygous mutations of either gene and lead to the increased penetrance of WS phenotypes. This dosage-dependent model is similar to the one reported previously in which double heterozygous mutations of SLC26A4 and its transcriptional regulatory gene FOXI1 lead to recessive hearing loss associated with non-syndromic enlarged vestibular aqueduct or Pendred syndrome (21).

Our study for the first time confirmed the genetic modification of WS phenotypes in human beings. The case presented here is relatively extreme; in that, the epistatic modification comes from a disease-causing mutation of PAX3 gene that leads to a significant decrease of MITF expression level. Most phenotypic variations observed in the WS patients, however, is more likely mediated by genetic variations with less impact, along with other environmental and stochastic factors. Nevertheless, based on this correlation model of MITF expression level, variations in transcription regulator genes of MITF are good candidates for...
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genetic modifiers of WS. Identification of such modifier loci may allow prediction and possible treatment of relevant clinical phenotypes.

Acknowledgements

This study was supported by grants from the National Basic Research Program of China to H. W. (2011CB504501), the Science and Technology Commission of the Shanghai Municipality, China to T. Y. (09DJ1400604, 11PJ1407000) and H. W. (08DZ1980100), the National Science Foundation of China to H. W. (30973307), T. Y. (30971596) and Z. W. (30801286), and the ‘Shu Guang’ project of the Shanghai Municipal Education Commission and Shanghai Education Development Foundation to T. Y. (09SG19).

References