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Homozygous *EDNRB* mutation in a patient with Waardenburg syndrome type 1

Noriko Morimoto [a], Hideki Mutai [b], Kazunori Namba [b], Hiroki Kaneko [c], Rika Kosaki [d], Tatsuo Matsunaga [b,e,*]

[a] *Division of Otolaryngology, National Center for Child Health and Development, 2-10-1 Ohkura, Setagaya, Tokyo 157-8535, Japan*
[b] *Division of Hearing and Balance Research, National Institute of Sensory Organ, National Hospital Organization Tokyo Medical Center, 2-5-1 Higashigaoka, Meguro, Tokyo 152-8902, Japan*
[c] *The Institute of Natural Sciences, College of Humanities and Sciences, Nihon University, 3-25-40 Sakurajosui, Segataya, Tokyo 156-8550, Japan*
[d] *Division of Medical Genetics, National Center for Child Health and Development, 2-10-1 Ohkura, Setagaya, Tokyo 157-8535, Japan*
[e] *Medical Genetics Center, National Hospital Organization Tokyo Medical Center, 2-5-1 Higashigaoka, Meguro, Tokyo 152-8902, Japan*

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ABSTRACT

Objective: To examine and expand the genetic spectrum of Waardenburg syndrome type 1 (WS1).
Methods: Clinical features related to Waardenburg syndrome (WS) were examined in a five-year old patient. Mutation analysis of genes related to WS was performed in the proband and her parents. Molecular modeling of EDNRB and the p.R319W mutant was conducted to predict the pathogenicity of the mutation.
Results: The proband showed sensorineural hearing loss, heterochromia iridis, and dystopia canthorum, fulfilling the clinical criteria of WS1. Genetic analyses revealed that the proband had no mutation in *PAX3* which has been known as the cause of WS1, but had a homozygous missense mutation (p.R319W) in endothelin receptor type B (*EDNRB*) gene. The asymptomatic parents had the mutation in a heterozygote state. This mutation has been previously reported in a heterozygous state in a patient with Hirschsprung's disease unaccompanied by WS, but the patient and her parents did not show any symptoms in gastrointestinal tract. Molecular modeling of EDNRB with the p.R319W mutation demonstrated reduction of the positively charged surface area in this region, which might reduce binding ability of EDNRB to G protein and lead to abnormal signal transduction underlying the WS phenotype.
Conclusions: Our findings suggested that autosomal recessive mutation in *EDNRB* may underlie a part of WS1 with the current diagnostic criteria, and supported that Hirschsprung's disease is a multifactorial genetic disease which requires additional factors. Further molecular analysis is necessary to elucidate the gene interaction and to reappraise the current WS classification.

1. Introduction

Waardenburg syndrome (WS) is an inherited disease caused by abnormal generation of neural crest cells [1–3]. The major clinical manifestations of WS include sensorineural hearing loss and pigmentary abnormalities such as gray hair, heterochromia iridis and vitiligo. WS is classified into four types

* Corresponding author at: Division of Hearing and Balance Research, National Institute of Sensory Organs, Medical Genetics Center, National Hospital Organization Tokyo Medical Center 2-5-1 Higashigaoka, Meguro, Tokyo 152-8902, Japan. Fax: +81 3 3412 9811.
E-mail address: matsunagatatsuo@kankakuki.go.jp (T. Matsunaga).

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based on the clinical presentation and six genes have been reported as summarized in the following description [1–3]: WS type 1 (WS1) is characterized by dystopia canthorum with most cases caused by mutations in the paired box 3 (*PAX3*) gene in an autosomal dominant manner. WS type 2 (WS2) differs from WS1 in that it is not associated with dystopia canthorum. Mutations in the microphthalmia-associated transcription factor (*MITF*) gene and SRY (sex determining region Y)-box 10 (*SOX10*) gene were identified in patients with WS2 by 15% each in an autosomal dominant manner, while mutations in endothelin receptor type B (*EDNRB*) and endothelin 3 (*EDN3*) is expected to be identified in less than 5% of WS2 patients in an autosomal dominant manner. In addition, mutations in the snail family zinc finger 2 (*SNAI2*) gene is also expected to be identified in less than 5% of WS2 patients in an autosomal recessive pattern. WS type 3 (WS3) is similar to WS1, but it has additional musculoskeletal abnormalities caused by mutations in *PAX3* in autosomal dominant or autosomal recessive manner. WS type 4 (WS4) is associated with Hirschsprung's disease in addition to the features of WS2. In WS4 patients, *SOX10* mutations have been identified by 50% in an autosomal dominant manner, and *EDNRB* or *EDN3* mutations have been identified by in 20–30% in autosomal dominant or autosomal recessive manner.

Here, we report a pediatric patient clinically diagnosed with WS1. Genetic testing revealed an absence of *PAX3* mutation but a homozygous p.R319W mutation in *EDNRB*. The p.R319W mutation in EDNRB has been reported as a heterozygote in a patient with only Hirschsprung's disease [4,5]. The patient's parents also had the heterozygous p.R319W mutation in *EDNRB* but had no symptoms related to WS or Hirschsprung's disease. To date, there are no reports of *EDNRB* mutation in a homozygous manner in relation to WS1, suggesting that in some cases other types of recessive mutations in *EDNRB* may be involved.

2. Materials and methods

2.1. Subjects and clinical evaluation

The proband was a five-year-old girl (Fig. 1, III-4). When the proband was three years old, her parents noticed that she did not respond to loud noises and sought medical evaluation to a local hospital. She was diagnosed of hearing loss because of no responses to clicking sounds at 105 dB bilaterally by ABR, and

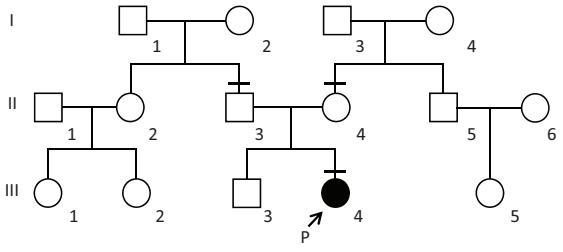

Fig. 1. Pedigree of the family. Horizontal lines above the symbols indicate individuals who underwent genetic analyses. The Roman numerals denote successive family generations. P, proband.

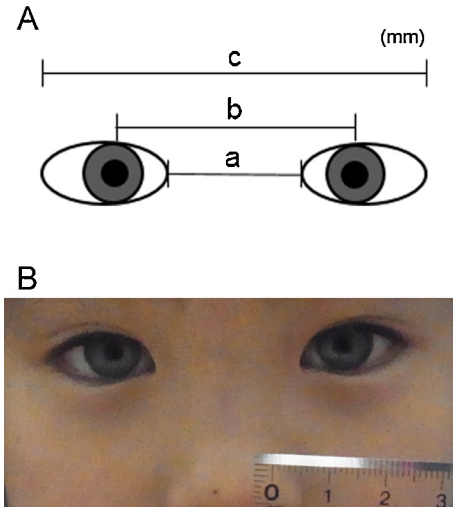

Fig. 2. Heterochromia irides and dystopia canthorum identified in the patient. (A) Schematic diagram of the inner canthal (a), interpupillary (b) and outer canthal distances (c) for calculation of W-index. The diagram was reproduced from Farrer et al. [6]. (B) Pictures of the eyes of the patient.

was referred to our hospital. At our hospital, the parents of the proband were interviewed about the status of hearing loss, possible causes, comorbidity, and family history. Physical and otolaryngological examinations were also performed. Hearing was evaluated by auditory steady-state response (ASSR), distortion-product otoacoustic emissions (DPOAEs), and conditioned play audiometry (CPA). W-index, which is the index for dystopia canthorum, was calculated by direct measurement of inner canthal distance, interpupillary distance, and outer canthal distance. Computed tomography (CT) was performed to investigate malformation of the temporal bones. W-index was calculated as follows (Fig. 2A) [1,6]: inner canthal distance (a), interpupillary distance (b), and outer canthal distance (c). $X = (2a - (0.2119c + 3.909))/c$, $Y = (2a - (0.2479b + 3.909))/b$, $W = X + Y + a/b$. This study was approved by the institutional ethics review board at the National Hospital Organization Tokyo Medical Center and Ethical Committee of National Center for Child Health and Development. All the procedures were carried out after obtaining a written informed consent from the parents of the children.

2.2. Genetic analysis

Genetic analysis was performed on the proband and her parents. Genomic DNA was extracted from peripheral blood DNA using the Gentra Puregene Blood kit (QIAGEN, Hamburg, Germany). Mutation analysis of *EDNRB*, *SOX10, MITF,* and *PAX3* was performed by bidirectional Sanger sequencing of their coding exons together with the flanking intronic regions. Data was analyzed using SeqScape 2.6 software (Applied Biosystems, CA, USA) and DNASIS Pro (Hitachisoft, Tokyo, Japan). Primers used to amplify each exon are shown (Online Resource 1). Multiplex ligation-dependent probe amplification analysis was performed for *SOX10, MITF*, and *PAX3* (SALSA MLPA kit, MRC-Holland, Amsterdam, Netherlands) according to the manufacturer's protocol.

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