






## A rare case of Waardenburg syndrome with unilateral hearing loss caused by nonsense variant c.772C>T (p.Arg259\*) in the *MITF* gene in Yakut patient from the Eastern Siberia (Sakha Republic, Russia)

Nikolay A. Barashkov <sup>a,b</sup>, Georgii P. Romanov <sup>a,b</sup>, Uigulaana P. Borisova<sup>b</sup>, Aisen V. Solovyev<sup>a,b</sup>, Vera G. Pshennikova <sup>a,b</sup>, Fedor M. Teryutin <sup>a,c</sup>, Alexander A. Bondar <sup>d</sup>, Igor V. Morozov<sup>d,e</sup>, Elza K. Khusnutdinova<sup>f,g</sup>, Olga L. Posukh<sup>e,h</sup>, Tatiana E. Burtseva<sup>ij</sup>, Jon Øyvind Odland<sup>k</sup> and Sardana A. Fedorova<sup>a,b</sup>

<sup>a</sup>Laboratory of Molecular Genetics, Yakut Science Centre of Complex Medical Problems, Yakutsk, Russia; <sup>b</sup>Laboratory of Molecular Biology, M.K. Ammosov North-Eastern Federal University, Yakutsk, Russia; <sup>c</sup>Department of Professional Pathology, Republican Hospital №2 - Center for Emergency Medical Aid, Yakutsk, Russia; <sup>d</sup>Genomics Core Facility, Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia; <sup>e</sup>Novosibirsk State University, Novosibirsk, Russia; <sup>f</sup>Laboratory of Human Molecular Genetics, Ufa Federal Research Center of Russian Academy of Sciences, Institute of Biochemistry and Genetics, Ufa, Russia; <sup>g</sup>Department of Genetics and Fundamental Medicine, Bashkir State University, Ufa, Russia; <sup>h</sup>Federal Research Center Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia; <sup>i</sup>Department of Pediatrics and Child Surgery, M.K. Ammosov North-Eastern Federal University, Yakutsk, Russia; <sup>j</sup>Laboratory of monitoring the children health and medico-environmental research, Yakut Science Centre of Complex Medical Problems, Yakutsk, Russia; <sup>k</sup>Department of Public Health and Nursing, Faculty on Medicine and Health Sciences, NTNU The Norwegian University of Science and Technology, Trondheim, Norway

### ABSTRACT

Waardenburg syndrome (WS) is an orphan genetic disease with autosomal dominant pattern of inheritance characterised by varying degrees of hearing loss accompanied by skin, hair and iris pigmentation abnormalities. Four types of WS differing in phenotypic characteristics are now described. We performed a Sanger sequencing of coding regions of genes *PAX3*, *MITF*, *SOX10* and *SNAI2* in the patient with WS from a Yakut family living in the Sakha Republic. No changes were found in the *PAX3*, *SOX10* and *SNAI2* coding regions while a previously reported heterozygous transition c.772C>T (p.Arg259\*) in exon 8 of the *MITF* gene was found in this patient. This patient presents rare phenotype of WS type 2: congenital unilateral hearing loss, unilateral heterochromia of irises, and absence of skin/hair depigmentation and dystopia canthorum. Audiological variability in WS type 2, caused by the c.772C>T (p.Arg259\*) variant in the *MITF* gene, outlines the importance of molecular analysis and careful genotype–phenotype comparisons in order to optimally inform patients about the risk of hearing loss. The results of this study confirm the association of pathogenic variants in the *MITF* gene with WS type 2 and expanded data on the variability of audiological features of the WS.

### ARTICLE HISTORY

Received 2 April 2019  
Revised 31 May 2019  
Accepted 3 June 2019



### KEYWORDS


Waardenburg syndrome;  
*MITF*; Sakha Republic;  
Eastern Siberia; Russia

## Introduction

Waardenburg syndrome (WS) is an orphan genetic disease with an autosomal dominant pattern of inheritance, leading to hearing loss accompanied by skin, hair and iris pigmentation abnormalities [1–4]. The incidence of WS is estimated as 1 on 42,000 among Caucasian populations, or 2–5% among patients with congenital deafness, and 0.9–2.8% among adults with hearing impairment [2,3,5–7]. Four types of WS differing in phenotypic characteristics are now described. The WS type 1 (MIM 193,500) and the WS type 2 (MIM 193,510) share similar main symptoms (pigmentation and hearing abnormalities). However, the patients with WS type 1 additionally have a dystopia canthorum (lateral displacement of the inner canthi of

the eyes with the normal interpupillary distance) while this feature is absent in patients with WS type 2. The WS type 3 or Waardenburg–Klein syndrome (MIM 148,820) includes upper limbs anomalies in addition to main WS symptoms. The WS type 4 (also known as Waardenburg–Shah syndrome, MIM 277,580) has main WS features accompanied by Hirschprung disease [1–13]. Detailed and correct characterisation of identified WS cases is very important since phenotypical features of the WS often vary even among members of one family [1–13]. Mutations in the *PAX3*, *MITF*, *SOX10* and *SNAI2* genes are known as main causes for WS [9,10]. Pathogenic variations in any of these genes disrupt normal development of melanocytes, altering pigmentation of skin, hair, eyes and lead to sensorineural hearing impairment [1–13].

**CONTACT** Nikolay A. Barashkov  [barashkov2004@mail.ru](mailto:barashkov2004@mail.ru)  Head of Laboratory of Molecular Genetics, Yakut Science Centre of Complex Medical Problems, Sergelyahskoe shosse, 4, Yakutsk 677000, Russia

 Supplemental data for this article can be accessed [here](#).

© 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

In this report, we present a rare case of Waardenburg syndrome with unilateral hearing loss caused by nonsense variant c.772C>T (p.Arg259\*) in the *MITF* gene in a Yakut patient from the Sakha Republic (Eastern Siberia, Russia).

## Materials and methods

### Patient

The case of this study was 17-year-old male patient with WS phenotype (II:4 in Figure 1) from a Yakut family living in the Sakha Republic (Eastern Siberia, Russia). His father (I:2) had the WS type 2 features (iris heterochromia with unilateral hearing loss) while the proband's mother (I:1) had profound bilateral hearing loss and no signs of WS, and his brother (II:3) had normal hearing (Figure 1(a)).

### DNA-extraction

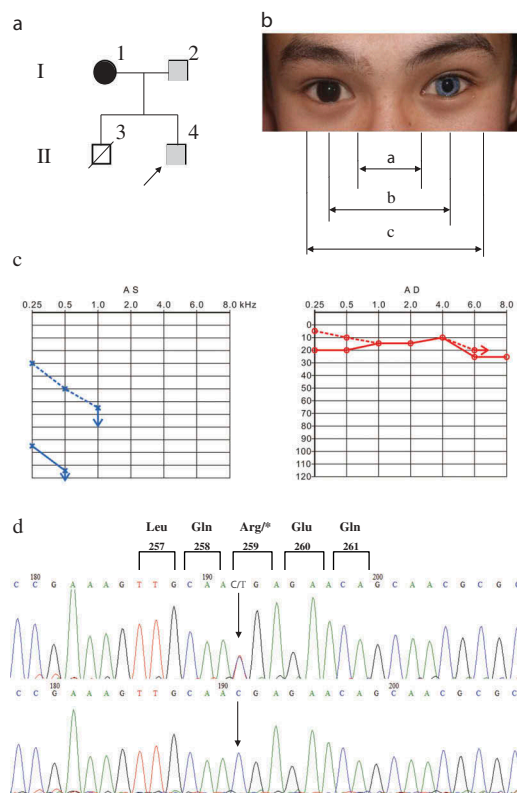
Isolation of genomic DNA was performed by standard phenol-chloroform extraction from the venous blood leukocytes and was quantified by the P330 spectrophotometer (Implen, Germany). The DNA was subsequently stored at 20°C until further use (the DNA Biobank "Genome of Yakutia", BRK 0556–2017-0003).

### PCR and Sanger sequencing

For mutation analysis we performed a Sanger sequencing of 25 fragments of the protein coding regions of the *PAX3* (9 exons), *MITF* (9 exons), *SOX10* (4 exons) and *SNAI2* (3 exons) genes. The amplification reaction was set in a total volume of 25 µl containing *Taq* polymerase (Sileks, Russia), x10 Mg<sup>2+</sup> PCR buffer (Sileks, Russia) and Betaine (Sigma-Aldrich, Inc., USA) with previously described primers [10] (Supplementary Table). The reaction was performed for 30 cycles: denaturation at 95°C for 30 sec, primer annealing at 56°C to 60°C for 30 sec and elongation at 72°C for 30 sec on a T200 thermal cycler (Bio-Rad, USA). The PCR products sequencing with the same primers was performed on an ABI Prism 3130XL automatic sequencer (Applied Biosystems, USA) with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The obtained nucleotide sequences of the investigated DNA fragments were compared to reference sequences of the *PAX3*, *MITF*, *SOX10* and *SNAI2* genes from the GenBank database.

### Case report

No changes were found in the *PAX3*, *SOX10* and *SNAI2* coding regions, while a c.772C>T transition in exon 8 of the *MITF* gene (Reference sequence: NM\_000248.3) was



**Figure 1.** The case of the WS type 2 with variant c.772C>T (p.Arg259\*) in the *MITF* gene.

(a) pedigree of Yakut family with WS type 2. Arrow points on proband (II:4). Filled symbols represent family members with hearing loss: bilateral hearing loss is shown in black, unilateral hearing loss – in grey. (b) Proband II:4: unilateral iris heterochromia (right eye is dark brown, left – diamond blue with some brown pigmentation). Dystopia canthorum index (W) was calculated according to [2] –  $W = X + Y + a/b$ ; where  $X = (2a - 0,2119c - 3,909)/c$ ,  $Y = (2a - 0,249b - 3,909)/b$ ; a – the distance between the inner corners of the eyes (mm), b – the distance between the pupils (mm), c – the distance between the outer corners of the eyes (mm). Index W for proband II:4 was calculated as 1.66 ( $0.47 + 0.69 + 32/64$ ) that indicates the absence of dystopia canthorum ( $< 1.95$  cm). (c) Audiograms of proband in range from 0.25 to 8 kHz, AD – on the right, AS – on the left. (d) Partial sequence chromatogram of *MITF* fragment (exon 8): upper panel – arrow shows the location of heterozygous variant c.772C>T (p.Arg259\*) detected in proband II:4, lower panel – normal *MITF* sequence (Reference sequence: NM\_000248.3).

found in heterozygous state in proband II:4 (Figure 1(d)). The c.772C>T transition leads to premature stop-codon (p.Arg259\*) terminating translation of melanocyte inducing transcription factor (MITF). Description of phenotype of proband II:4 is presented in Table 1. This patient had no dystopia canthorum (index  $W = 1.66$  cm) (Figure 1(b)). Thus, this patient is more likely to present phenotypic features of WS type 2: iris heterochromia (right eye is dark brown, left – diamond blue) (Figure 1(b)) and sensorineural hearing loss. His hearing loss was unilateral: profound (118 dB) – in the left ear, normal hearing (15 dB) – in the right ear (Figure 1(c)). The patient is a student in

**Table 1.** Phenotype of observed patient with Waardenburg syndrome.

Phenotypic features	Proband II:4
Gender	Male
Age	17 years old
Ethnicity	Yakut
Type of inheritance	AD
Hearing loss	Unilateral
Degree of hearing loss	Profound on the left (>90 dB in speech range), normal hearing on the right
Communication	Speech/Sign language
Eyes	Unilateral iris heterochromia (right eye is dark brown, left – diamond blue with some brown pigmentation)
Skin	Normal pigmentation
Hair	Normal pigmentation
W index	1.66 cm
WS type	2

Age is stated as on the moment of examination (May 2015); speech range (0.5, 1.0, 2.0, 4.0 kHz); dB – decibels; AD – autosomal dominant; W index – dystopia canthorum index: value over 1.95 cm signs on presence of dystopia canthorum; WS type – Waardenburg syndrome type.

a regular school and has no limitations in communication, because he uses both oral speech and sign language (Table 1).

Close relatives of proband II:4 were not thoroughly clinically examined. Nevertheless, his mother (I:1) has profound bilateral hearing loss and no signs of WS while his father (I:2) has the WS type 2 features: iris heterochromia and unilateral hearing loss (profound hearing loss in one ear, normal hearing in another ear) and his brother had normal hearing (Figure 1(a)).

## Discussion

The *MITF* gene (MIM 156,845) is located on chromosome 3 and consists of 9 exons. This gene encodes a transcription factor MITF (melanocyte inducing transcription factor) involved in melanocytes development [14,15]. MITF is a basic helix-loop-helix (hHLH)-leucine zipper protein that plays an important role in the development of various cell types, including neural crest-derived melanocytes and optic cup-derived retinal pigment epithelial cells [16]. According to the ClinVar database (March 2019), there are ~80 variants in *MITF* associated with WS and Tietz syndrome (MIM 103,500) [17], one variant associated with skin melanoma and renal cell carcinoma (MIM 614,456), and other (~30) variants in *MITF* have unknown or no clinical significance. In our study proband II:4 had no features of Tietz syndrome and no skin pigmentation impairment or melanoma. The c.772C>T (p.Arg259\*) variant in the *MITF* gene is not reported in the 1000 Genomes Project, the dbSNP, the ExAC (the Exome Aggregation Consortium) and the ClinVar database. However, we found information about c.772C>T (p.Arg259\*) in the HGMD (Human Genome Mutation Database) [18] with reference to Nobukuni et al. [14]. In this study, the c.772C>T (p.Arg259\*) variant was revealed in a family with 13 individuals having WS type 2 phenotype [14]. All affected members of this family have a typical WS type 2 anomalies with sensorineural hearing loss, heterochromia iriditis, white forelock and early greying.

None of the family members showed dystopia canthorum [14]. However, in the paper of Nobukuni et al. [14], detailed audiological features of these 13 patients were not described.

In our study patient with the c.772C>T (p.Arg259\*) variant in the *MITF* gene presents rare WS type 2 phenotype: congenital unilateral hearing loss (profound deafness on the left side, normal hearing on the right side), unilateral iris heterochromia (right eye is dark brown, left – diamond blue) and absent of dystopia canthorum (index W = 1.66 cm.) (Table 1). Meta-analysis of the WS cases (417 patients) in different regions of the world showed that pathogenic variants in *MITF* were detected in 28% out of all examined WS cases and in 89.6% – among patients with the WS type 2 [9]. Moreover, almost 90% of patients with WS type 2 having pathogenic *MITF* variants presented bilateral sensorineural hearing loss [9].

In conclusion, the results of this study confirm the association of pathogenic variants in the *MITF* gene with the WS type 2 and enrich current information on the clinical manifestation of this rare syndrome worldwide. In addition, obtained data provide important targeted information to genetic counselling of families affected by the WS type 2 and will be useful for medical practitioners working with such patients.

## Acknowledgments

We thank all patients and blood sample donors who have contributed to this study.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This work was supported by the Ministry of Education and Science of the Russian Federation (the Project #6.1766.2017); NEFU in Yakutsk (the Project #0794-2017-0019, FSRG-2017-

0019); Russian Foundation for Basic Research (grants #17-29-06-016\_ofi\_m, #18-015-00212\_A, #18-013-00738\_A, #18-05-600035\_Arctica) and the Program “Genome of Yakutia” YSC CMP (BRK 0556-2017-0003)

## Ethical approval

Written informed consent was obtained from all individuals. This study was approved by the local Committee on Biomedical Ethics of the Yakut Science Centre of Complex Medical Problems (Yakutsk, Russia, Protocol No 16, 16 April 2015).

## ORCID

Nikolay A. Barashkov  <http://orcid.org/0000-0002-6984-7934>  
 Vera G. Pshennikova  <http://orcid.org/0000-0001-6866-9462>  
 Fedor M. Teryutin  <http://orcid.org/0000-0002-8659-0886>  
 Alexander A. Bondar  <http://orcid.org/0000-0001-9181-0487>

## References

- [1] Arias S, Mota M. Apparent non-penetrance for dystopia in Waardenburg syndrome type 1 with some hints on the diagnosis of dystopia canthorum. *J Genet Hum.* 1978;26:103–131.
- [2] Reed WB, Stone VM, Boder E, et al. Pigmentary disorders in association with congenital deafness. *Arch Dermatol.* 1967;95:176–186.
- [3] Read AP, Newton VE. Waardenburg syndrome. *J Med Genet.* 1997;34:656–665.
- [4] Waardenburg PJ. A new syndrome combining developmental anomalies of the eyelids, eyebrows and nose root with pigmentary defects of the iris and head hair and with congenital deafness. *Am J Hum Genet.* 1951;3(3):195–253.
- [5] Farrer LA, Grundfast KM, Amos J, et al. Waardenburg syndrome (WS) type I is caused by defects at multiple loci, one of which is near ALPP on chromosome 2: first report of the WS consortium. *Am J Hum Genet.* 1992;50:902–913.
- [6] Hodgkinson CA, Nakayama A, Li H, et al. Mutation at the ocular white locus in Syrian hamsters: haploinsufficiency in the MITF gene mimics human Waardenburg syndrome type 2. *Hum Mol Genet.* 1998;7:703–708.
- [7] Nayak CS, Isaacson G. Worldwide distribution of Waardenburg syndrome. *Ann Otol Rhinol Laryngol.* 2003;112:817–820.
- [8] Liu XZ, Newton VE, Read AP. Waardenburg syndrome type II: phenotypic findings and diagnostic criteria. *Am J Med Genet.* 1995;55:95–100.
- [9] Song J, Feng Y, Acke FR, et al. Hearing loss in Waardenburg syndrome: a systematic review. *Clin Genet.* 2016;89:416–425.
- [10] Yang S, Dai P, Liu X, et al. Genetic and phenotypic heterogeneity in Chinese patients with Waardenburg syndrome type II. *PLoS One.* 2013;10:e77149.
- [11] Bertolotto C, Busca R, Abbe P, et al. Different cis-acting elements are involved in the regulation of TRP1 and TRP2 promoter activities by cyclic AMP: pivotal role of M boxes (GTCATGTGCT) and of microphthalmia. *Mol Cell Biol.* 1998;2:694–702.
- [12] Yasumoto K, Yokoyama K, Shibata K, et al. Microphthalmia-associated transcription factor as a regulator for melanocyte-specific transcription of the human tyrosinase gene. *Mol Cell Biol.* 1994;12:8058–8070.
- [13] Chen Y, Yang F, Zheng H, et al. Clinical and genetic investigation of families with type II Waardenburg syndrome. *Mol Med Rep.* 2016;3:1983–1988.
- [14] Nobukuni Y, Watanabe A, Takeda K, et al. Analyses of loss-of-function mutations of the MITF gene suggest that haploinsufficiency is a cause of Waardenburg syndrome type 2A. *Am J Hum Genet.* 1996;59:76–83.
- [15] Fuse N, Yasumoto K, Takeda K, et al. Molecular cloning of cDNA encoding a novel microphthalmia-associated transcription factor isoform with a distinct amino-terminus. *J Biochem.* 1999;126:1043–1051.
- [16] Tassabehji M, Newton VE, Liu XZ, et al. The mutational spectrum in Waardenburg syndrome. *Hum Mol Genet.* 1995;4:2131–2137.
- [17] Grill C, Bergsteinsdóttir K, Ogmundsdóttir MH, et al. MITF mutations associated with pigment deficiency syndromes and melanoma have different effects on protein function. *Hum Mol Genet.* 2013;22:4357–4367.
- [18] Stenson PD, Mort M, Ball EV, et al. The Human Gene Mutation Database: 2008 update. *Genome Med.* 2009;1:13.1–13.6.