

**SHORT REPORT**

# Locus and allelic heterogeneity and phenotypic variability in Waardenburg syndrome

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Waardenburg syndrome (WS) is a disorder of neural crest cell migration characterized by auditory and pigmentary abnormalities. We investigated a cohort of 14 families (16 subjects) either by targeted sequencing or whole-exome sequencing. Thirteen of these families were clinically diagnosed with WS and one family with isolated non-syndromic hearing loss (NSHL). Intra-familial phenotypic variability and non-penetrance were observed in families diagnosed with WS1, WS2 and WS4 with pathogenic variants in *PAX3*, *MITF* and *EDNRB*, respectively. We observed gonosomal mosaicism for a variant in *PAX3* in an asymptomatic father of two affected siblings. For the first time, we report a biallelic pathogenic variant in *MITF* in a subject with WS2 and a biallelic variant in *EDNRB* was noted in a subject with WS2. An individual with isolated NSHL carried a pathogenic variant in *MITF*. Blended phenotype of NSHL and albinism was observed in a subject clinically diagnosed to have WS2. A phenocopy of WS1 was observed in a subject with a reported pathogenic variant in *GJB2*, known to cause isolated NSHL. These novel and infrequently reported observations exemplify the allelic and genetic heterogeneity and show phenotypic diversity of WS.

**KEYWORDS**

DNA copy number variation, hearing loss, heterochromia iridis, Hirschsprung disease, mosaicism, penetrance, phenotypic variability, Waardenburg syndrome

## 1 | INTRODUCTION

Waardenburg syndrome (WS) is a neurocristopathy.<sup>1</sup> It is a clinically and genetically heterogeneous disorder. The characteristic clinical features include sensorineural hearing loss (SNHL), heterochromia iridis and pigmentary abnormalities of hair and skin.<sup>2</sup> Prevalence of WS is estimated to be 1 in 42 000 and it accounts for approximately 2% to 5% of congenital hearing loss population.<sup>3,4</sup>

Clinically WS is classified into four subtypes (WS1-4). WS1 (MIM #193500) and WS3 (MIM #148820) are characterized by common findings of telecanthus, sensorineural hearing loss, heterochromia iridis and hypopigmentation of skin and/or hair. In addition, WS3 has musculoskeletal abnormalities of the upper limbs. WS2 clinically differs from WS1 by the absence of telecanthus. WS4 or Shah-WS is associated with Hirschsprung disease (HD).

WS1 is caused by heterozygous variants in *PAX3* (MIM \*606597). Heterozygous as well as biallelic variants in *PAX3* have been reported for WS3. Heterozygous variants in *MITF* (MIM \*156845), *SOX10* (MIM \*602229) cause WS2 (MIM #193510, MIM #611584). Homozygous and less frequently heterozygous variants in *EDNRB* and *EDN3* cause WS4A (MIM #277580) and WS4B (MIM #613265), respectively. Heterozygous variants in *SOX10* have been reported to cause WS4C (MIM #613266).<sup>5</sup>

WS is characterized by significant intra- and inter-familial phenotypic variability and reduced penetrance.<sup>5</sup> We herein provide novel phenotypic and genetic insights into various subtypes of WS.

## 2 | MATERIALS AND METHODS

Subjects presenting with SNHL and heterochromia iridis or hypopigmentation of hair/skin with or without additional abnormalities were included in the study. Sixteen subjects from fourteen families were recruited. Genetics testing was performed by Sanger sequencing or whole-exome sequencing (WES). Copy number variations (CNV) analysis was performed using ExomeDepth algorithm. Quantitative polymerase chain reaction (qPCR) was used to validate CNV detected by ExomeDepth algorithm. Detailed methodology including molecular testing strategy is described in Appendix S1, Supporting Information. Written informed consents for the use of photographs and research findings were obtained from all participants. The study was approved by Kasturba Medical College and Kasturba Hospital Institutional Ethics Committee.

## 3 | RESULTS AND DISCUSSION

In the present study, we report 15 subjects clinically diagnosed with WS and one subject with congenital NSHL. We identified 11 single nucleotide variants (SNV) and one CNV in genes known to cause WS. Clinical diagnosis, pathogenic variants, in silico prediction, allele frequency and final molecular diagnosis are provided in Table 1. Detailed clinical summary findings, pedigree of the families and molecular findings of respective families are provided in Appendix S1. We

observed phenotypic variability, non-penetrance and novel molecular insights among WS families.

WS1 is caused by pathogenic variants in *PAX3*, which encodes for paired box transcription factor.<sup>5</sup> In our cohort, we identified two pathogenic variants in *PAX3* in two families. A novel missense variant, c.256A > T [p.(Ile86Phe)] in *PAX3* in heterozygous state was identified in similarly affected siblings (II-1, II-2) in family 8. The variant was inherited from clinically asymptomatic father (I-1) who harbored the variant in a gonosomal mosaic state. This molecular mechanism has not been previously reported in *PAX3* related WS. In family 9, proband (III-1), was found to have a novel nonsense variant, c.1230C > G [p.(Tyr410Ter)] in heterozygous state in *PAX3*. His father (II-6) was also heterozygous for the same variant and had telecanthus and premature graying of hair. He did not have hearing loss or heterochromia iridis suggesting variable phenotypic expressivity as reported previously for *PAX3* related WS.<sup>5-7</sup>

WS2A is caused by pathogenic variants in *MITF*, which encodes for a basic helix-loop-helix leucine zipper (bHLH-Zip) protein.<sup>8</sup> Heterozygous variants in *MITF* are known to cause WS/Tietz syndrome.<sup>5</sup> In our cohort, we noted five subjects from four families with pathogenic variants in *MITF*. In family 3, a novel nonsense variant, c.979G > T [p.(Glu327Ter)] occurring in the HLH domain of *MITF* protein was observed in the proband III-1) and similarly affected mother (II-2). In family 10, a previously reported canonical splice site variant, c.1013 + 1G > A was observed in proband (III-1) and affected sibling (III-2). However, mother (II-2) harbored the same variant was non-penetrant for the phenotype.

We identified a novel missense variant, c.1021C > G [p.(Arg341-Gly)] in *MITF* in homozygous state in the proband (III-1) of family 5. Clinical features in him were suggestive of WS2/Tietz syndrome. Previously, biallelic variants in *MITF* have been associated with a severe phenotype, COMMAD syndrome (MIM #617306) characterized by microphthalmia, coloboma, macrocephaly, sensorineural hearing loss, osteopetrosis, albinism and facial dysmorphism. The severity seen in these individuals was attributed to the dominant negative effect of variants present in the DNA binding domain of *MITF*.<sup>9</sup> However, the variant in the proband (III-1) was located in bHLH domain of the *MITF* protein which is involved in dimerization of *MITF* protein.<sup>8</sup>

Pathogenic variants in *MITF* are also reported to cause NSHL.<sup>10</sup> In the proband (III-8) of family 14, we identified a known pathogenic nonsense variant, c.1066C > T [p.(Arg356Ter)] in *MITF*. Targeted sequencing showed presence of this variant in heterozygous state in II-9 with unilateral hearing loss, III-5 with bilateral hearing loss and also in other unaffected members (III-7, III-6, and II-8). This variant was previously reported in a family with WS2.<sup>11</sup>

Pathogenic SNVs and CNVs (small and large heterozygous deletions) in *SOX10* are known to cause both WS2E and WS4C.<sup>5</sup> Two novel variants, c.1169C > G and c.415G > T in heterozygous state in *SOX10* were identified in two probands (II-2 and III-1) of family 7 and 12 diagnosed with WS2, respectively. Variants in *SOX10* have a high de novo rate as reported by recent study.<sup>12</sup> However, because of non-availability of parental samples segregation analysis could not be performed.

In family 6, proband (V-1) with clinical diagnosis of WS4 had a de novo heterozygous deletion of approximately 0.178 Mb spanning

**TABLE 1** Pathogenic variants, in silico predictions, allele frequency and final molecular diagnosis observed in the cohort

Family ID	Subject ID	Gene	Transcript	Nucleotide change	Protein change	Zygoty	Mutation Taster	In silico prediction				Allele frequency			Final molecular diagnosis
								Polyphen2	CADD score	gnomAD	1000 genomes	514 Indian exome (in-house)			
F8	III-1	PAX3	NM_181459.3	c.256A > T	p.(Ile86Phe)	Het	D	D	32	0	0	0	0	0	WS1
	III-2														
F9	III-1	PAX3	NM_181459.3	c.1230C > G	p.(Tyr410Ter)	Het	D	NA	24.6	0	0	0	0	0	WS1
F3	III-1	MITF	NM_198159.2	c.979G > T	p.(Glu327Ter)	Het	D	NA	44	0	0	0	0	0	WS2A
F5	III-1	MITF	NM_198159.2	c.1021C > G	p.(Arg341Gly)	Hom	D	D	34	0	0	0	0	0	WS2A
F10	III-1	MITF	NM_198159.2	c.1013 + 1G > A	-	Het	D	NA	27.4	0	0	0	0	0	WS2A
	III-2														
F14	III-8	MITF	NM_198159.2	c.1066C > T	p.(Arg356Ter)	Het	D	NA	41	0	0	0	0	0	HL due to MITF variant
F7	II-2	SOX10	NM_006941.3	c.1169C > G	p.(Ser390Ter)	Het	D	NA	40	0	0	0	0	0	WS2E
F12	III-1	SOX10	NM_006941.3	c.415G > T	p.(Gly139Cys)	Het	D	D	33	0	0	0	0	0	WS2E
F6	V-1	SOX10	-	CNV	-	Het	NA	NA	NA	0	0	0	0	0	WS4C
F4	II-1	EDNRB	NM_001201397.1	c.327C > A	p.(Cys109Ter)	Het	D	NA	35	0	0	0	0	0	WS4A
F13	III-1	EDNRB	NM_001201397.1	c.673G > A	p.(Gly225Ser)	Hom	D	D	25	0.000004	0	0	0	0	WS4A
F1	II-3	EDN3	NM_207032.2	c.332G > T	p.(Cys111Phe)	Hom	D	D	31	0	0	0	0	0	WS4B
F2	IV-1	GJB2	NM_004004.5	c.71G > A	p.(Trp24Ter)	Hom	D	NA	36	0.000608	0.000399	0.0191	0.0191	0.0191	DFNB1A
F11	III-2	ADGRV1	NM_032119.3	c.1608C > G	p.(Tyr536Ter)	Hom	D	NA	35	0	0	0	0	0	USH2C
		TYR	NM_000372.4	c.575C > A	p.(Ser192Tyr)	Hom	P	D	25	0.2545	0.123	0.0865	0.0865	0.0865	Reduced tyrosinase activity

Abbreviations: D, damaging; DFNB1A, deafness, autosomal recessive 1A; Het, heterozygous; Hom, homozygous; HL, hearing loss; LB, likely benign; NA, not applicable; NSHL, non-syndromic hearing loss; P, polymorphism; USH2C; Usher syndrome type 2C.

11 genes and 1 pseudogene (*H1F0*, *GCAT*, *GALR3*, *ANKRD54*, *MIR658*, *MIR659*, *EIF3L*, *MICALL1*, *C22orf23*, *POLR2F*, *SOX10* and *RNU6-900P*). None of the above mentioned genes, except *SOX10* are known to contribute to the phenotype. Previous studies have shown CNVs can range from single exon deletion of *SOX10* to 6 Mb deletion around the *SOX10* locus causing WS2E or WS4C.<sup>13,14</sup>

WS4A and WS4B are caused by pathogenic variants in *EDNRB* and *EDN3*, respectively. *EDNRB* and *EDN3* encode for endothelin receptor type B and its ligand endothelin, respectively. In Family 1, proband (II-3) was diagnosed with WS4. We identified a novel missense variant, c.332G > T [p.(Cys111Phe)] in homozygous state in *EDN3*. The variant occurs within the endothelin domain of the *EDN3* protein, which is essential for binding to its receptor, *EDNRB*.

In family 4, a novel variant, c.327C > A [p.(Cys109Ter)] in *EDNRB* was observed in heterozygous state in proband (II-1) and father (I-1). Proband showed features suggestive of WS1 including hearing loss, heterochromia irides and telecanthus (Figure S4) without HD. Father was clinically asymptomatic and non-penetrant. Another novel variant, c.673G > A [p.(Gly225Ser)] in *EDNRB* was observed in proband (III-1) of family 13 in homozygous state. He had features of WS2 without any evidence of HD. His asymptomatic parents were heterozygous carriers for the variant. Incomplete penetrance has been observed among few families with heterozygous variants in *EDNRB*.<sup>15</sup> Homozygous variants in *EDNRB* have been reported with WS1.<sup>16</sup> Heterozygous variants in *EDNRB* have been described in individuals with WS without HD and also with isolated HD.<sup>15,17</sup> Although variants in *EDNRB* have been associated with WS4, these findings further validate that both heterozygous as well as homozygous variants in *EDNRB* can cause other subtypes of WS.

In our cohort, two individuals fulfilling diagnostic criteria for WS did not have any pathogenic variants in genes known to cause WS. In family 11, proband (III-2) was noted to have a novel pathogenic variant, c.1608C > G [p.(Tyr536Ter)] in homozygous state in *ADGRV1* (MIM \*602851). *ADGRV1* is known to cause Usher syndrome 2C (MIM #605472). It is clinically characterized by SNHL and progressive retinitis pigmentosa. *ADGRV1* variants are also infrequently associated with NSHL according to Deafness Variation Database. Our subject had SNHL, heterochromia iridis and premature graying of hair and albinotic fundus without any evidence of retinitis pigmentosa. In addition, he had a missense variant c.575C > A (p.Ser192Tyr) in homozygous state in *TYR* which is associated with reduced tyrosinase enzyme activity. This variant has a minor allele frequency of 0.2545 in gnomAD Exomes. However, Indian Genome Variation Consortium genotyped this variant in 1871 normal healthy Indians and found this variant to be under represented among Dravidians as compared to Indo-Europeans.<sup>18</sup> It has also been reported as cause of oculocutaneous albinism type 1 in a family with three affected siblings.<sup>19</sup> Therefore, this variant is the probably cause of hypopigmentation noted in him.

In Family 2, proband (IV-1) had a known pathogenic variant, c.71G > A [p.(Trp24Ter)] in homozygous state in *GJB2* (MIM \*121011).<sup>20</sup> Biallelic pathogenic variants in *GJB2* causes non-syndromic hearing loss, *DFNB1A* (MIM #220290). This variant explains only the hearing loss phenotype observed in the proband but

not the heterochromia iridis, telecanthus and synophrys (Figure S2) seen in her.

To summarize, our cohort demonstrates intra- and inter-familial phenotypic variability and non-penetrance in families with WS. We report gonosomal mosaicism in WS1 and biallelic variants in *MITF* and *EDNRB* causing WS2. We observed NSHL because of a pathogenic variant in *MITF*. Blended phenotype of NSHL and albinism mimicked WS. A phenocopy of WS1 was seen in a subject with a pathogenic variant in *GJB2*. With these, our study adds to the spectrum of molecular and phenotypic findings and further understanding of WS.

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## CONFLICTS OF INTEREST

Authors declare no conflict of interest.

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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