Waardenburg Syndrome Panel

PANEL GENE LIST

EDN3, EDNRB, KIT, KITLG, MITF, PAX3, SNAI2, SOX10

CLINICAL FEATURES

Waardenburg syndrome consists of a group of conditions associated with hearing loss and pigmentation abnormalities. Waardenburg syndrome type I (WS1) is estimated to occur in 1 in 20,000-40,000 individuals, and to account for approximately 3% of cases of congenital hearing loss. Waardenburg syndrome type II (WS2) is the next most common form, while Waardenburg syndrome type III (WS3) and Waardenburg syndrome type IV (WS4) are rare.[1] WS1 and WS2 are characterized by sensorineural hearing loss and pigmentation anomalies including complete or segmental iris heterochromia or hypoplastic blue irides, hair hypopigmentation such as a white forelock orpremature gray hair, and congenital leukoderma. The hearing loss associated with Waardenburg syndrome is often congenital, profound and bilateral, although unilateral and mild hearing loss have also been described. Additional features may include high nasal root and medial eyebrow flare and temporal bone abnormalities. Dystopia canthorum (lateral displacement of the inner canthi, which can cause the appearance of hypertelorism) is characteristic of WS1 but absent in WS2. WS3 includes the typical features of WS1 and the additional finding of upper limb abnormalities (such as hypoplasia of the musculoskeletal system, contractures of the limb muscles or joints, carpal bone fusion or syndactyly). Finally, WS4 is characterized by sensorineural hearing loss and pigmentation abnormalities, with the additional finding of Hirschsprung disease.[1] Although penetrance is high, the clinical findings and severity of Waardenburg syndrome are highly variable, both within and between families, with some individuals presenting only with subtle clinical features.

GENETICS

WS1 and WS3 are inherited in an autosomal dominant manner due to pathogenic variants in the *PAX3* gene. Reported variants include missense, nonsense, splice site and frameshift variants, as well as large deletions predicted to result in haploinsufficiency of the resulting protein.[1,2,3,4] Bi -allelic variants in *PAX3* have been reported in individuals with severe symptoms of WS3, with features suggestive of WS1 in their heterozygous parents, suggesting recessive or semi-dominant inheritance of a severe WS3 phenotype.[5,6,7]A single pathogenic variant (p.N47K) in *PAX3* has been reported in individuals with Craniofacial-Deafness-Hand syndrome, an autosomal dominant condition which shares some features of WS1 and WS3 but is thought to represent a distinct and separate clinical disorder.[8]

Most cases of WS2 are autosomal dominant, due to pathogenic variants in the *MITF* or *SOX10* genes. Pathogenic variants in *MITF* are predicted to lead to reduced or absent DNA-binding and transcription activity.[9] The majority of pathogenic variants in *SOX10* are protein-truncating, although missense variants resulting in abnormal protein function have also been reported.[10] Autosomal recessive inheritance of WS2 has been suggested due to the observance of homozygous deletions of the entire *SNAI2* gene in two unrelated patients with WS2.[11] Heterozygous full gene deletions and a variant in the regulatory region of *SNAI2* have been observed in individuals with piebaldism.[12,13]

WS4 is also due to pathogenic variants in the *SOX10* gene, which show autosomal dominant inheritance.[4,10,14] A more severe phenotype of peripheral demyelinating neuropathy, central dysmyelination, Waardenburg syndrome, and Hirschsprung disease (PCWH) is also due to heterozygous pathogenic *SOX10* variants.[10,14] Pathogenic variants in the *EDN3* and *EDNRB* genes have also been associated with WS4, exhibiting complex inheritance patterns. The majority of families with *EDN3* variants show autosomal recessive transmission, but in some families, heterozygous individuals may have pigmentation abnormalities and hearing loss without Hirschsprung disease. Autosomal recessive and dominant inheritance has also been observed with *EDNRB* variants, with heterozygous pathogenic variants exhibiting incomplete penetrance in some cases.[4]

The *KIT* and *KITLG* genes are also included on this panel as differential diagnoses. Pathogenic variants in the *KIT* gene are associated with autosomal dominant piebaldism, which is characterized by unpigmented hair or skin, while pathogenic variants in the *KITLG* gene have been reported in association with both autosomal dominant pigmentation abnormalities and sensorineural hearing loss.[15,16,17]

TEST METHODS

Using genomic DNA from the submitted specimen, the complete coding regions and splice site junctions of the genes on this panel are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

The technical sensitivity of sequencing is estimated to be > 99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size. For the KIT gene, sequencing but not deletion/duplication analysis is performed.

CLINICAL SENSITIVITY

The clinical sensitivity of sequencing and deletion/duplication analysis of the genes included in this panel depends in part on the patient's clinical phenotype and family history. Additional information about the general clinical sensitivity of each gene is included in the table below.

Gene	Protein	Inheritance	Disease Associated	Sensitivity
EDN3	Endothelin-3	AD/AR	WS4B	10% of individuals diagnosed with WS4[18]
EDNRB	Endothelin receptor type B	AD/AR	WS4A/ACBD syndrome	19% of individuals diagnosed with WS4[18]
KIT	Mast/stem cell growth factor receptor Kit	AD	Piebaldism	75% of individuals with piebaldism[19,20]
KITLG	Kit ligand	AD	AD deafness, unilateral or asymmetric / FPHH	Rare in AD deafness (1/64 families with asymmetric HL)17; 6/9 families with FPHH[16,17]
MITF	Microphthalmia-associated transcription factor	AD	WS2A / Tietz Albinism-Deafness Syndrome / COMMAD	15% of individuals with WS2[4,21]

PAX3	Paired box protein Pax-3	AD	WS 1 / WS3	>90% of WS1 for sequencing, ~6% of WS1 for del/dup[1]
SNAI2	Zinc finger protein SNAI2	AD/AR	WS2D / Piebaldism	<5% of individuals with WS2[4]; rare in piebaldism (3/17 individuals without a KIT variant)[12]
SOX10	Transcription factor SOX-10	AD	WS4C / WS2E / PCWH	15% of individuals with WS2[4]; 40-50% of WS4[4,14]

Abbreviations: ABCD - Albinism, Black Lock, Cell Migration Disorder of the Neurocytes of the Gut, and Deafness; AD – Autosomal Dominant; AR – Autosomal Recessive; COMMAD - Coloboma, Osteopetrosis, Microphthalmia, Macrocephaly, Albinism, and Deafness; FPHH – Familial Progressive Hyperpigmentation with or without Hypopigmentation; PCWH - Peripheral Demyelinating Neuropathy, Central Dysmyelination, Waardenburg Syndrome, and Hirschsprung Disease; WS – Waardenburg Syndrome,

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