

Clinical manifestations and novel pathogenic variants in *SOX10* in eight Danish probands with Waardenburg syndrome

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Abstract

The SRY-box 10-gene (*SOX10*), located on 22q13.1, encodes a member of the SOX family of transcription factors involved in the regulation of embryonic development and in the determination of cell fate and differentiation. *SOX10* is one of the six causal genes for Waardenburg syndrome, which is a dominantly inherited auditory-pigmentary disorder characterized by sensorineural hearing impairment and abnormal pigmentation of the hair, skin and iris. Waardenburg syndrome is categorized into four subtypes based on clinical features (WS1-WS4). Here we present eight families (eleven patients) harboring pathogenic variants in *SOX10*. The patients displayed both allelic and clinical variability: bilateral profound hearing impairment (11/11), malformations of the semicircular canals (5/11), motor skill developmental delay (5/11), pigmentary defects (3/11) and Hirschsprung's disease (3/11) were some of the clinical manifestations observed. The patients demonstrate a spectrum of pathogenic *SOX10* variants, of which six were novel (c.267del, c.299_300insA, c.335T>C, c.366_376del, c.1160_1179dup, and exon 3-4 deletion), and two were previously reported (c.336G>A and c.422T>C). Six of the variants occurred *de novo* whereas two were dominantly inherited. The pathogenic *SOX10* variants presented here add novel information to the allelic variability of Waardenburg syndrome and illustrate the considerable clinical heterogeneity.

Keywords: *SOX10*; Waardenburg syndrome; hearing impairment; semicircular canals; CHARGE syndrome.

Introduction

The SRY-related HMG box (*SOX*) gene family encodes a group of transcription factors known as SOX-proteins. Depending on the presence of various structural domains, the SOX family proteins are divided into subgroups A-J (1). SOX10 (OMIM 602229), alongside SOX8 and SOX9, belongs to the SOXE-subfamily which is heavily involved in the specification, multipotency and survival of neural crest cells in vertebrates (2). Moreover, SOX10 has a crucial role as a transcription factor in glial cell development and myelin formation and maintenance, as suggested by knockout mouse studies (3-6). Importantly, *SOX10* is one of the six causal genes (*EDN3*, *EDNRB*, *MITF*, *PAX3*, *SNAI2*, *SOX10*) for Waardenburg syndrome (WS), which is a dominantly inherited auditory-pigmentary disorder with main characteristics sensorineural hearing impairment (HI) and abnormal pigmentation of the hair, skin and iris (7). WS affects ~1 in 42,000 people and accounts for 2% of congenital deafness cases, making it the most common cause of autosomal dominant syndromic HI (8).

WS is categorized into four subtypes (WS1-WS4) based on clinical features and genetic etiology (Table 1). According to the diagnostic criteria proposed by the Waardenburg Consortium (9), the diagnostic features of WS are classified as either *major*, *minor* or *rare*. A person must have two major or one major plus two minor criteria to be diagnosed with WS1 (OMIM 193500) (9). Some of the features observed in WS1 include HI, white forelock, premature greying of hair, heterochromia iridis, synophrys, congenital leukoderma and dystopia canthorum (lateral displacement of the inner canthi) (7). WS2 (OMIM 193510) is characterized by more frequent occurrence of sensorineural HI and heterochromia iridis but absence of dystopia canthorum (10). Liu et. al. proposed that to establish a diagnosis of WS2, two major criteria should be fulfilled and dystopia canthorum be replaced with premature graying of hair as one of the major criteria (7, 10). WS3 (OMIM 148820) is similar to WS1 with additional musculoskeletal abnormalities (7). If WS is seen in combination with

Hirschsprung's disease (HSCR) a diagnosis of WS4 (OMIM 277580) can be made (reviewed in (11)). HSCR, sometimes referred to as congenital aganglionic megacolon, is a birth defect characterized by the presence of aganglionic segments in various parts of the intestine (12). WS4 is further divided into subgroup A-C., where WS4C (OMIM 613266) is associated with pathogenic variants in *SOX10*. It is estimated that pathogenic variants in *SOX10* account for ~15% of all WS2 cases and ~50% of all WS4 cases (11). Moreover, *SOX10* mutations are occasionally associated with a more severe phenotype named PCWH: Peripheral demyelinating neuropathy, Central dysmyelinating leukodystrophy, WS, and HSCR (OMIM 609136) (13).

Inner ear deformities, such as agenesis of the semicircular canals (SSCs), have previously been reported to be linked to *SOX10* associated WS (8, 14). Furthermore, hypogonadism is occasionally observed in adult patients harboring pathogenic variants in *SOX10* (15, 16). Thus far, no clear genotype-phenotype correlation exists for patients with pathogenic variants in *SOX10*. The vast majority of patients with *SOX10* variants have profound HI (8).

WS needs to be distinguished from other causes of congenital sensorineural HI. The combination of HI, SSC malformations and/or hypogonadotropic hypogonadism are features characteristic also for CHARGE syndrome (OMIM 214800), caused by pathogenic variants in *CHD7* (OMIM 608892). Additional clinical signs associated with this condition are developmental delay and growth deficiency (17). Hence, since CHARGE syndrome represents a significant differential diagnosis to WS, appropriate genetic testing schemes are required to distinguish these entities.

Materials and methods

Patients

A cohort of eleven patients from eight families, diagnosed through genetic examination at the Kennedy Center, Glostrup, Denmark, were included in this retrospective study. They demonstrate a spectrum of both *de novo* and dominantly inherited pathogenic variants with very variable clinical abnormalities. The patients were identified among individuals referred for genetic examination at the department and suspected of having WS or CHARGE syndrome. The associated clinical information was variable at referral, but if they presented with malformation of the SSCs and for example showed normal sequence of the *CHD7* gene, investigations for pathogenic variants in the *SOX10* gene were conducted. Complete or partial agenesis of the SSCs was a critical clue to suspect the involvement of the *SOX10* gene.

Genetic and molecular analysis

DNA was extracted from peripheral blood samples, obtained from the patients during the routine molecular diagnostic process.

For Sanger sequencing analysis the 4 exons of *SOX10* (RefSeq no. NM_006941.3) and flanking intronic regions, were PCR amplified. Primer sequences and PCR conditions are available upon request. Amplicons were sequenced using the Big Dye Terminator Kit v1.1 (Applied Biosystems) on an ABI 3130XL genetic analyzer (Applied Biosystems) according to the manufacturer's instructions.

For NGS analysis coding exons and 5'-UTRs of 106 genes for non-syndromic and syndromic HI were investigated using a HaloPlex custom designed targeted gene-panel (gene list is available upon request). Enrichment library from patient DNA was created using HaloPlex Target Enrichment system (Agilent Technologies, Santa Clara, CA, USA) and sequenced using MiSeq v2 (2×250 bp) chemistry (Illumina Inc, San Diego, USA) on a MiSeq instrument (Illumina). FASTQ files were analyzed using SureCall v.3.5.1.46 (Agilent Technologies) using default settings.

Identified variants were evaluated using: Alamut Visual, version 2.12; gnomAD (<https://gnomad.broadinstitute.org/>); ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>), OMIM (<https://www.ncbi.nlm.nih.gov/omim?db=OMIM&itool=toolbar>) and HGMD® Professional. Variant nomenclature follows HGVS guidelines (<http://www.hgvs.org>) with cDNA numbering with +1 = A of start ATG codon in the *SOX10* cDNA reference sequence (RefSeq no. NM_006941.3).

The probands in families 1 and 2 were investigated for copy number variations in *SOX10*, *MITF* and *PAX3* using the Multiplex Ligation-dependent Probe Amplification (MLPA)-PAX3-MITF-SOX10 kit (P186) according to the manufacturer's instructions (MRC-Holland, <https://www.mrcholland.com/>). Amplification products were identified and quantified by capillary electrophoresis on an ABI 3130XL genetic analyzer (Applied Biosystems, Foster City, CA, USA) and peak analysis was performed by comparing the peak profiles using Coffalyser (MRC-Holland).

Results

Genetic investigations of the patients resulted in identification of a spectrum of *SOX10* variants of which six were novel (c.267del (p.(Met90Cysfs*19)), c.299_300insA (p.(Ser100Argfs*34)), c.335T>C (p.(Met112Thr), c.366_376del (p.(Ala123Profs*7)), c.1160_1179dup (p.(Ser394Thrfs*115)), and deletion of exon 3-4) and two were previously reported (c.336G>A (p.(Met112Ile) and c.422T>C (p.(Leu141Pro))). Six of the variants occurred *de novo* and two were found to be dominantly inherited. Both familial cases presented with novel *SOX10* variants (Table 2). A schematic view of the *SOX10* gene with localization of the frameshift and missense variants detected in the cohort is provided in Figure 2.

Patients

Family 1, case 1 is a 29-year-old female with profound HI, HSCR and autism spectrum disorder. The parents, brother and sister were unaffected (for pedigree see Figure 1). Case 1 was born at term. Birthweight was 3200 grams. Case 1 sat without support at the age of 10 months and walked without support at the age of 1 year. When examined at the age of 5 years, case 1 had low muscle tone, balance problems and slightly delayed motor skill development. No dysmorphic features were observed. Case 1 did not have cochlear implants (CI) and was never MRI- or CT- scanned. Array CGH (400K, Agilent Technologies) showed a heterozygous deletion of about 8 kb located on 22q13.1 (chr22:36.697.726-36.705.448 (hg18)) including the *SOX10* gene. The MLPA-PAX3-MITF-SOX10 kit (P186) was employed and a heterozygous *SOX10* deletion of exon 3-4 was detected. Parental testing did not detect the *SOX10* variant.

Family 2, case 2 is a 13-year-old male with congenital bilateral profound HI, HSCR and growth delay. The parents and younger sibling were unaffected. Case 2 was born after labor induction at a gestational age of 42+2. Apgar score 10/1, 10/5. Three days old case 2 was hospitalized due to abdominal distention and bile colored vomit. Abdominal x-ray showed ileus accompanied by pneumatosis in the small intestine. Biopsy revealed hypotrophy of ganglia and nerve cells. When case 2 was 1 month old, a partial colectomy was performed, and he suffered from subsequent sequelae until the age of 7 years. At the age of 11- and 12-months case 2 got CI on the right and left side, respectively. Prior to the operations a CT- scan was performed which showed symmetrically wide *pori acustici interna* on both sides. Cochleae and the SCCs were normal. As an infant, case 2 was described as having brilliant blue eyes, pale skin and blonde hair. Case 2 had enophthalmos, mild to moderate left-sided plagiocephaly and slightly posterior rotated ears. Case 2 walked without support at the age of 14-15 months old. Language development was delayed (6-8 words per sentence at the age of 5 years). The clinical picture of HI and HSCR led to the investigation of WS associated genes. MLPA of *PAX3*, *MITF* and

SOX10 was normal. Sanger sequencing of *SOX10* detected a heterozygous frameshift variant in exon 4 (c.1160_1179dup, p.(Ser394Thrfs*115), ClinVar ID SCV001573794). Parental testing did not detect the *SOX10* variant.

Family 3 is a two-generation family. The proband (**case 3**), her son (**case 4**) and her daughter (**case 5**) presented with profound HI and they were harboring the same *SOX10* variant. The sister and brother of the proband, as well as her parents were unaffected. The proband was born 18 days overdue after labor induction. Case 3 had cleft palate, which was successfully treated with surgery. No pigmentary defects, musculoskeletal abnormalities or additional dysmorphic features were reported. Case 3 walked without support at the age of 18 months. The first 4 years of life case 3 had recurrent cystitis with hematuria, with one case of acute glomerulonephritis. Ultrasound of the kidneys was normal. CT- scan and MRI- scan was carried out, which showed symmetrically wide *pori acustici interni*. The son of the proband had profound HI, white forelock, pale eyelashes and eyebrows, blue eyes and pigment defects on his face and abdomen. Case 4 got bilateral CI at the age of 1 year. Case 4 had balance problems and walked without support when he was 21 months old. Language development, motor skill development and growth were delayed. Case 5, the daughter of the proband, had congenital bilateral profound HI and got CI at the age of 1 year. Case 5 walked without support at the age of 15 months. The father of case 4 and case 5 had unilateral congenital profound HI and pigmentary defects, although he did not harbor the *SOX10* variant. Further, his sister had unilateral profound HI. NGS gene-panel sequencing was performed with targeted analysis of WS associated genes. The results indicated a variant in *SOX10*, however, in a region which had a low coverage. Sanger sequencing in this region revealed a heterozygous frameshift variant in exon 2 of the *SOX10* gene (c.366_376del, p.(Ala123Profs*7), ClinVar ID SCV001573793). The parents of the proband did not harbor the *SOX10* variant.

Family 4 is a family with an affected proband (**case 6**) and her half- sister (**case 7**). The sisters had congenital bilateral profound HI and malformations of the SCCs. Both sisters had CI. MRI-scan of case 6 revealed that the lateral SCCs were bilaterally dysplastic, the posterior SSCs were bilaterally aplastic, whereas the superior SCCs were normal. The ventricular system was said to be widened, however, this finding could be due to benign macrocephaly. MRI- scans of the sister (case 7) detected aplastic posterior SCCs on both sides. The mother of case 6 and 7 had unilateral profound HI, and on the maternal side, her uncle and grandmother as well as her two sisters were anecdotally known to have profound HI (Figure 1). Based on SCC abnormalities, Sanger sequencing of *SOX10* was performed which revealed a heterozygous frameshift variant in exon 2 (c.299_300insA, p.(Ser100Argfs*34), ClinVar ID SCV001573797). The mother of case 6 and case 7 was not available for genetic testing.

Family 5, case 8 is a 22-year-old female with bilateral profound HI, heterochromia iridis and bilateral aplastic posterior SCCs. Case 8 got CI on the left side at the age of 5,5 years and on the right side at the age of 17 years. CT- scans of the temporal bone showed slightly widened vestibulum in addition to the SCCs malformation. On clinical examination it was noted that the proband had bilateral prominent ear lobes. Case 8 had a mild degree of concentration problems. NGS gene-panel sequencing was performed with targeted analysis of WS associated genes, and a heterozygous single base deletion was found in exon 2 of the *SOX10* gene (c.267del, p.(Met90Cysfs*19), ClinVar ID SCV001573798). Parental testing did not detect the *SOX10* variant.

Family 6, case 9 is a 5-year-old female with bilateral profound HI and bilateral aplastic posterior SCCs. The parents and younger sibling of case 9 were unaffected. Case 9 got bilateral

CI at the age of 2 years. The operation was successful, and on the 7 months post-CI check-up case 9 scored equivalent to 2 years post-CI. Case 9 had early onset dentition (at the age of 4 months) and late onset tear production. Both fine and gross motor skill development were according to age. Case 9 sat without support at the age of 6 months and walked without support at the age of 13 months. Growth wise case 9 followed a normal development. No dysmorphic features or other clinical manifestations were observed. Based on SCC abnormalities, Sanger sequencing of *SOX10* was performed which revealed a heterozygous missense variant in exon 2 (c.335T>C, p.(Met112Thr), ClinVar ID SCV001573792). Parental testing did not detect the *SOX10* variant. According to ACMG criteria this variant is classified as likely pathogenic (PM2, PM5, PM6, PP3, PP4) (18).

Family 7, case 10 is a 10-year-old male with bilateral profound HI, hypermetropia, developmental delay and growth delay. The mother, father and brothers of case 10 were unaffected. Case 10 was born at gestational age 41 weeks. Birthweight was 2970 grams. At an early age case 10 was described as having blue eyes, blonde hair, slightly low-set ears and a hint of plagiocephaly. Additionally, case 10 had bilateral flat feet. Examination of eyes and vision demonstrated darker iridis with Brushfield spots and hypermetropia. At the age of 1 year, case 10 got bilateral CI. Prior to the CI-operation an MRI-scan was performed which showed bilateral dysplastic superior and posterior SCCs, with normal lateral SCCs. Case 10 had early onset dentition and xerostomia. When CHARGE syndrome was excluded, Sanger sequencing of *SOX10* was performed due to the inner ear malformation. The analysis revealed a known disease-causing missense variant in exon 2 of the *SOX10* gene (c.422T>C, p.(Leu141Pro), ClinVar ID SCV001573796). Parental testing did not detect the *SOX10* variant.

Family 8, case 11 is a 15-year-old male with bilateral profound HI, mildly impaired vision, developmental, growth and pubertal delay and HSCR. Case 11 was born from healthy parents, at term, and with normal growth parameters. Shortly after birth, case 11 showed failure to thrive. At the age of 4 months, case 11 was diagnosed with HSCR, and a total colectomy was performed. Case 11 had pigmentary abnormalities, including vitiligo patches on hands, right leg and a pigment patch on his forehead. Furthermore, case 11 had a white forelock and five *café au lait* patches located on his back and abdomen. At the age of 6 months, case 11 had rotatory nystagmus which later normalized. HI was detected at the nationwide neonatal hearing screening and he got bilateral CI at the age of 1-2 years. Language development after CI was adequate. No inner ear malformations were reported. Case 11 had slightly poor balance and some of the expected milestones in motor skills were delayed. Case 11 had a micropenis and was followed by the clinic for growth and reproduction. A diagnosis of hypogonadotropic hypogonadism was confirmed, and case 11 received testosterone treatment for a while. The mother and older brother were unaffected. The father of case 11 was healthy, however both case 11 and his father carry a balanced translocation between chromosome 3 and chromosome 10. Sanger sequencing of *SOX10* revealed a *de novo* known disease-causing missense variant in exon 2 (c.336G>A, p.(Met112Ile), ClinVar ID SCV001573795). The pathogenic variant in *SOX10* with additional clinical information regarding the patient was published in Pingault et al. 2014 (19).

Discussion

WS is a genetically heterogeneous disease with several genes implicated. Clinical features observed can vary both within and between families, and currently no clear genotype-phenotype correlation exists. The highly variable degree in the severity of the associated abnormalities makes a reliable prognosis for newly diagnosed infants hard to establish. Several clinical

findings, especially malformations of the SCCs overlap with CHARGE syndrome. HI, mental and pubertal delay are other shared features, whereas pigmentary abnormalities and HSCR are relative unique to WS and coloboma and heart malformations are typical in a large fraction of CHARGE syndrome patients.

The malformations of the SCC's led to sequencing of the *SOX10* gene in some of the present cases after *CHD7* sequencing and MLPA analysis were found normal. This work, alongside others, propose a clinically relevant relationship between *SOX10* variants and (isolated) agenesis of the SCCs (11, 14, 20). Strikingly, one patient (case 11) had normal anatomy of the inner ear and no malformation of the SCCs, but several other serious hallmarks of WS, including severe HSCR.

Case 1 presented with HI, HSCR and autism spectrum disorder. Cognitive delay, mental health problems and/or autism spectrum disorder have previously been observed in individuals with WS. The prevalence is unknown and the evidence for the association is limited (21). Whether the development of mental health problems and/or autism spectrum disorder is due to the syndrome itself or the associated clinical manifestations is not clear. Furthermore, the extent of the deletion in case 1, may stretch downstream exon 4 of the *SOX10* gene and might therefore affect other genes or regulatory elements on chromosome 22q13.1.

Pathogenic variants in *SOX10* are rarely recurrent (22). In this study we describe two patients each harboring recurrent variants; (c.422T>C, p.(Leu141Pro)) and (c.336G>A, p.(Met112Ile)), respectively. The c.422T>C (p.(Leu141Pro)) variant was previously reported in a Chinese patient, as well as in an Iranian family (23, 24). The latter of which the father of two affected children presented with WS phenotype (sensorineural HI, hypoplastic blue eyes, white forelock and chronic constipation), however, he was not subjected to genetic analysis. The son was referred to as a *full presentation* of WS4 (WS phenotype in addition to HSCR).

His sister had an *intermediate presentation* including WS phenotype and chronic obstipation, but not HSCR. Case 10 in family 7, harboring the same variant, presented with profound HI, malformations of the SCC, Brushfield's spots on the iris, hypermetropia, developmental delay and growth delay. Notably, case 10 did not have HSCR or problems with chronic obstipation. The c.336G>A (p.(Met112Ile)) variant has previously been reported in several independent cases. In 2011 Chaoui et. al. reported two *de novo* cases (c.336G>A, p.(Met112Ile)) and one familial case (c.336G>C, p.(Met112Ile)). The two *de novo* cases were male individuals aged 1 year and 7 months, respectively. The 1-year-old presented with PCWH, depigmentation, HI, neurological deficits and HSCR. The 7-month-old reportedly had PCW or PCWH, unilateral HI, neurological deficits and intermittent constipation. The familial case consisted of a female and her daughter, both harboring WS2 phenotype with depigmentation and HI. Neither of them had HSCR (22). In 2014, Pingault et. al. reported phenotypic similarities such as pigmentation defects and neurological symptoms, but differences in gastrointestinal symptoms, in four patients harboring the p.(Met112Ile) variant (19). One of them is referred to as case 11 in this report.

The findings presented here underscore the clinical intra- and interfamilial phenotypic heterogeneity seen in WS in patients with identical *SOX10* mutation.

The *SOX10* gene harbors a remarkable spectrum of mutations of which the majority (146/191) are predicted to cause either truncation or complete absence of the *SOX10* protein. Thus far, 84 pathogenic single nucleotide variants have been reported, of which roughly half of them are of the nonsense type. Furthermore, small and gross deletions account for 77 out of 191 pathogenic variants identified this far (Human Gene Mutation Database (HGMD) professional, as of April 2021). In addition, a smaller number of substitutions, indels, and complex rearrangements are known.

SOX10 contains a DNA-binding HMG (high mobility group) domain, a dimerization region right upstream to the HMG domain, a conserved domain in the center, and a trans-activation (TA) domain at the extreme C-terminus (11). The HMG domain consists of a highly conserved motif of 80 amino acids, involved in regulation of intracellular transport, as well as transcriptional regulations involving multi-transcription factors important for, among other things, oligodendrocyte differentiation as well as regulation of the onset of CNS myelination and remyelination (25). Pathogenic missense variants in *SOX10* are mainly, if not only, affecting the HMG domain. This underscores the importance of the HMG domain in *SOX10* function as amino acid side chain alterations tend not to be accepted. This notion is supported by population studies demonstrating that the HMG domain contains very few nonsynonymous variants as compared to elsewhere in *SOX10* (The Genome Aggregation Database; gnomAD).

Six of the eight disease causing variants presented here affect the HMG domain, either directly as missense mutations, or indirectly as truncating variants within or preceding the coding sequence of the HMG domain (Figure 2).

Conclusion

The results presented here add six novel pathogenic variants: one deletion, one missense and four frameshift variants, to the spectrum of pathogenic variants in *SOX10*. Further, we present two recurrent missense variants. The clinical and genetic data presented in this study supports that a clinical picture consisting of HI and malformations of the SCCs should raise a suspicion of WS related to *SOX10* deficiency. In line with the increasing routine to use NGS in the evaluation of patients with syndromes, the genetic differentiation between CHARGE and WS is likely to be solved, but awareness towards the different clinical and developmental implications of the two syndromes are still essential in order to offer proper follow-up and rehabilitation. Lastly, the pathogenic *SOX10* variants and associated clinical findings presented

here expand the spectrum of mutations and illustrate the considerable clinical variability in WS.

Ethical aspects

Written consent was obtained from all families included in the study. Investigations were performed in a diagnostic setting, with the aim of a more correct diagnosis for the involved patients. The included families have received genetic counselling at the Department of Clinical Genetics, Rigshospitalet / The Kennedy Centre, Glostrup, Denmark.

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Figure titles and Legends

Table 1 Summary of the most prominent characteristics of subtype WS1-WS4, with associated genes.

Table 2 Summary of clinical and genetic data in the patient cohort. NI= no information.

Figure 1 Pedigrees of the Waardenburg syndrome families. Individuals with bilateral hearing impairment are shaded. Individuals with unilateral hearing impairment are half shaded. The star

symbol “*” indicates that the individual is genetically examined. Individuals marked with “N/N” had normal analysis of *SOX10*. Some individuals not clinically examined are indicated as affected with hearing impairment. This information is based on family anamnesis.

Figure 2 Schematic view of the *SOX10* gene with localization of the frameshift and missense variants detected in the cohort. The grey bars indicate the coding region of the HMG domain. The deletion of exon 3-4, detected in case 1 (family 1) is not included in the figure, as breakpoints have not been characterized. Nomenclature of mutations is based on cDNA sequence, NM_006941.3. Numbering of nucleotides: +1 = A of start ATG codon.

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Supplemental data:

Figure S1. Representative sequence chromatograms for each of the pathogenic *SOX10* variants. The arrows indicate the nucleotide changes of the heterozygous variants.

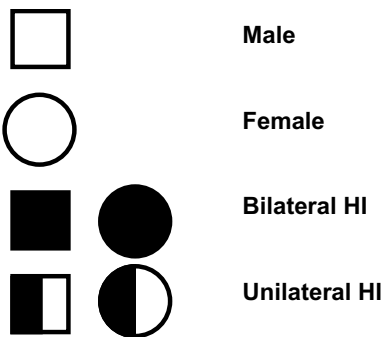
Table 1 Summary of the most prominent characteristics of subtype WS1-WS4, with associated genes.

Subtype	WS1	WS2	WS3	WS4
Clinical characteristics	Dystopia canthorum, hearing impairment, heterochromia iridis, white forelock, congenital leukoderma, synophrys, broad high nasal root, hypoplasia of alae nasi, premature greying of hair (7, 9).	Similar characteristics as WS1, but without dystopia canthorum (7, 10).	Similar characteristics as WS1, but with additional upper limb abnormalities, such as hypoplasia of limb muscles and contractures of elbows and fingers (7).	Similar characteristics as WS2, but with additional Hirschsprung's disease (7).
Associated genes	<i>PAX3</i> (11).	<i>MITF</i> , <i>SNAI2</i> , <i>SOX10</i> , (<i>EDN3</i> , <i>EDNRB</i>) (11).	<i>PAX3</i> (11).	<i>EDN3</i> , <i>EDNRB</i> , <i>SOX10</i> (11).

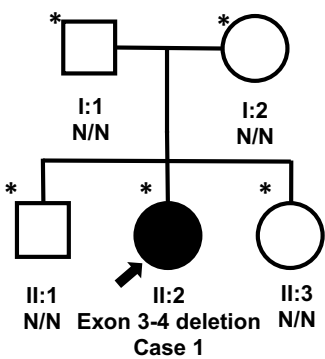
Table 2 Summary of clinical and genetic data in the patient cohort. NI= no information.

Family number	Case number	Gender	Age (year of birth)	Bilateral profound hearing impairment	Bilateral cochlear implants	Malformation of the semicircular canals	Other temporal bone abnormalities	Hirschsprung's disease	White forelock	Abnormal pigmentation of the skin	Heterochromia irides	Plagiocephaly	Vision impairment	Growth and/ or pubertal delay	Motorskill developmental delay	Mutation (c.DNA)	Predicted protein	Coding effect	Origin	Novelty
1	1	F	29 (1991)	+	-	NI	NI	+	-	-	-	-	-	-	+	Exon 3-4 deletion			De novo	Novel
2	2	M	13 (2007)	+	+	-	+	+	-	-	-	+	-	+	+	c.1160_1179dup	p.(Ser394Thrfs*115)	Frameshift	De novo	Novel
3	3	F	28 (1992)	+	+	-	+	-	-	-	-	-	NI	NI	-	c.366_376del	p.(Ala123Profs*7)	Frameshift	Familial	Novel
	4	M	9 (2011)	+	+	-	-	-	+	+	-	-	NI	+	+					
	5	F	6 (2014)	+	+	NI	NI	-	-	-	-	-	NI	NI	-					
4	6	F	11 (2009)	+	+	+	+	-	-	-	-	-	NI	NI	-	c.299_300insA	p.(Ser100Argfs*34)	Frameshift	Familial	Novel
	7	F	6 (2014)	+	+	+	-	-	NI	NI	-	-	NI	NI	NI					
5	8	F	22 (1998)	+	+	+	+	-	-	-	+	-	-	-	-	c.267del	p.(Met90Cysfs*19)	Frameshift	<i>De novo</i>	Novel
6	9	F	5 (2015)	+	+	+	-	-	-	-	-	-	NI	-	-	c.335T>C	p.(Met112Thr)	Missense	<i>De novo</i>	Novel
7	10	M	10 (2010)	+	+	+	-	-	-	-	-	+	+	+	+	c.422T>C	p.(Leu141Pro)	Missense	<i>De novo</i>	Known (23)
8	11	M	15 (2005)	+	+	-	-	+	+	+	-	-	+	+	+	c.336G>A	p.(Met112Ile)	Missense	<i>De novo</i>	Known (22)

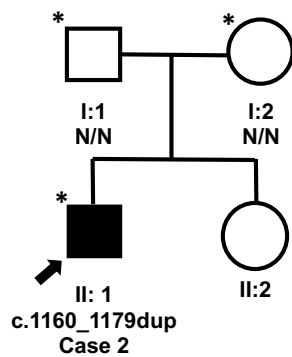
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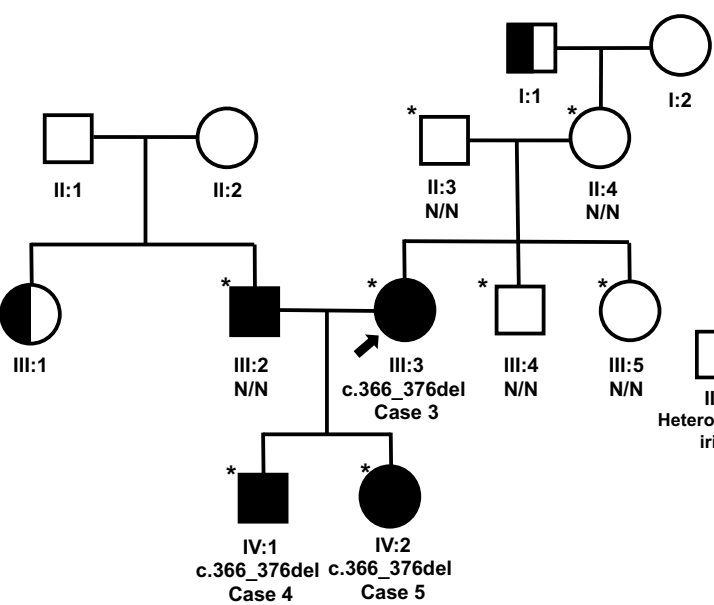
Family 1



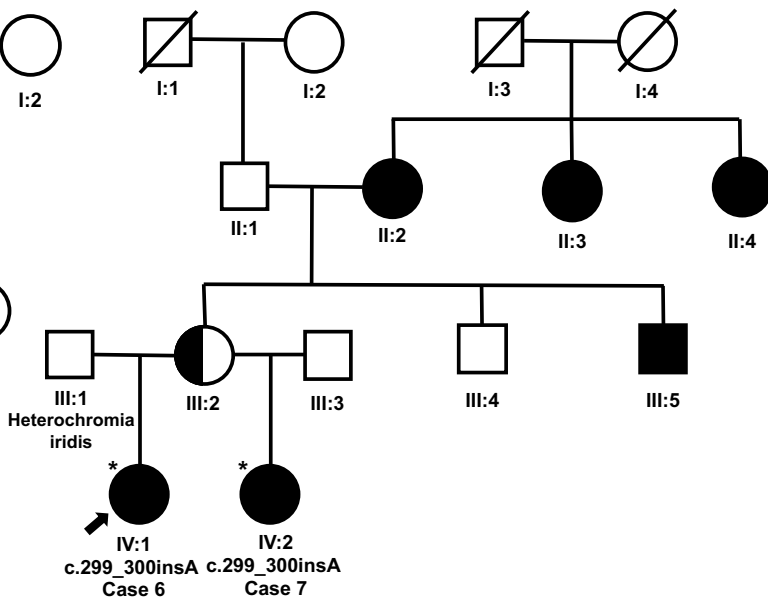
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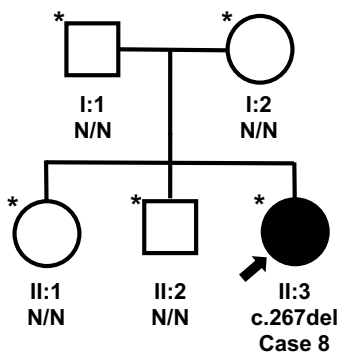
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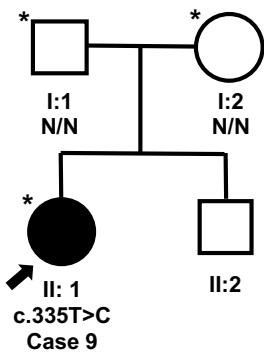
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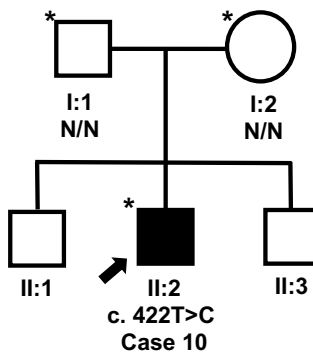
Family 5



Family 6



Family 7



Family 8

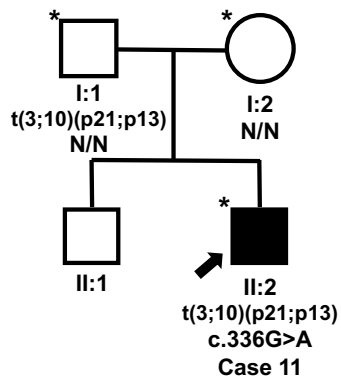


Figure 1.

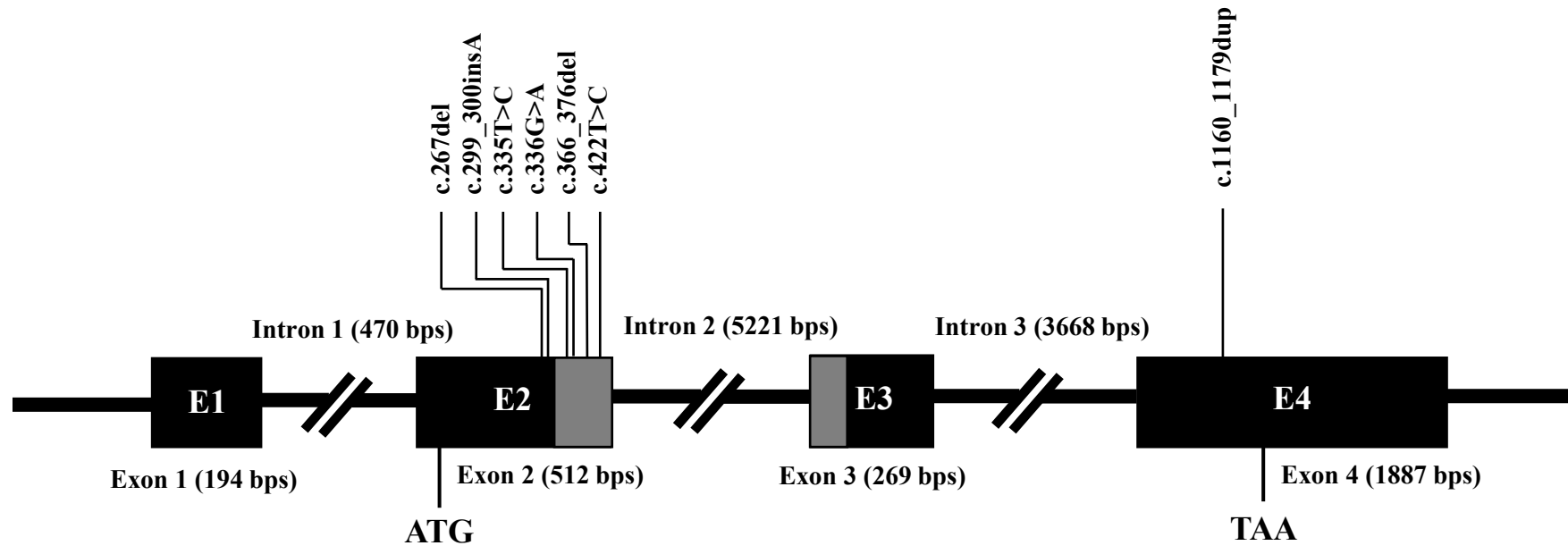


Figure 2.