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CASE REPORT Open Access

Three novel variants in *SOX10* gene: Waardenburg and PCWH syndromes

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Abstract

Background: Waardenburg syndrome (WS) is a rare genetic disorder characterized by musculoskeletal abnormalities, deafness and hypopigmentation of hair and skin. This article's aim is to investigate clinical and genetic characteristics of WS in three unrelated Caucasian individuals.

Case presentation: The first patient was a 25-year-old female with congenital bilateral hearing loss, bright-blue-eyes, hypopigmentation of hair and skin, megacolon, language retardation, tenosynovitis and neuromas. The second case was an infant symptomatic from birth, with dysphagia, Hirschsprung disease and neurological abnormalities. The third patient was a 14-year-old boy with congenital bilateral hearing loss and ileocolic Hirschsprung disease. In order to identify variants in potentially causal genes of the patients' phenotype, genetical testing was conducted: targeted clinical exome, targeted exome and trio exome, respectively. We identified three novel variants spread throughout the coding sequence of *SOX10*. The c.395C>G variant identified de novo in patient 1 was a single nucleotide substitution in exon 2. The c.850G>T variant identified as heterozygous in patient 2 was a loss-of-function variant that generated a premature stop codon. The c.966dupT variant identified in patient 3 was a duplication that generated a premature stop codon. It had been identified in his father, arising a possible germinal mosaicism. According to in silico predictors the variant identified in patient 1 was considered as pathogenic, whereas the other two were classified as likely pathogenic.

Conclusions: An exact description of the mutations responsible for WS provides useful information to explain clinical features of WS and contributes to better genetic counselling of WS patients.

Keywords: Hereditary motor and sensory neuropathy, Waardenburg syndrome, PCWH syndrome, *SOX10*

Background

Waardenburg syndrome (WS), also known as auditory-pigmentary syndrome, is a rare genetic disorder typically characterized by musculoskeletal abnormalities, deafness and hypopigmentation of the skin. Basic clinical symptoms of WS include dystopia of the canthus; abnormal pigmentation of the skin, hair, and eyes; different degrees of unilateral or bilateral sensorineural deafness; and high and wide nasal base [1].

WS is inherited in an autosomal dominant manner and has an incidence of 1 in 20,000. Four distinct clinical subtypes of WS (WS1-4) are defined based on the presence or absence of additional symptoms. Facial dysmorphic features are present in WS1 (MIM#193500) and WS3 (Klein-Waardenburg syndrome; MIM#148820) patients. The absence of additional features characterizes WS2 (MIM#193510), whereas an association with Hirschprung disease (HD, aganglionic megacolon or gastrointestinal atresia) defines WS4 (MIM#277580), also known as Shah-Waardenburg syndrome or Waardenburg-Hirschprung disease [2].

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WS is both phenotypically and genetically heterogeneous [3]. Mutations in six genes have been identified to be associated with WS: paired box gene 3 (*PAX3),* melanocyte inducing transcription factor *(MITF),* snail family transcriptional repressor 2 (*SNAI2*), SRY-box 10 (*SOX10*), endothelin 3 *(EDN3)* and endothelin receptor type B *(EDNRB).* De novo mutations in the *SOX10* gene are rarely observed, even if heterozygous *SOX10* mutations have been described in 15% and 45–55% of WS2 and WS4 patients respectively. Distinct classes of *SOX10* mutations are responsible for a more severe phenotype of this condition evolving peripheral and central nervous system known as PCWH syndrome, that comprises peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome and Hirschsprung disease [4, 5].

This article's aim is to investigate clinical and genetic characteristics (using Sanger and whole-exome sequencing) of WS and PCWH in three unrelated Caucasian individuals. Interpretation of the variants was based on the guidelines of American College of Medical Genetics and Genomics (ACMG) [6].

Case presentation

We identified three unrelated Spanish individuals with an unusual but similar clinical phenotype. They did not have family history of congenital or neurological disorders, and their parents were non-consanguineous. A comprehensive clinical history was gathered and clinical examinations were meticulously performed for each affected individual. Written informed consent was obtained from patients included in this study or their parents.

Patient 1

A 25-year-old female with congenital bilateral hearing loss (Fig. 1A), chronic constipation due to megacolon, gastroesophageal reflux, diabetes and mild language retardation, was referred for asymmetric pain in the lateral aspect of the legs, triggered by exercise and relieved by rest, which was attributed to tenosynovitis.

On examination, bright blue eyes with partial heterochromia iridis, hypopigmentation of hair (white forelock) and skin (café-au-lait spots on the right hand, breasts, right thigh, knees and scalp, Fig. 1B) and pes cavus, were found. Neuromas were present in the left arm, right leg and forehead.

On neurological examination, tendon reflexes were 1/4 in upper limbs and absent in the lower ones. Muscle bulk and power, stance, gait and pain and vibration sense were preserved. Coordination and cranial nerves were normal.

An electrophysiological study revealed decreased sensory (31 m/s) nerve conduction velocity (NCV) in the right median nerve, and absent action potentials in both sural nerves. Motor NCV was decreased in the median, peroneal and tibial nerves, while increased motor latencies were found in the median nerve.

Referred for genetic study by Multiplex Ligation-dependent Probe Amplification (MLPA) of the *PMP22* gene and obtaining a negative result, she then went through a massive sequencing study (targeted clinical exome, ExoNIM®, NIMGenetics, Spain) with the aim of identifying variants in 103 selected genes associated with Charcot-Marie Tooth and other HMSN. Four variants of uncertain significance were obtained.

As so, genetic testing was amplified with a massive sequencing of the complete human exome of the patient and his parents (ExoNIM® Trio Approach, NIMGenetics, Spain). The c.395C>G p.(Ala132Gly) variant was identified de novo in the case sample consisting in a single nucleotide substitution in exon 2 of the *SOX10* gene (transcript ID: LRG_271). According to the ACMG variant classification guidelines, this *missense* variant was classified as pathogenic since PS2, PM1, PM2_supporting, PP2, PP3 and PP4 criteria were met.

Patient 2

Infant symptomatic from birth, with dysphagia, a diagnosis of Hirschprung disease and carrier of gastrostomy and ileostomy. Neurologically, abnormal tendon reflexes, rhythmic and horizontal head jerks, limb tremor and rotating eye movements were observed from day 15 of life. The patient died aged 20 months due to complications derived from intestinal obstruction.

A brainstem auditory evoked potential (BAEP) test showed severely prolonged latencies bilaterally.

With these clinical features, we were asked to carry out an array comparative genomic hybridization (aCGH, qChip® Post, qGenomics, Spain), with no relevant findings. Then, we amplified the genetical study by conducting a targeted exome (ExoNIM® Clinical, NIMGenetics, Spain) in order to identify variants in 6102 selected genes that may present an association with our case's phenotype. The heterozygous c.850G>T p.(Glu284*) variant identified in *SOX10* (transcript ID: LRG_271) in the patient's sample was a *nonsense* variant classified by ACMG as likely pathogenic (PVS1_strong, PM2_supporting and PP4 criteria).

Patient 3

A 14-year-old boy with congenital bilateral hearing loss, required cochlear implants at one year of age. He had no heterochromia iridis or skin hypopigmentation. The patient was diagnosed with ileocolic Hirschprung disease at 6 months, with ileostomy discharge at 15 months. A colectomy was performed to remove the non-functional

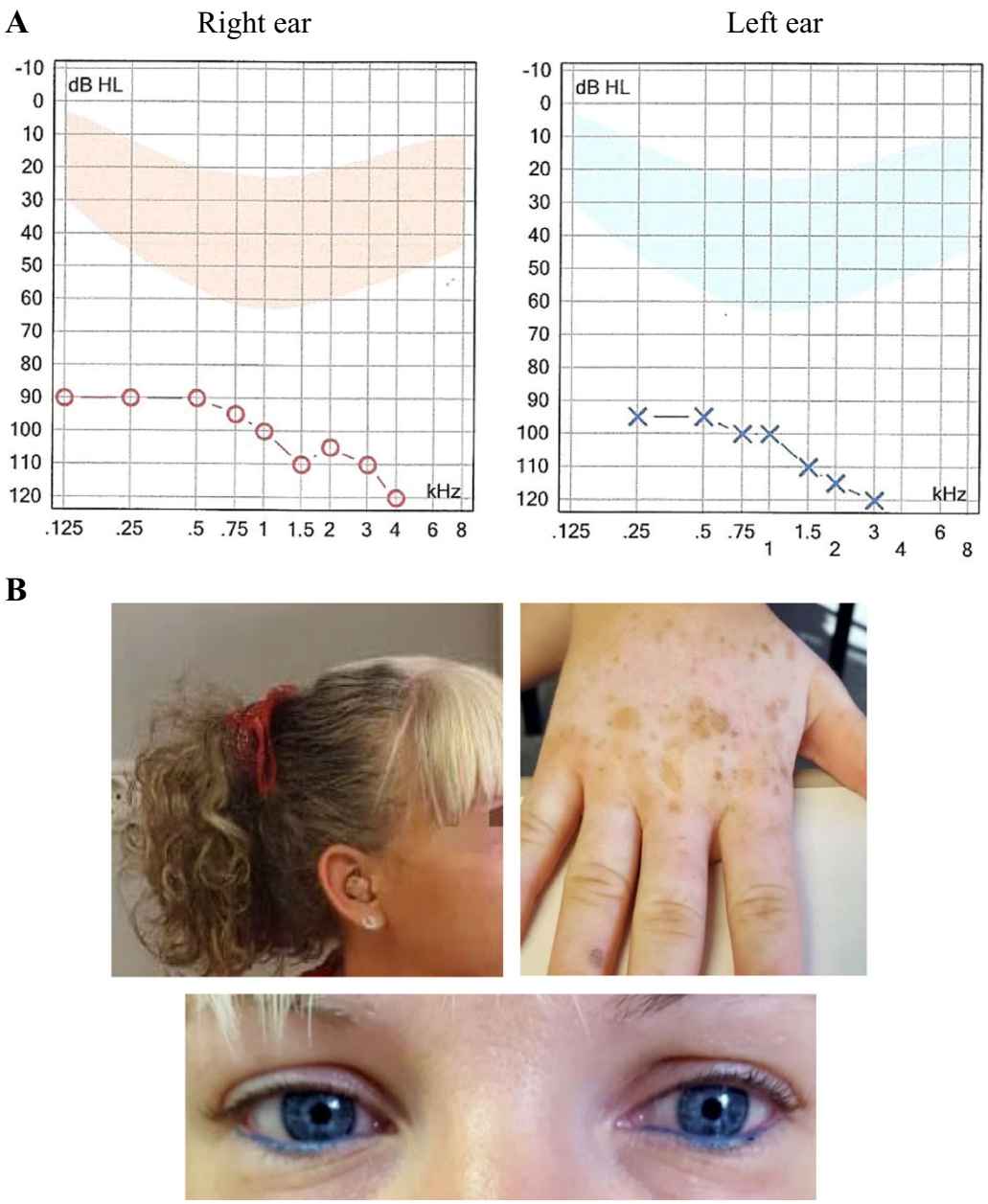

Fig. 1 **A** Phenotypic characteristics in patient 1 and **B** pure tone audiogram demonstrating bilateral hearing loss

segment of the intestine (Duhamel's technique), but he retains a residual aganglionic colon segment. Later colonoscopic follow-up revealed histopathological lesions and an anastomotic ulcer consistent with moderate-severe active chronic pouchitis.

Nowadays, he experiences frequent episodes of diarrhea, rectal bleeding and dehydration, requiring multiple antibiotic regimens due to bacterial overgrowth. A neurological evaluation was normal: without executive dysfunction or clinical signs of peripheral neuropathy or central neurological involvement.

In this clinical context, a genetic study was carried out by massive sequencing of the complete human exome of the patient and his parents (ExoNIM® Trio Approach, NIMGenetics, Spain) in order to identify variants in potentially causal genes of the patient's phenotype. The c.966dupT p.(Ala323fs) variant identified in *SOX10* (transcript ID: LRG_271) in the samples from the patient and his father was catalogued as pathogenic because it met

PVS1_strong, PS2, PM2_supporting and PP4 ACMG criteria.

Discussion

Three novel *SOX10* variants were identified including c.850G>T, c.966dupT and c.395C>G (Table 1). The *SOX10* gene (SRY (sex-determining region Y)- box 10) is located on the human chromosome 22q13.1. It is composed of 4 exons, being the last three coding for a 466-aa peptide (~ 50 kDa) belonging to the *SOX* family of the transcription factors [1]. SOX10 protein possesses an N-terminal dimerization domain (DIM), a high-mobility group (HMG) domain functionating as a DNA-binding site, a SOX E-specific (or K2) domain having context-dependent transactivation activity, and the distal transactivation (TA) domain (Fig. 2). SOX10 is first expressed in the dorsal neural tube at the early stage of neural crest cell (NCC) migration, regulating the development and maintenance of neural crest derivatives including Schwann cells, melanocytes and enteric ganglion cells, and also of oligodendrocytes [4, 7, 8]. SOX10, in synergy with PAX3, acts as a transcription factor of the *MITF* gene, playing a key role in the development of melanocytes [9]. Moreover, the hearing loss in WS may relate to abnormal proliferation, survival, differentiation or migration of NCC-derived melanocytes (a type of intermedia cells of stria vascularis) [10]. Lastly, SOX10 may play roles in activating several previously undescribed genes, including genes involved in integrin and focal adhesion kinase signaling [11]. All this may explain the wide range of phenotypic abnormalities caused by reduced *SOX10* expression.

To date, more than 150 *SOX10* pathogenic mutations related to WS and PCWH have been reported, according to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and the Leiden Open Variation Database (https://grenada.lumc.nl/LOVD2/WS/home). Of this, 55% are truncating mutations (*nonsense*, *frameshift*, and *splicing* defect) and null alleles (gross rearrangement or deletion), while the remainder represent *missense* variants.

In our study, we identified three novel variants spread throughout the entire coding sequence of *SOX10* in three patients with WS4 (patient 3)/PCWH (patients 1 and 2). The pathogenicity of the variants was supported by in silico predictors, sequence conservation analysis and cosegregation studies of the variants within families. Two of the identified variants were in exon 4 (c.850G>T and c.966dupT), while the other was found in exon 2 (c.395C>G). Previous studies have suggested that more severe phenotypes, including PCWH, were associated with truncating mutations in the last coding exon (exon 4), because the resulting mRNAs escaped NMD, leading to production of the mutant protein and triggering of a potent dominant-negative process. In contrast, truncating variants with mutations in the first exons (exons 2 and 3) were considered to activate the NMD machinery, resulting in less-severe phenotypes. The fact that *missense* mutations are not subject to NMD are use in favor or other mechanism at the origin of the phenotype variability. Earlier results suggested that the phenotypes could not actually be predicted based on the genotype alone and that additional information from in vitro studies is essential for making genotype–phenotype predictions [9, 12].

Table 1 Variants of the *SOX10* gene identified in massive sequencing studies of the human exome in peripheral blood (ExoNIM®, NIMGenetics, Spain) of the three patients

Patient	Gene	Variant nomenclature	Exon	Zygosity	Effect	Variant classification (ACMG criteria)	Inheritance	Patient's phenotype
1	*SOX10*	c.395C>G p.(Ala132Gly)	2	Het	*missense*	PV (PS2, PM1, PM2_supporting, PP2, PP3, PP4)	AD	Bright blue eyes (partial heterochromia iridis), hypopigmentation of hair and skin, pes cavus and hearing loss. Megacolon and gastroesophageal reflux. Peripheral neuropathy, language retardation, asymmetric pain and neuromas
2	*SOX10*	c.850G>T p.(Glu284*)	4	Het	*nonsense*	LPV (PVS1_strong, PM2_supporting, PP4)	AD	Hirschprung disease and dysphagia. Abnormal reflexes, head jerks, limb tremor and rotating eye movements
3	*SOX10*	c.966dupT p.(Ala323fs*79)	4	Het	*frameshift*	PV (PVS1_strong, PS2, PM2_supporting, PP4)	AD	Hirschprung disease and hearing loss. Normal neurological evaluation

Het heterozygous, *VUS* variant of uncertain significance, *LPV* likely pathogenic variant, *PV* pathogenic variant, *AD* Autosomal dominant, *WS* Waardenburg syndrome

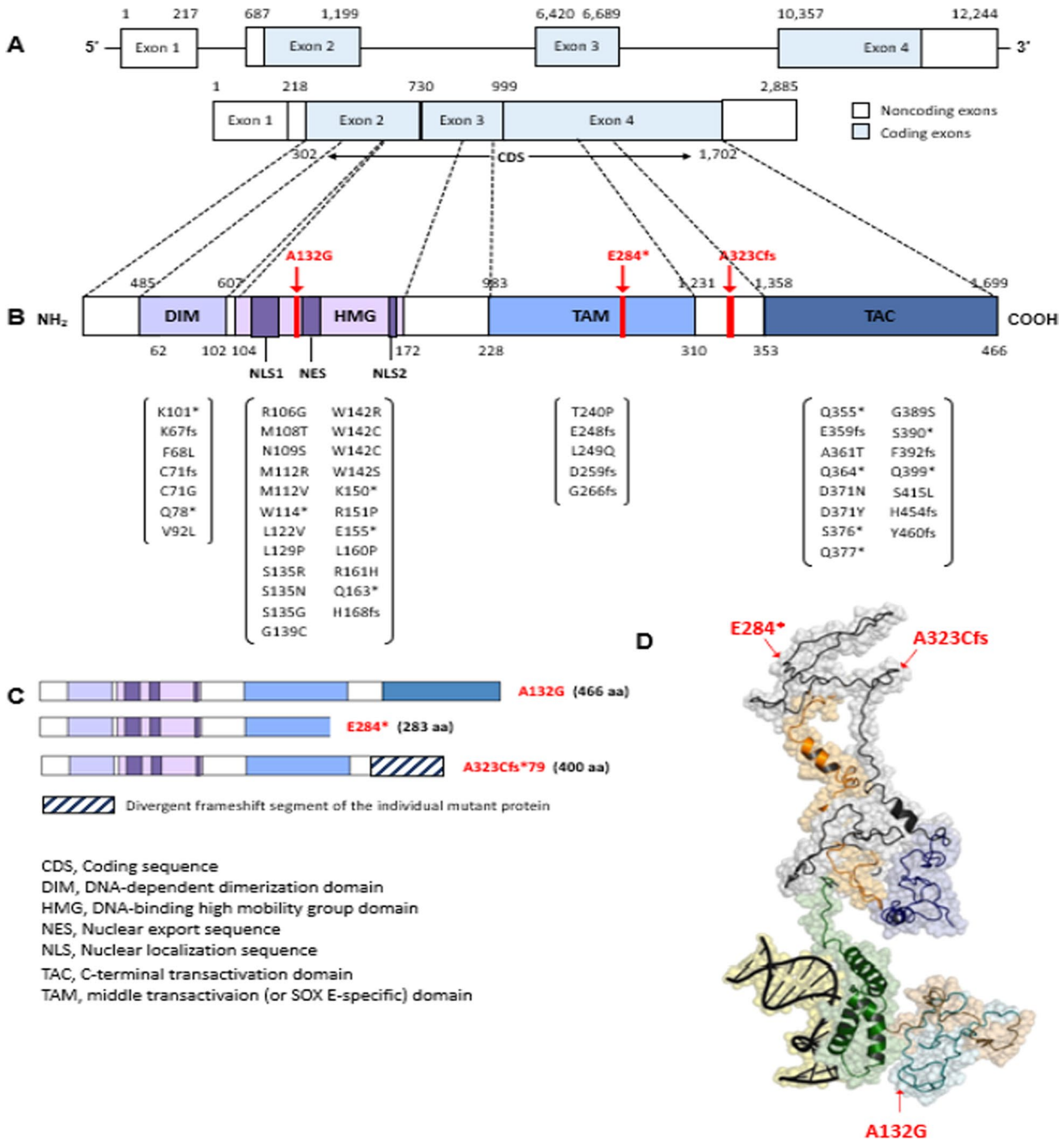

Fig. 2 Proteomic and genomic organization of SOX10 including variants found in the patients. **A** Diagram of *SOX10* gene. Nucleotide position of each exon is indicated by number above the diagram and the amino acids encoded by the respective exons are indicated by number below diagram. **B** Linear representation of matured SOX10 protein illustrating the different domains and amino acids of this molecule. Variants described in the literature are included with the p.(Ala132Gly), p.(Glu284*) and p.(Ala323fs) variants described in this article highlighted in red. **C** Linear representation of the three mutant SOX10 proteins, product of each variant described. **D** 3D molecular model of SOX10 showing in red the three variants identified in the patients studied

In our case, the c.395C>G p.(Ala132Gly) variant identified de novo in the patient 1 sample was a single nucleotide substitution in exon 2 of the *SOX10* gene, which changed the amino acid Ala132 for a Gly. This *missense* variant, without associated population frequency, had not been previously registered in the literature or in

the consulted databases associated with a specific phenotype. However, it was located in a hotspot region in which pathogenic *missense* variants had been described in adjacent codons associated with WS and PCWH [12–14]. According to splice AI, HSF, and MaxEnt tools, the c.395C>G variant could possibly create a new donor splice site, which would lead to a frameshift in the spliced mRNA. Based on the results of several in silico prediction tools (16/21), conservation analysis, population frequency, and de novo character of the variant, it was considered a pathogenic variant.

The *SOX10* c.850G>T p.(Glu284*) variant identified as heterozygous in the patient 2 sample was a loss-of-function variant that generated a premature stop codon, resulting in an early termination of the coding protein sequence at amino acid position 283. This change could have made the mutant messenger RNA (mRNA) escape the nonsense-mediated mRNA decay (NMD) pathway, a cellular surveillance mechanism for mRNA that prevents the expression of truncated or erroneous proteins; or generated a truncated protein with a negative dominant effect. This variant had not been previously registered in the literature or in the consulted databases associated with a specific phenotype. Thus, it was considered a likely pathogenic variant according to all the consulted in silico predictors (9/9), conservation scores and population frequency.

The *SOX10* c.966dupT p.(Ala323Cysfs*79) variant identified in the samples from the patient 3 and his asymptomatic father was a duplication that caused a change in the reading pattern, generating a premature stop codon and resulting in a mutant protein consisting of a sequence of 400 amino acids, with a divergent frameshift segment. This change escaped the NMD mechanism as it was located in the last exon of the gene, although variants of loss of function in posterior positions have been previously described as pathogenic in the ClinVar database (Variation ID: 7402, 518422, 805532). Likewise, this variant had been identified in the father with an allelic frequency of 7%, arising a possible germinal mosaicism, previously described in the literature [13, 15]. Given the absence of the variant in the control population and its effect on the protein, the obtained in silico predictions and the conservation analysis, pending further studies, it was considered a likely pathogenic variant.

Conclusions

These three cases highlight the importance of detailed clinical phenotyping in cases in which HMSN are suspected, since non-neuropathic phenotypic characteristics warn of possible rare causes and should be taken into account in the performance and interpretation of genetic tests, especially when massive sequencing techniques are used.

Mutation detection in WS is important, as this syndrome is genetically heterogeneous. In addition, *SOX10* mutations give a recurrence risk of 50% for offspring. As information on the functional characterization of *SOX10* variants is limited, and phenotypes cannot be predicted accurately based on the genotype alone, further investigation is needed. In vitro studies of each variant are necessary to provide a better understanding of the pathogenic mechanisms and better phenotype predictions, while in vivo analysis would further improve our understanding of the phenotypic effects of *SOX10* variants. An exact description of the mutations responsible for WS provides useful information to explain clinical features of WS and contributes to better genetic counselling of WS patients.

Abbreviations

ACMG: American College of Medical Genetics and Genomics; BAEP: Brainstem auditory evoked potential; DIM: *N*-Terminal dimerization domain; HD: Hirschprung disease; HMG: High-mobility group domain; HMSN: Hereditary motor sensory neuropathy; *MITF*: Melanocyte inducing transcription factor; MLPA: Multiplex Ligation-dependent Probe Amplification; NCC: Neural crest cell; NCV: Nerve conduction velocity; NMD: Nonsense-mediated mRNA decay; PCWH: Peripheral demyelinating neuropathy-central dysmyelinating leukodystrophy Waardenburg syndrome-Hirschsprung disease; SOX10: Sex-determining region Y-box 10; WS: Waardenburg syndrome.

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Authors' contributions

PSB, NGR and SIA drafted and critically reviewed the manuscript with literature research. JG and IRA were the principal physicians in patient's cases. SA contributed to data collection and literature search. JG helped to draft the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Written informed consent was obtained from the patient for publication of case report and any accompanying images (patient 1). A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests

The authors declare that they have no competing interests.

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