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Phenotypic continuum between Waardenburg syndrome and Idiopathic Hypogonadotropic Hypogonadism in humans with *SOX10* mutations

Rebecca A. Rojas, BS[1], **Anna A. Kutateladze, MS**[1], **Lacey Plummer, MS**[1], **Maria Stamou, MD, PhD**[1], **David L. Keefe Jr, BA**[1], **Kathyrn B. Salnikov, BS**[1], **Angela Delaney, MD**[2], **Janet E. Hall, MD**[3], **Ruslan Sadreyev, PhD**[4], **Fei Ji, PhD**[4], **Eric Fliers, MD**[5], **Katarina Gambosova, MD**[6], **Richard Quinton, MD FRCP (Edin)**[7], **Paulina M Merino, MD**[8], **Veronica Mericq, MD**[8], **Stephanie B Seminara, MD**[1], **William F. Crowley Jr, MD**[1], **Ravikumar Balasubramanian, MBBS, PhD, MRCP(UK)**[1]

[1]Harvard Reproductive Sciences Center, The Reproductive Endocrine Unit and The Endocrine Unit of the Department of Medicine, Massachusetts General Hospital, Boston, MA 02114, USA [2]Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland [3]National Institute of Environmental Health Sciences, Research Triangle, NC, USA [4]Department of Molecular Biology, Massachusetts General Hospital, Boston, United States [5]Amsterdam University Medical Center, location AMC, Dept. of Endocrinology and Metabolism 1105 AZ Amsterdam, The Netherlands [6]Stormont-Vail Health, Cotton O'Neil Diabetes and Endocrinology, Topeka, KS, USA [7]Translational and Clinical Research institute, University of Newcastle-upon-Tyne, UK [8]Institute of Maternal and Child Research, University of Chile, Santiago, Chile

Abstract

Purpose: *SOX10* mutations previously implicated in Waardenburg syndrome (WS), have now been linked to Kallmann Syndrome [KS], the anosmic form of idiopathic hypogonadotropic hypogonadism (IHH). We investigated whether *SOX10*-associated WS and IHH represent

Address for correspondence: Ravikumar Balasubramanian, M.D., Ph.D., M.R.C.P. (UK), Harvard Reproductive Endocrine Sciences Center, Massachusetts General Hospital, Bartlett Hall Extension, 5th Floor, 55 Fruit Street, Boston, Massachusetts 02114., rbalasubramanian@mgh.harvard.edu.

Author Contributions

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Ethics Declaration

All subjects provided written informed consent and research activities were approved by the Human Research Committee at the MGH, Boston, MA.

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Data Availability

Data and materials will be made available by the authors individually upon request subject to the data sharing plan and consent provided by the study participants.

elements of a phenotypic continuum within a unifying disorder or if they represent phenotypically distinct allelic disorders.

Methods: Exome sequencing from 1309 IHH subjects (KS: 632; normosmic idiopathic hypogonadotropic hypogonadism [nIIHH:677) were reviewed for *SOX10* rare sequence variants (RSVs). The genotypic and phenotypic spectrum of *SOX10*-related IHH (this study & literature) and *SOX10*-related WS cases (literature) were reviewed and compared with *SOX10*-RSV spectrum in gnomAD population.

Results: Thirty-seven *SOX10*-associated IHH cases were identified: Current study:16 KS; 4 nIHH; literature:16 KS; 1 nIHH. Twenty-three IHH cases (62%; all KS), had ≥1 known WS-associated feature(s). Moreover, five previously reported *SOX10*-associated WS cases showed IHH-related features. Four *SOX10* missense RSVs showed allelic overlap between IHH-ascertained and WS-ascertained cases. The SOX10-HMG domain showed an enrichment of RSVs in disease-states *vs.* gnomAD.

Conclusions: *SOX10* mutations contribute to both anosmic (KS) and normosmic (nIHH) forms of IHH. IHH and WS represent *SOX10*-associated developmental defects that lie along a unifying phenotypic continuum. The *SOX10*-HMG domain is critical for the pathogenesis of SOX10-related human disorders.

INTRODUCTION

Allelic heterogeneity refers to instances when several causal alleles within one gene results in a single unifying disorder with shared mutational mechanisms and with phenotypes that lie across a continuum [1]. Alternatively, in some instances, distinct alleles within a single gene can result in phenotypically non-overlapping allelic disorders secondary to differential mutational mechanisms (e.g. loss-of-function vs. gain-of-function) [2]. Human mutations in the *SOX10* gene represents an excellent example to study such genetic heterogeneity and phenotypic pleiotropy. The *SOX10* gene encodes for the SOX10 (SRY [sex determining region Y]-box 10) protein, a transcription factor regulating neural crest development [3]. To-date, two separate syndromic phenotypes have been linked to *SOX10* mutations in humans: Waardenburg syndrome (WS [type 2 and 4]) [3], an auditory-pigmentary disorder; and Kallmann Syndrome (KS) [4], a reproductive disorder. These observations raise the question whether WS and KS are examples of a phenotypic continuum or if they are two non-overlapping allelic disorders.

KS is a rare genetic disorder defined clinically by a syndromic association of idiopathic hypogonadotropic hypogonadism (IHH) and olfactory dysfunction, i.e. hyposmia/anosmia [5]. The candidacy of *SOX10* as a KS gene emerged from the overlapping observation that agenesis of the olfactory bulb (a marker of anosmia) was seen in some WS individuals [6]. Hence, Pingault *et al* examined 17 KS subjects exhibiting one or more WS-features (primarily hearing loss) for *SOX10* mutations and identified 5 KS subjects (~30%), all with hearing loss, harboring pathogenic *SOX10* mutations [4]. Additional reports have subsequently implicated *SOX10* variants in KS subjects with hearing loss [7–9]. The co-segregation of KS and hearing loss (a WS-feature) supports the hypothesis that *SOX10*-related WS and KS may actually represent phenotypically overlapping disorders. However,

Pingault *et al* also identified one KS individual with a *SOX10* variant without overt WS features hinting that disease-specific alleles suggestive of an allelic disorder may also exist [4]. Hence, a systematic comparative genotypic and phenotypic analysis of a large cohort of *SOX10*-related IHH patients is required to fully validate either hypothesis (phenotypic continuum vs. distinct allelic disorders). Finally, some KS genes have also been known to contribute to normosmic forms of IHH [nIHH] [5]; however apart from a single nIHH reported to harbor a *SOX10* RSV [10], the contribution of *SOX10* mutations to nIHH is not known.

To address these questions, a large IHH cohort (with both KS & nIHH) was examined for *SOX10* rare sequence variants (RSVs) and their associated phenotypes defined. Additionally, previously reported *SOX10* RSVs identified in KS & WS cases from the published literature were catalogued and their genotypic and phenotypic spectra compared. All disease-associated *SOX10* RSVs were also compared against the Genome Aggregation Database (gnomAD) [11], a publicly available dataset to identify critical domains of the SOX10 protein relating to human disease.

METHODS:

Study Subjects

Study cohorts:

(i) MGH study cohort: A total of 632 KS (IHH + anosmia/hyposmia) subjects from the Harvard Reproductive Endocrine Sciences Center's IHH cohort were included in this study. We also included 677 nIHH subjects to determine any causal/contributory role for *SOX10* pathogenic variants in nIHH. Diagnostic criteria for IHH were applied as previously reported [5] and are described in Supplementary methods.

(ii) Previously published SOX10-associated WS and IHH patients: We catalogued the genotypes and phenotypes of *SOX10* RSVs previously reported in IHH and WS in the literature and in the Leiden Open Variation Database (LOVD) [12], a public repository of pathogenic *SOX10* variants. This search yielded additional 101 WS probands with *SOX10* RSVs and 17 IHH probands with *SOX10* RSVs (Table S1A & S1B).

Phenotypic evaluation for MGH IHH cohort: Clinical charts were reviewed for phenotypic evaluation. In IHH patients with *SOX10* RSVs, "Waardenburg syndrome-like features" (WS-like features) were deemed to be present if they exhibited at least one of the following WS-associated features: sensorineural hearing loss, atypical pigmentation, Hirschsprung's disease and/or features of PCWH (**P**eripheral demyelinating neuropathy, **C**entral demyelinating leukodystrophy, **W**aardenburg syndrome, and **H**irschsprung disease syndrome) [13].

Phenotypic evaluation for WS/IHH cases reported in the literature: All text/tables of IHH subjects with *SOX10* RSVs that were previously reported (LOVD and published literature) were reviewed for WS-like features (as described above) (Table S1A and S1B; Supplemental References). For previously reported WS patients with *SOX10* RSVs, reproductive phenotypes suggestive of IHH was deemed present if they exhibited at

least one of the following IHH-related phenotypes: micropenis or cryptorchidism (boys), absent puberty in both sexes, primary amenorrhea (girls), and/or a biochemical observation of hypogonadotropic hypogonadism.

Genetic analysis

Whole Exome Sequencing (WES) methodology and variant annotation for the MGH IHH cohort have been described previously [14]. WES data was queried for *SOX10* (RefSeq: NM_006941.3) rare sequence variants [RSVs, defined as: variants with <0.1% minor allele frequency (MAF) in gnomAD database] and for RSVs in other IHH genes (Supplemental Methods). All RSVs were confirmed by Sanger sequencing and pedigree segregation analysis was performed for determining mode of inheritance whenever possible. We also catalogued all *SOX10* RSVs with <0.1% minor allele frequency (MAF) in the gnomAD database (Table S1C).

Functional annotation of *SOX10* Rare Sequence Variants

Functional effects of *SOX10* RSVs identified in the MGH cohort was examined using the following *in silico* programs: PolyPhen-2 [15], Sorting Intolerant from Tolerant [SIFT] [16], Combined Annotation Dependent Depletion [CADD] [17], and Rare Exome Variant Ensemble Learner [REVEL] [18]. All *SOX10* RSVs were also categorized using the American College of Medical Genetics (ACMG) Standard and Guidelines for interpretation of sequence variants [19]. The amino acid sequence of the SOX10 high mobility group (HMG) protein domain has a very strong similarity (95% amino acid identity) to the sequence of the *SOX9* HMG domain, which has a solved 3D structure (PDB ID 4s2q) [20]. Based on the pairwise sequence alignment of these two close homologs, the *SOX10* variants were mapped on the structure of the *SOX9* HMG domain.

Statistical analysis: Gene-burden testing, including domain-based testing was performed using Fischer's exact test. Only filtered variant allele counts from WES data were considered for burden testing. Relative proportions of mutation classes (loss-of-function [LOF] and missense variants) between IHH, WS and gnomAD variants were compared using Chi-square test with Yates correction. P value of <0.05 was considered as significant.

RESULTS

SOX10 RSVs are associated with both KS and nIHH forms of idiopathic hypogonadotropic hypogonadism

In the MGH IHH cohort, we identified 18 heterozygous *SOX10* RSVs: 5 loss-of-function (LoF) variants and 13 missense variants, in 20 unrelated (13M:7F) subjects (Figure 1, Tables 1, 2, and S2). Of these 20 subjects, 16 IHH subjects manifested KS, while 4 subjects were normosmic (Figure 1, Table S2). RSVs that were deemed likely pathogenic/pathogenic by ACMG criteria were inferred as pathogenic. RSVs that were deemed likely benign/variant of unknown significance by ACMG criteria were inferred to be pathogenic if they were assessed as deleterious in any one of the four *in silico* prediction programs (Table S2). Of the 18 RSVs only 1 variant (p.Ala28Ser) was deemed benign by the above criteria. Segregation analysis in informative pedigrees for the *SOX10* RSVs was consistent with an

autosomal dominant mode of inheritance with significant variable penetrance and/or expressivity (Figure 1). In keeping with the known oligogenic basis of IHH pathogenesis [21], 6/20 *SOX10*+ probands also harbored additional RSVs in known IHH genes (Table 1, Figure 1). Review of the literature revealed 17 additional IHH subjects (16 KS; 1 nIHH) with published *SOX10* RSVs (Figure 2; Tables 1, 2, S1B & S2). *In lieu* of cellular studies, since there was a congregation of IHH-associated variants within the SOX10 HMG domain (see below), the functional impact of these variants was examined using SOX9-based homology modeling analysis. The majority of the IHH-associated *SOX10* HMG domain mutated residues were predicted to be directly involved in DNA binding and/or helix formation (Supplemental Figure 1). Overall, these results confirm the previously reported causal association of *SOX10* RSVs in KS and, in addition, demonstrate a previously underappreciated role for *SOX10* variants in nIHH.

Phenotypic analyses show that *SOX10*-related IHH and WS represent phenotypic constituents of a unifying SOX10-related human syndrome

To determine if *SOX10*-related IHH and WS represented a phenotypic continuum vs. distinct allelic disorders, we performed *two* complementary phenotypic analyses based on case ascertainment:

(a) Phenotypic analysis of *SOX10* cases ascertained by the IHH phenotype: We first reviewed the phenotypic spectrum of individuals harboring *SOX10* RSVs in the MGH cohort. In the MGH cohort, 10/20 IHH subjects (all with the KS form of IHH) exhibited ≥1 WS-associated feature(s) (Table 1). Next, we reviewed the 17 previously reported IHH patients with *SOX10* RSVs in the literature and found that 13/17 KS subjects also exhibited ≥1 WS-associated feature(s) (Table 1). Thus, in a significant majority of *SOX10*-IHH cases, WS-like features were present, suggesting that IHH represents a constituent phenotype that becomes evident through the natural history of an underlying unifying clinical syndrome. However, in a small number of *SOX10*-IHH pedigrees, WS-like phenotypes were not readily evident (Table 2). This observation raises three possibilities: (i) incomplete phenotypic information or cryptic phenotypes not readily evident; (ii) variable expressivity of the constituent phenotypes within a singular syndrome; or (iii) a small subset of patients may represent a distinct “allelic disorder” with no phenotypic overlap.

(b) Phenotypic analysis of previously reported *SOX10* cases ascertained by WS phenotypes: For a majority of reports, since subjects were typically pre-pubertal, reproductive phenotypes suggestive of IHH were not reported. However, we identified five individuals who had an initial diagnosis of WS, in whom at least one IHH-related phenotype was already documented (Table 3).

Taken together, the above data supports the notion that IHH and WS represent phenotypic constituents positioned along a singular *SOX10*-associated human syndromic spectrum.

Genotypic analyses show *SOX10*-related IHH and WS represent phenotypic constituents of a unifying syndrome

To further examine the phenotypic continuum *vs.* distinct allelic disorder hypothesis, we compared the mutational spectrum and allelic overlap between *SOX10* RSVs identified in subjects ascertained by an initial IHH diagnosis (MGH cohort and published literature) and those ascertained by an initial WS diagnosis (published literature). This analysis showed that the *SOX10* allelic spectrum in IHH-ascertained cases (Tables 1, 2, & Table S1B) and WS-ascertained cases (Table 3, Table S1A) included *both* loss-of-function [LOF] mutations [frameshifting indels, nonsense and splice-altering] and missense mutations that spanned the entire length of the SOX10 protein. Furthermore, we noted that four *SOX10* RSVs (p.Met112Ile; p.Trp114Arg; p.Leu145Pro) and p.Val92Leu) showed allelic overlap – i.e. identical RSVs were identified in cases ascertained by IHH or WS independently. This latter observation strongly suggests that IHH and WS represent phenotypic tenets of a single disorder. However, cumulatively, *SOX10* LOF alleles were proportionally more prevalent in WS-ascertained cases (79% LOF [n=81]; 21% missense [n=21]) while missense variants were proportionally more prevalent in IHH-ascertained cases (30% LOF [n=11]; 70% missense [n=26]) ($p<0.001$) (Figure 2). This observation suggests that, within this single disorder, the mutational severity may partly account for the variable penetrance and/or variable phenotypic expressivity of IHH- or WS- features exhibited by individual patients.

We then performed a SOX10 protein domain based mutational enrichment analysis to identify any specific domains that conferred phenotypic specificity. This approach revealed that there was a no significant difference in the proportional prevalence of RSVs in any specific SOX10 domain between IHH- and WS-ascertained cases ($p=0.07$). However, since a large proportion of *SOX10* missense RSVs congregated within the HMG domain across both IHH (n=15) and WS (n=18), we then compared the specific mutated HMG domain residues in IHH- and WS-ascertained cases. This analysis showed that the mutated HMG domain residues in IHH-ascertained cases were largely *non-overlapping* with the missense residues in WS-ascertained cases except for three overlapping residues (M112, W114 and L145) (Tables 1, 2, S1A, and S1B). However, review of phenotypes among IHH cases harboring these distinct HMG residues showed that the vast majority of these distinct HMG residue variants resulted in WS-features (Table 1). This observation strongly suggests that there may be no phenotypic specificity conferred by specific HMG domain residues and that the HMG domain is critical for pathogenesis of both phenotypes. This notion is further substantiated by the observation of a significant proportional enrichment for *SOX10* missense mutations within the HMG domain across both disease-states when compared to their distribution in the gnomAD population database (70% in disease-states vs. 6% in gnomAD; $p<0.001$) (Figure 2, Tables 1, 2, S1A and S1C). Intriguingly, a total of 6 gnomAD variants, including 2 HMG domain variants (R151 and R161) overlapped with disease-associated variants; however, no phenotypic details were available for the gnomAD subjects (Table S1D).

DISCUSSION

With the advent of next-generation sequencing, several pleiotropic phenotypes resulting from mutations in a single gene are increasingly recognized [22]. Phenotypic continuum *vs.*

distinct allelic disorders represent two dichotomous explanations for the observed pleiotropy. Among the SOX10-associated human phenotypes, WS-related features (e.g. hearing loss, Hirschsprung disease, skin pigmentation) are often recognizable early in childhood. In contrast, IHH is typically recognized later in adolescence. This study took advantage of this "temporal gap" in phenotypic manifestations (i.e. the natural history) and systematically examined SOX10-related IHH patients for the presence or absence of WS-like phenotypes that would have manifested in childhood. This approach allowed us to demonstrate that WS features co-segregated in a significant majority of cases ascertained by the IHH phenotype. These findings strongly suggest that IHH and WS represent constituent phenotypes within a single human SOX10-related continuum of developmental defects rather than representing distinct allelic disorders. These findings have important implications for clinical care of children harboring pathogenic *SOX10* alleles as longitudinal follow up for incipient IHH during adolescence is imperative to allow early recognition of pubertal phenotypes and enable timely institution of hormonal therapy to avoid physical and psychological effects of IHH.

Three key observations provide validity to our conclusion that WS and IHH relate to a phenotypic continuum:

(i) Overlapping phenotypes independent of case ascertainment:

Amongst patients with *SOX10* mutations ascertained by the IHH phenotype, a majority (62%) exhibited one or more WS features and nearly 25% of subjects harbored 2 or more WS-features (Table 1). Both in this report and the literature [4], hearing loss is almost ubiquitous in IHH individuals with pathogenic *SOX10* RSVs. In this report, WS-like features such as pigmentation abnormalities and Hirschsprung's disease also co-segregated with IHH further substantiating the IHH-WS phenotypic continuum hypothesis (Tables, 1, 2 & S1B). Similarly, in a small number of previously reported *SOX10* individuals ascertained by the WS phenotype, IHH-related phenotypes were present (Table 3). However, the prevalence of IHH features in WS-ascertained individuals is certainly an underestimate since a vast majority of these subjects were pre-pubertal children in whom reproductive phenotypes are typically quiescent. In addition, the majority of these reports were published before *SOX10* was causally linked to reproductive phenotypes and hence these phenotypes were not sought.

(ii) Shared mutational spectrum, mutational mechanisms, and overlapping alleles in *SOX10*-related IHH and WS.

We noted that the *SOX1*0 mutational spectrum of IHH and WS was comprised of both LOF and missense alleles. The LOF mutations resulting in IHH or WS typically occurred in the heterozygous state and spanned the entire length of the SOX10 protein; hence it is likely that SOX10 haploinsufficiency is a shared underlying mechanism for these LOF alleles. In terms of missense alleles, no regional enrichment within the SOX10 protein was evidently different between IHH and WS. In contrast, there was a significant enrichment for missense alleles in the SOX10 HMG domain in disease cohorts compared to gnomAD, suggesting a shared importance of this domain for both phenotypes. Furthermore, functional cellular studies of WS and IHH associated *SOX10* HMG domain missense variants have been shown

to be hypomorphic/LOF alleles [3,23–25] with no discernible dominant-negative activity [3]. Finally, in this study, three HMG-domain missense RSVs (p.Met112Ile; p.Trp114Arg; p.Leu145Pro) and one non-HMG domain missense variant (p.Val92Leu) showed allelic overlap independent of the phenotypic ascertainment (IHH or WS). This finding adds further certitude to the shared mutational mechanisms – phenotypic continuum hypothesis.

(iii) Shared developmental biology of *SOX*10-related IHH and WS:

The key clinical features of *SOX10*-related WS have been shown to result from distinct defects in neural crest derived cells such as melanocytes (skin pigmentation, deafness) and enteric nervous system (Hirschsprung's disease) [3]. Studies in *Sox10*-mutant mice confirm a critical role for SOX10 protein in the development of a distinct subset of neural crest derived glial cells called 'olfactory ensheathing cells' (OEC) and when OECs are absent, migration of GnRH cells is imparied [4,26]. Furthermore, emerging data using lineage tracing approaches in both zebrafish [27] and mice [28] support a dual origin of GnRH neuroendocrine cells, one cohort arising from olfactory placodal ectoderm and the other from neural crest progenitors. Thus, association of human *SOX10* variants with IHH and their phenotypic overlap with WS provide human validation to the neural crest origins for GnRH cells and confirm that *SOX10*-related IHH also represents a neurocristopathy [29].

The next question relates to the precise timing of targeted reproductive phenotyping in *SOX10*+ patients. Although puberty is initiated at adolescence, the reproductive axis is known to be active in the first few months of life in both sexes, a developmental window termed as the "minipuberty of infancy" [30]. While the relevance of minipuberty in girls is poorly understood, hypogonadotropism during this period in boys can result in micropenis and/or cryptorchidism (undescended testes). Timely laboratory assessment of gonadotropin levels during this developmental window can permit a definitive diagnosis of congenital hypogonadotropic hypogonadism [30] allowing early treatment in boys (*e.g.* testosterone/hCG therapy to promote penile growth; hCG and FSH therapy to promote sertoli cell proliferation and thereby improve future fertility potential [31]). Similarly, during adolescence, early identification of hypogonadotropism in both boys and girls who are *SOX10*+ will allow initiation of treatment to induce more timely development of secondary sexual characteristics, optimize bone maturation to allow height growth, skeletal maturation and prevention of psychological scars from delayed puberty. A longitudinal report of a female patient with WS2 case nicely illustrates the importance of longitudinal follow up in *SOX10*+ children [32]. A female infant was initially diagnosed *SOX10*-related WS when she presented with iris hypopigmentation, bilateral sensorineural deafness and a white forelock due to causal *SOX10* LOF mutation (SOX10 p.P169fsX117) [33]. Subsequently, during longitudinal follow up, she displayed delayed puberty at adolescence and endocrine evaluation revealed findings consistent with IHH necessitating hormonal therapy [32]. This exemplary case report fully substantiates this study's findings and argue for careful follow up of *SOX10*+ patients in order to discern the full phenotypic spectrum including incipient reproductive phenotypes. This is particularly important since *SOX10*+ patients may be encountered in multiple medical specialist domains (audiology, colorectal surgery, dermatology, endocrinology) and these providers must be cognizant of these co-segregating phenotypes in order to avoid a diagnostic odyssey and delayed recognition and treatment of other related phenotypes.

Despite the strengths of our observations presented herein suggesting that WS and IHH lie along a SOX10-related continuum of developmental phenotypes, there are some notable exceptions. First, a very rare, but severe *SOX10*-related neurological phenotype (PCWH) resulting from distinct *SOX10* RSVs affecting the last coding exon has been described. These variants have been shown to result in a stable *SOX10* mutant mRNA that escapes nonsense mediated decay with resultant mutant protein that possesses a potent dominant-negative activity [25]. In another PCWH patient, disruption of the *SOX10* native stop codon extended the SOX10 protein into the 3' untranslated region to create a SOX10 mutant fusion protein that was shown to exert a toxic gain-of-function activity [24]. Similarly, Chaoui *et al* have also demonstrated that specific *SOX10* missense pathogenic variants may also exert a dominant-negative activity leading to a PCWH phenotype. Second, a biallelic *SOX10* deletion [34] resulting in a severe arthrogryposis syndrome in a stillborn infant at 32 weeks has also been described. In contrast, IHH- and WS- related *SOX10* pathogenic LOF variants occur in the heterozygous state, spare the last coding exon, and do not exhibit dominant-negative or gain-of-function activity. Thus, PCWH phenotype resulting from dominant-negative or gain-of-function mechanisms and the arthrogryposis syndrome resulting from biallelic *SOX10* deletions may represent distinct *SOX10*-related allelic disorders that have differential underlying mechanisms. Finally, some IHH subjects in this study did not manifest WS-like features raising the possibility that this subset of patients may represent an allelic disorder. However, the mutational types and locations were similar to those IHH subjects who exhibited WS-like features; and hence it is more likely that these cases represent examples of variable expressivity within a single disorder.

In this study, *in lieu* of cellular studies for determining pathogenicity, we utilized a combination of *in silico* prediction algorithms, variant classification using the ACMG criteria and applied human population genetic data to help determine RSV pathogenicity. This composite analysis showed that almost all HMG domain missense variants in our cohort and the literature were likely to be pathogenic (Tables 1 & 2). Structural modeling analysis of the mutated HMG domain residues showed that almost all were predicted to disrupt DNA binding (Supplemental Figure 1). Furthermore, previous functional cellular studies on HMG domain *SOX10* missense variants have consistently shown that majority of these variants are deleterious [3]. However, individual variants within the HMG domain may show differential gradients of transcriptional activity, ranging from null alleles to mild hypomorphic alleles, thus resulting in variable phenotypic expressivity. Domain-specific burden analyses showed significant enrichment for disease-associated missense variants within the HMG domain (Figure 2) compared to gnomAD but no enrichment for other domains of the SOX10 protein. *In silico* analyses and ACMG variant classification of non-HMG variants in this study also showed variability in their predicted pathogenicity (Table S2). Furthermore, prior cellular studies have shown that non-HMG domain variants displayed preserved DNA binding and normal sub-cellular localization [3,8]. These findings suggest that *SOX10* variants affecting the non-HMG domains may not sufficient to be causal alleles on their own. Putative pathogenic variants in other IHH genes were seen in a subset of patients with *SOX10* variants (Table S3) suggesting potential oligogenic inheritance mechanisms. Furthermore, murine studies have previously suggested genetic modifiers of SOX10-related neurocristopathies[35] and similar mechanisms may also underlie human

SOX10-related disorders. However, detailed phenomic, genomic and transcriptomic studies in a large cohort of *SOX10*-variant positive families will be required to uncover such modifiers.

In this study, surprisingly, in addition to KS, we identified 4 nIHH subjects harboring putatively pathogenic *SOX10* RSVs: one LOF allele and 3 missense alleles, of which one was within the HMG domain (Table 2). Our results suggest that *SOX10* variants appear to contribute to both KS and nIHH forms. The precise neurodevelopmental basis for the preservation of normal olfactory phenotype in these *SOX10+* nIHH individuals remains unclear. Intriguingly, none of the nIHH subjects affected showed clear WS-like features (Table 2). This observation suggests that anosmia may be a key pathophysiologic link between WS and IHH and further studies will be required to corroborate this assertion.

This study has some limitations. First, for *SOX10*-WS variants, we relied on the previously reported limited phenotypic information and IHH-related phenotypes were only identifiable in 5 individuals. However, since most of the WS-cases in the literature are pre-pubertal and have not been updated with longitudinal follow up through puberty, it is likely that this is a gross underestimate. Hence, future studies in older subjects with *SOX10* variants will be needed to fully establish the prevalence of IHH in SOX10-related human disorders. Second, our genetic analysis focused primarily on single nucleotide variants (SNV)s copy number changes (CNVs) were not examined. Hence, the differential contribution of *SOX10* CNVs to SOX10-related phenotypic pleiotropy relating to IHH/WS remains to be studied.

In conclusion, this study demonstrates that IHH and WS represent *SOX10*-associated developmental defects that lie along a phenotypic continuum. With the increasing use of genotype-first approaches to study rare genetic diseases, this study provides a framework for studying pleiotropic single gene disorders and highlights the critical importance of using large well-phenotyped cohorts with natural history information to help (re)define Mendelian disease nosologies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Hormozdiari F, Zhu A, Kichaev G, et al. Widespread Allelic Heterogeneity in Complex Traits. Am J Hum Genet. 2017;100(5):789–802. [PubMed: 28475861]
2. Walsh CA, Engle EC. Allelic diversity in human developmental neurogenetics: insights into biology and disease. Neuron. 2010;68(2):245–253. [PubMed: 20955932]
3. Chaoui A, Watanabe Y, Touraine R, et al. Identification and functional analysis of SOX10 missense mutations in different subtypes of Waardenburg syndrome. Hum Mutat. 2011;32(12):1436–1449. [PubMed: 21898658]

4. Pingault V, Bodereau V, Baral V, et al. Loss-of-function mutations in SOX10 cause Kallmann syndrome with deafness. Am J Hum Genet. 2013;92(5):707–724. [PubMed: 23643381]
5. Balasubramanian R, Crowley WF, Jr. Isolated GnRH deficiency: a disease model serving as a unique prism into the systems biology of the GnRH neuronal network. Mol Cell Endocrinol. 2011;346(1–2):4–12 (Review). [PubMed: 21782888]
6. Elmaleh-Berges M, Baumann C, Noel-Petroff N, et al. Spectrum of temporal bone abnormalities in patients with Waardenburg syndrome and SOX10 mutations. AJNR Am J Neuroradiol. 2013;34(6):1257–1263. [PubMed: 23237859]
7. Suzuki E, Izumi Y, Chiba Y, et al. Loss-of-Function SOX10 Mutation in a Patient with Kallmann Syndrome, Hearing Loss, and Iris Hypopigmentation. Horm Res Paediatr. 2015;84(3):212–216. [PubMed: 26228106]
8. Dai W, Wu J, Zhao Y, et al. Functional analysis of SOX10 mutations identified in Chinese patients with Kallmann syndrome. Gene. 2019;702:99–106. [PubMed: 30914325]
9. Vaaralahti K, Tommiska J, Tillmann V, et al. De novo SOX10 nonsense mutation in a patient with Kallmann syndrome and hearing loss. Pediatr Res. 2014;76(1):115–116. [PubMed: 24769923]
10. Amato LGL, Montenegro LR, Lerario AM, et al. New genetic findings in a large cohort of congenital hypogonadotropic hypogonadism. Eur J Endocrinol. 2019;181(2):103–119. [PubMed: 31200363]
11. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020;581(7809):434–443. [PubMed: 32461654]
12. Fokkema IF, Taschner PE, Schaafsma GC, Celli J, Laros JF, den Dunnen JT. LOVD v.2.0: the next generation in gene variant databases. Hum Mutat. 2011;32(5):557–563. [PubMed: 21520333]
13. Touraine RL, Attie-Bitach T, Manceau E, et al. Neurological phenotype in Waardenburg syndrome type 4 correlates with novel SOX10 truncating mutations and expression in developing brain. Am J Hum Genet. 2000;66(5):1496–1503. [PubMed: 10762540]
14. Guo MH, Plummer L, Chan YM, Hirschhorn JN, Lippincott MF. Burden Testing of Rare Variants Identified through Exome Sequencing via Publicly Available Control Data. Am J Hum Genet. 2018;103(4):522–534. [PubMed: 30269813]
15. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet. 2013;Chapter 7:Unit7 20.
16. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009;4(7):1073–1081. [PubMed: 19561590]
17. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res. 2019;47(D1):D886–D894. [PubMed: 30371827]
18. Ioannidis NM, Rothstein JH, Pejaver V, et al. REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. Am J Hum Genet. 2016;99(4):877–885. [PubMed: 27666373]
19. Biesecker LG, Harrison SM, ClinGen Sequence Variant Interpretation Working G. The ACMG/AMP reputable source criteria for the interpretation of sequence variants. Genet Med. 2018;20(12):1687–1688. [PubMed: 29543229]
20. Vivekanandan S, Moovarkumudalvan B, Lescar J, Kolatkar PR. Crystallization and X-ray diffraction analysis of the HMG domain of the chondrogenesis master regulator Sox9 in complex with a ChIP-Seq-identified DNA element. Acta Crystallogr F Struct Biol Commun. 2015;71(Pt 11):1437–1441. [PubMed: 26527273]
21. Sykiotis GP, Plummer L, Hughes VA, et al. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(34):15140–15144 (Research Support, N.I.H., Extramural). [PubMed: 20696889]
22. Chong JX, Buckingham KJ, Jhangiani SN, et al. The Genetic Basis of Mendelian Phenotypes: Discoveries, Challenges, and Opportunities. Am J Hum Genet. 2015;97(2):199–215. [PubMed: 26166479]

23. Chaoui A, Kavo A, Baral V, et al. Subnuclear re-localization of SOX10 and p54NRB correlates with a unique neurological phenotype associated with SOX10 missense mutations. Hum Mol Genet. 2015;24(17):4933–4947. [PubMed: 26060192]
24. Inoue K, Ohyama T, Sakuragi Y, et al. Translation of SOX10 3' untranslated region causes a complex severe neurocristopathy by generation of a deleterious functional domain. Hum Mol Genet. 2007;16(24):3037–3046. [PubMed: 17855451]
25. Inoue K, Khajavi M, Ohyama T, et al. Molecular mechanism for distinct neurological phenotypes conveyed by allelic truncating mutations. Nat Genet. 2004;36(4):361–369. [PubMed: 15004559]
26. Barraud P, St John JA, Stolt CC, Wegner M, Baker CV. Olfactory ensheathing glia are required for embryonic olfactory axon targeting and the migration of gonadotropin-releasing hormone neurons. Biol Open. 2013;2(7):750–759. [PubMed: 23862023]
27. Whitlock KE, Wolf CD, Boyce ML. Gonadotropin-releasing hormone (GnRH) cells arise from cranial neural crest and adenohypophyseal regions of the neural plate in the zebrafish, Danio rerio. Dev Biol. 2003;257(1):140–152. [PubMed: 12710963]
28. Forni PE, Taylor-Burds C, Melvin VS, Williams T, Wray S. Neural crest and ectodermal cells intermix in the nasal placode to give rise to GnRH-1 neurons, sensory neurons, and olfactory ensheathing cells. J Neurosci. 2011;31(18):6915–6927. [PubMed: 21543621]
29. Noisa P, Raivio T. Neural crest cells: from developmental biology to clinical interventions. Birth Defects Res C Embryo Today. 2014;102(3):263–274. [PubMed: 25226872]
30. Grumbach MM. A window of opportunity: the diagnosis of gonadotropin deficiency in the male infant. The Journal of clinical endocrinology and metabolism. 2005;90(5):3122–3127 (Review). [PubMed: 15728198]
31. Kohva E, Huopio H, Hietamaki J, Hero M, Miettinen PJ, Raivio T. Treatment of gonadotropin deficiency during the first year of life: long-term observation and outcome in five boys. Hum Reprod. 2019;34(5):863–871. [PubMed: 31067328]
32. Izumi Y, Musha I, Suzuki E, et al. Hypogonadotropic hypogonadism in a female patient previously diagnosed as having waardenburg syndrome due to a sox10 mutation. Endocrine. 2015;49(2):553–556. [PubMed: 25273316]
33. Iso M, Fukami M, Horikawa R, Azuma N, Kawashiro N, Ogata T. SOX10 mutation in Waardenburg syndrome type II. Am J Med Genet A. 2008;146A(16):2162–2163. [PubMed: 18627047]
34. Stevenson RE, Vincent V, Spellicy CJ, Friez MJ, Chaubey A. Biallelic deletions of the Waardenburg II syndrome gene, SOX10, cause a recognizable arthrogryposis syndrome. Am J Med Genet A. 2018;176(9):1968–1971. [PubMed: 30113773]
35. Matera I, Watkins-Chow DE, Loftus SK et al. A sensitized mutagenesis screen identifies Gli3 as a modifier of Sox10 neurocristopathy. Hum Mol Genet. 2008; 17(14): 2118–2131. [PubMed: 18397875]
36. Maione L, Brailly-Tabard S, Nevoux J, Bouligand J, Young J. Reversal of congenital hypogonadotropic hypogonadism in a man with Kallmann syndrome due to SOX10 mutation. Clin Endocrinol (Oxf). 2016;85(6):988–989. [PubMed: 27616149]
37. Shin SJ, Sul Y, Kim JH, et al. Clinical, endocrinological, and molecular characterization of Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism: a single center experience. Ann Pediatr Endocrinol Metab. 2015;20(1):27–33. [PubMed: 25883924]
38. Siomou E, Manolakos E, Petersen M, et al. A 725 kb deletion at 22q13.1 chromosomal region including SOX10 gene in a boy with a neurologic variant of Waardenburg syndrome type 2. Eur J Med Genet. 2012;55(11):641–645. [PubMed: 22842075]
39. Bondurand N, Dastot-Le Moal F, Stanchina L, et al. Deletions at the SOX10 gene locus cause Waardenburg syndrome types 2 and 4. Am J Hum Genet. 2007;81(6):1169–1185. [PubMed: 17999358]
40. Korsch E, Steinkuhle J, Massin M, Lyonnet S, Touraine RL. Impaired autonomic control of the heart by SOX10 mutation. Eur J Pediatr. 2001;160(1):68–69. [PubMed: 11195028]

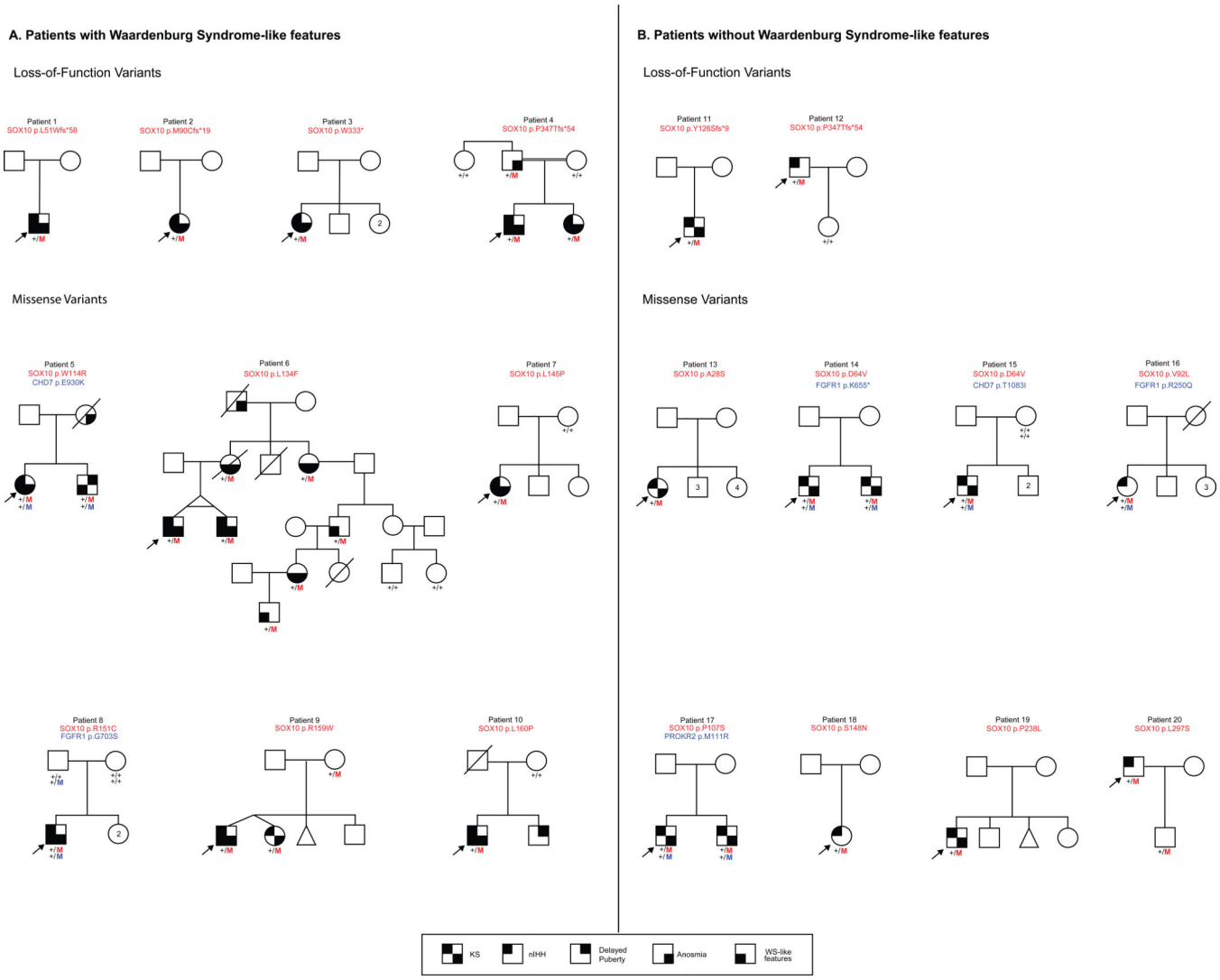

Figure 1: Family pedigrees of probands with *SOX10* RSVs identified in the MGH IHH cohort.
Pedigrees are sorted by presence (Panel A) or absence (Panel B) of WS-associated features. Probands are identified by arrows. "+" indicates Wild-type (WT) allele and M indicates the mutant allele.

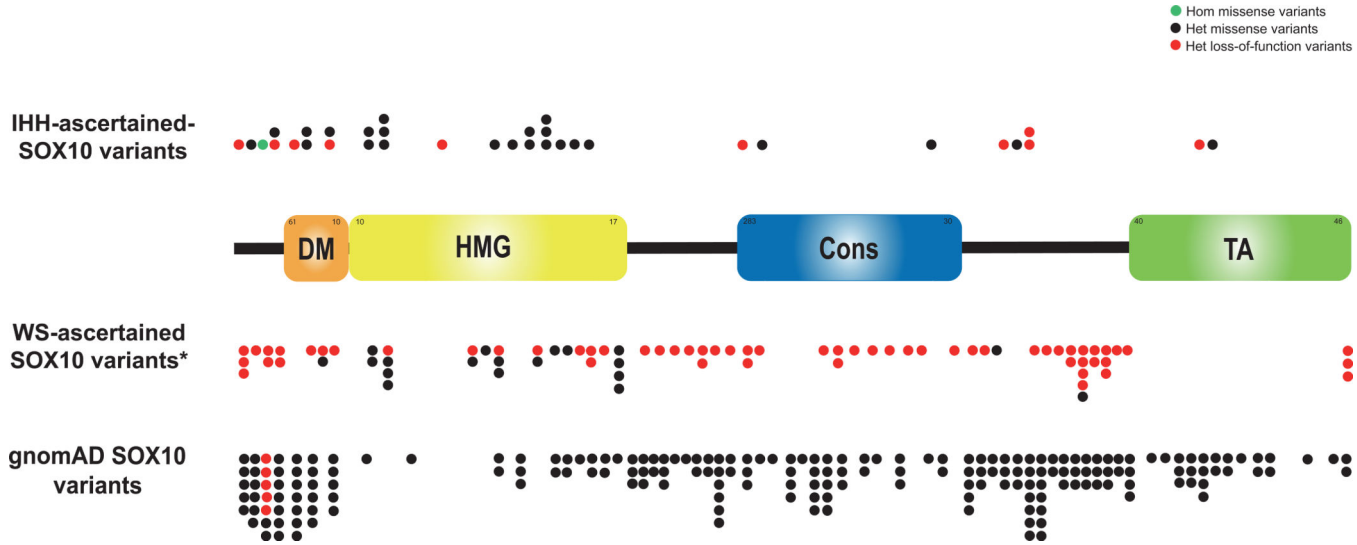

Figure 2: SOX10 protein domains and positions of *SOX10* RSVs identified in IHH, WS and gnomAD.

Heterozygous loss-of-function alleles are shown in red circles; heterozygous missense alleles are shown in black circles; homozygous missense alleles are shown in green circles. *Only single nucleotide variants associated with WS are shown and *SOX10* structural variants are not depicted. DM, Dimerization Domain; HMG, High Mobility Group; Cons, Conserved in SOX-E Family; TA, Transactivation Domain

Table 1.

SOX10 RSVs in IHH patients with Waardenburg syndrome-related features

Patient	Sex	*SOX10* Variant	IHH diagnosis	Waardenburg phenotypes	Other phenotypes	Other IHH Variants	Reference
1	M	p.Leu51Trpfs*58 (Het)[a]	KS	HL	N/D	–	This study
2	F	p.Met90Cysfs*19 (Het)[a]	KS	HL	–	–	This study
3	F	p.Trp333* (Het)[a]	KS	HL	Hypertelorism, ptosis, small ears, high arched palate, scoliosis, pectus excavatum, asymmetrical face	–	This study
4	M	p.Pro347Thrfs*54 (Het)[a]	KS	DP	High arched palate, flat feet, pectus excavatum,	–	This study
5	F	p.Trp114Arg (Het)	KS	HL, DP	Developmental delay	*CHD7* p.Glu930Lys (Het)	This study
6	M	p.Leu134Phe (Het)[a]	KS	HL, DP	Intellectual disability	–	This study
7	F	p.Leu145Pro (Het)	KS	HL, PN	Morbid Obesity, Grave's disease, joint hypermobility	–	This study
8	M	p.Arg151Cys (Het)	KS	HD	N/D	*FGFR1* p.Gly703Ser (Het)	This study
9	M	p.Arg159Trp (Het)[a]	KS	HL[c]	N/D	–	This study
10	M	p.Leu160Pro (Het)[a]	KS	HL, HD	N/D	–	This study
N/A	F	Complete deletion of SOX10 (Het)	KS	HL	N/D	*POLR3B* p.Val337Ala (Het)	[10]
N/A	F	c.698–1G>C; p.spl? (Het)	KS	HL	N/D	–	[4]
N/A	M	c.2T>G; p.? (Het)	KS	HL, DP	N/D	–	[28]
N/A	M	p.Glu62* (Het)	KS	HL, DP	broad nasal bridge, joint hypermobility	–	[9]
N/A	M	p.Ser431Argfs*71 (Het)	KS	HL, DP	–	*SEMA3A* p.Val435Ile (Het)	[4]
N/A	M	p.Gly41Val (Hom)	KS	HL	N/D	–	[8]
N/A	M	p.Leu80Val (Het)	KS	HL (unilateral)	N/D	–	[8]
N/A	M	p.Met108Thr (Het)	KS	HL (partial)	Ptosis	–	[4]
N/A	M	p.Met108Thr (Het)	KS[b]	HL, DP	Ptosis	–	[36]
N/A	F	p.Phe111Val (Het)	KS	HL	Ptosis	–	[4]
N/A	N/D	p.Met112Ile (Het)	KS	HL	N/D	–	[37]
N/A	F	p.Trp142Arg (Het)	KS	HL	Morbid obesity	–	[4]
N/A	M	p.Leu145Pro (Het)	KS	HL, DP	N/D	–	[7]

KS: Kallmann Syndrome; nIHH: normosmic Idiopathic Hypogonadotropic Hypogonadism; Key Waardenburg Phenotypes: HL = Hearing Loss, HD = Hirschsprung's Disease, DP = Distinctive Pigmentation on body, hair, and/or eyes, PN = Peripheral Neuropathy; N/A = Not applicable; N/D = Not Determined; "-" = None; Het = Heterozygous mutation; Hom = Homozygous mutation; p.? = Initiation Codon mutation; p.spl? = Predicted Splice Variant.

[a] Novel mutation identified in this study.

[b] Reversal of KS.

[c] Patient also reports noise damage.

Table 2:

SOX10 RSVs in IHH patients without overt Waardenburg syndrome-related features

Patient	Sex	*SOX10* Variant	IHH Diagnosis	Other Phenotypic Details	Other Variants	Reference
11	M	p.Tyr126Serfs*9 (Het)[a]	KS	–	–	This study
12	M	p.Pro347Thrfs*54(Het)[a]	nIHH	Flat feet, hypothyroidism	–	This study
13	F	p.Ala28Ser (Het)[a]	KS	High arched palate, deviated septum, flat feet, clinodactyly, syndactyly- 2nd and 3rd toes, short bones hands feet	–	This study
14	M	p.Asp64Val (Het)	KS	Obesity	*FGFR1* p.Lys655* (Het)	This study
15	M	p.Asp64Val (Het)	KS	N/D	*CHD7* p.Thr1083Ile (Het)	This study
16	F	p.Val92Leu (Het)	nIHH	Deviated septum, Diabetes type II, obesity	*FGFR1* p.Arg250Gln (Het)	This study
17	M	p.Pro107Ser (Het)[a]	KS	Diabetes type II	*PROKR2* p.Met111Arg (Het)	This study
18	F	p.Ser148Asn (Het)[a]	nIHH	–	–	This study
19	M	p.Pro238Leu (Het)[a]	KS	Flat feet	–	This study
20	M	p.Leu297Ser (Het)[a]	nIHH	Crowded teeth	–	This study
N/A	M	p.Ala44Gly (Het)	KS	–	N/D	[8]
N/A	F	p.Arg151Cys (Het)	KS	Macroscelia	–	[4]
N/A	F	p.Val340Met (Het)	nIHH	–	*OTX2* p.Ala55Ser (Het)	[10]
N/A	M	p.Arg433Gln (Het)	KS	Intellectual disability, dysmorphism, poly-malformation	–	[4]

KS: Kallmann Syndrome; nIHH: normosmic Idiopathic Hypogonadotropic Hypogonadism; N/A = Not Applicable; N/D = Not Determined;

"–" = None; Het = Heterozygous mutation.

[a]Novel mutation identified in this study.

Table 3:

SOX10 RSVs previously reported in WS patients in whom reproductive phenotypes were evident

Sex	*SOX10* Variant	Key Waardenburg features	Reproductive phenotypes	Other Phenotypes	Reference
M	chr22:38,202,740–38,927,438. 725 kb deletion of genomic material, includes 14 deleted genes including *SOX10* (Het)	HL, DP	Cryptorchidism	Hexadactyly, broad nasal root, nystagmus, profound intellectual disability, hypotonia, delayed psychomotor development	[38]
M	c.697–740_1085del ins CCT	HL, HD, DP	Cryptorchidism	N/D	[39]
M	g.(17,712,505_17,716,229)_(17,929,647_17,933,832) del (Het) p.Val92Leu (Het)	HL, HD, DP, PN, CD	KS	Hypotonia, delayed psychomotor development, mild developmental disability	[39]
F	p.Pro169fs*284 (Het)	HL, DP	KS	N/D	[33, 32]
M	p.Tyr313* (Het)	HL, HD, DP, PN, CD	nIHH	Delayed developmental milestones, nystagmus, hypotonia, cerebellar ataxia, autonomic heart dysregulation	[13, 40]

KS: Kallmann Syndrome; nIHH: normosmic Idiopathic Hypogonadotropic Hypogonadism; Key Waardenburg Phenotypes: HL = Hearing Loss, HD = Hirschsprung's Disease, DP = Distinctive Pigmentation on body, hair, and/or eyes, PN = Peripheral Neuropathy, CD = Central Demyelination;

N/D: Not Determined; Het = Heterozygous mutation