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22q11.2q13 Duplication Including *SOX10* causes Sex-reversal and Peripheral Demyelinating Neuropathy, Central Dysmyelinating Leukodystrophy, Waardenburg Syndrome and Hirschsprung Disease

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Abstract

Diagnosis of genetic syndromes may be difficult when specific components of a disorder manifest at a later age. We present a follow up of a previous report [Seeherunvong et al., 2004; *Ajmg* 127: 149–151], of an individual with 22q duplication and sex-reversal syndrome. The subject's phenotype evolved to include peripheral and central demyelination, Waardenburg syndrome type IV, and Hirschsprung disease (PCWH; MIM 609136). DNA microarray analysis defined the duplication at 22q11.2q13, including *SOX10*. Sequencing of the coding region of *SOX10* did not reveal any mutations. Our data suggest that *SOX10* duplication can cause disorders of sex development and PCWH, supporting the hypothesis that *SOX10* toxic gain-of-function rather than dominant negative activity underlies PCWH.

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CONFLICT OF INTEREST DISCLOSURES

Baylor College of Medicine (BCM) and Miraca Holdings Inc. have formed a joint venture with shared ownership and governance of the Baylor Genetics laboratory (BG), formerly the Baylor Miraca Genetics Laboratories (BMGL), which performs genomics testing including chromosomal microarrays (CMA) and clinical exome sequencing. JEP and JRL are employees of BCM. JRL derives support through a professional services agreement with the BG. JRL serves on the Scientific Advisory Board of the BG. JRL has stock ownership in 23 and Me, is a paid consultant for Regeneron Pharmaceuticals, has stock options in Lasergen, Inc and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases and bacterial genomic fingerprinting.

Keywords

22q11 duplication syndrome; *SOX10*; Waardenburg-Shah syndrome; WS4; peripheral demyelinating neuropathy; central demyelinating leukodystrophy; Waardenburg syndrome; and Hirschsprung disease; PCWH; Sex reversal; XX male syndrome; neurocristopathies; disorders of sex development

INTRODUCTION

The phenotypes of duplications of the short arm of chromosome 22 are variable, depending on the extent of the duplication, the genes involved, and interruption of the coding sequence or regulatory elements of those genes. Perhaps the best known duplication of this region is a recurrent 22q11.2 microduplication (MIM 608363), mediated by low copy repeats (LCRs) and reciprocal to the common 22q11.2 deletion underlying DiGeorge syndrome and velocardiofacial syndrome. Among the dosage-sensitive genes on 22q is the *SOX10* gene (MIM 602229), which belongs to the *SOX* (SRY-related HMG-box) family of genes located on chromosome 22q13.1 [Ito et al., 2015], centromeric to the common duplication. It is first expressed during embryonic development in cells of the neural crest, and has a critical role in regulation of development of cells in the neural crest lineage. Thus, dysregulation of *SOX10* results in abnormalities of neural-crest derived cells including Schwann cells, oligodendrocytes, melanocytes and enteric ganglia [Inoue et al., 2004] (Table S1).

Waardenburg syndrome type IV, also known as Waardenburg-Shah syndrome (WS4; MIM 277580) is an auditory-pigmentary syndrome characterized by pigmentary abnormalities of the eye, deafness, and Hirschsprung disease [Shah et al., 1981; Pingault et al., 1998; Inoue et al., 1999]. Abnormal formation of enteric nerves and melanocytes are key manifestations, and *SOX10* haploinsufficiency has been shown to be the underlying pathogenic mechanism [Inoue et al., 1999]. Disruption of highly conserved non-coding elements regulating *SOX10* has also been associated with WS4 [Bondurand et al., 2012]. The *SOX10* variant allele phenotype is known as peripheral demyelinating neuropathy, central demyelinating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease (PCWH; MIM #609132) [Inoue et al., 2004]. Both WS4 and PCWH are mostly caused by truncating (i.e., nonsense or frameshift) mutations of *SOX10*. However, the position of the premature termination codon (PTC) dictates the phenotype. *SOX10* truncating mutations of the upstream exons trigger nonsense mediated decay (NMD) resulting in *SOX10* loss-of-function alleles with haploinsufficiency of *SOX10* leading to the WS4 phenotype, whereas *SOX10* premature truncating mutations of the 3-prime region of the gene (i.e., last exon or 55 bp of the penultimate exon) escape NMD and cause the more severe PCWH phenotype. A potential dominant-negative interference mechanism was proposed initially for those alleles that escape NMD, although a gain-of-function effect could not be ruled out [Inoue et al., 2004].

Several patients with partial 22q duplication, including *SOX10*, have shown a sex reversal phenotype (XX male) (Aleck, Argueso, Erickson, Hackel, & Stone, 1999; Rajender et al., 2006; Nicholl et al., 1994). Those patients were not well defined clinically or cytogenetically, and there is controversy over the precise role of *SOX10* in the pathogenesis. Aleck et al. [1999] reported on a patient with partial 22q duplication and true

hermaphroditism. In 2003, the same group reported on another patient with an XX male phenotype and a typical 22q11.2 deletion. The authors concluded that the deletion and sex reversal phenotypes may have co-occurred in the same individual by chance, given the high frequency of the typical 22q11.2 deletion [Erickson et al., 2003]. Polanco et al. [2010], using a transgenic mouse approach, tested the hypothesis that *Sox10* overexpression causes XX sex reversal, proposing that human *SOX10* is involved in 22q duplication 46,XX male syndrome. They observed that transgenic expression of *Sox10* in gonads of XX mice resulted in development of testes and male physiology, in a dose-dependent manner. It was concluded that *SOX10* dosage is involved in the pathogenesis of 22q-linked disorders of sex development [Polanco et al., 2010].

We present clinical and molecular data on a previously reported patient with sex reversal and 22q11.2q13 duplication [Seeherunvong et al., 2004], with nearly two decades of clinical follow up. *SOX10* duplication in PCWH supports the hypothesis that PCWH is caused by a gain-of-function mechanism, in contrast to WS4 which is caused by *SOX10* haploinsufficiency.

CLINICAL REPORT

At the time of the initial report [Seeherunvong et al., 2004], the proband was a 12-month-old individual with a sex reversal syndrome (karyotype 46,XX yet phenotypically male) who presented with developmental delay, growth delay, hypotonia, skin pigmentation abnormalities, hearing loss, and recurrent pneumonia. At age 19 years he manifested profound intellectual disability, growth delay, microcephaly, telecanthus, hypertelorism, broad nasal bridge, thick lower vermilion, short neck and mandibular prognathism (Figure 1A–B). As the individual became older, the four components of PCWH became apparent: (1) peripheral neuropathy was diagnosed clinically by high-arched feet, muscle atrophy, undetectable peripheral reflexes, and distal contractures (Figure 1C–D); (2) central dysmyelination was observed on brain MRI; (3) Waardenburg syndrome was evidenced by a white fore-lock, telecanthus, and hearing loss; and (4) Hirschsprung disease was diagnosed, reportedly by biopsy during infancy. Additional features included renal failure diagnosed at 18 years, cataracts, delayed puberty, and G-tube dependency. Family history was unremarkable on the maternal side and unknown on the paternal side. Ancestry was African American on both sides of the family. At age 19 years, the patient succumbed to end-stage renal disease. Renal biopsy and work-up for renal transplantation were not pursued due to the patient's severe morbidity.

Cytogenetic analysis in infancy had revealed a female karyotype (46,XX) and a duplication on chromosome 22q. Fluorescence *in situ* hybridization (FISH), utilizing probes on chromosome 22, indicated duplication of 22q11.2 to 22q13, shown by microsatellite marker analysis to be derived from the paternal chromosome. *SRY* was undetectable by FISH [Seeherunvong et al., 2004]. DNA comparative genomic hybridization (CGH) array revealed a 20.62 Mb duplication in the long arm of chromosome 22 (22q12.1q13.31, maximum interval, 27691965–48315347, hg19), encompassing 216 OMIM genes. Among these, 16 OMIM genes have been documented to display a dominant inheritance pattern, including *SOX10* (Figure 1E). To determine whether the PCWH phenotype in the reported individual

could be attributed to the extra gene dosage of *SOX10* (i.e., the duplication), rather than a point mutation in *SOX10*, we undertook Sanger sequencing of the entire coding sequence of *SOX10*. No rare variants were detected. RNA was not available for analysis of transcript levels.

MATERIALS AND METHODS

The family of the deceased individual provided renewed research consent, including photo consent, according to the Baylor-Hopkins Center for Mendelian Genomics (BHCMG) research protocol H-29697, approved by the Institutional Review Board (IRB) at Baylor College of Medicine (BCM). Array CGH was performed in a certified diagnostic lab (Cytogenetics and Molecular Diagnostic Laboratory, University of Miami Miller School of Medicine) (Figure 1E). PCR amplification and Sanger sequencing of the coding sequence of *SOX10* was amplified from genomic DNA of the affected individual (see Supplemental Information).

DISCUSSION

Here we provide a follow-up description of an individual with a 22q11.2q13 duplication, who presented with sex reversal in infancy [Seehervunvong et al., 2004] and developed a PCWH phenotype as he grew older. We present microarray data (Figure 1) defining the copy number variant, and rule out *SOX10* exonic single nucleotide variants (SNVs) as contributors to the phenotype.

22q duplication with concomitant disorders of sex development has been previously noted in multiple individuals [Rajender et al., 2006; Nicholl et al., 1994; Aleck et al., 1999], and is supported by studies of *Sox10* overexpression in the mouse [Polanco et al., 2010]. *SOX10* belongs to the SoxE subfamily. Duplication and overexpression of other members of this gene family, namely *SOX3* and *SOX9*, have been associated with sex reversal [Vidal et al., 2001; Bergstrom et al., 2000]. Similarly, disorders of sex development have been described with CNVs involving *NROB1*, encoding DAX1. Both *NROB1/DAX1* duplication [Swain et al., 1996] as well as a deletion upstream of *NROB1*, hypothesized to lead to loss of regulatory sequences and consequent up-regulation of DAX1 expression, have been associated with male-to-female sex reversal [Smyk et al., 2007]. Together, these studies suggest that dosage effects of genes involved in sex development have the potential to cause sex reversal. We cannot rule out the contribution of additional genes in the duplicated interval, or interruption of regulatory elements to the sex reversal phenotype.

PCWH is typically caused by *SOX10* truncating mutations in the 3-prime region of the *SOX10* gene that escape NMD. It has been proposed that the encoded abnormal SOX10 protein has a toxic effect by either a dominant-negative (antimorphic) effect or a gain-of-function (neomorphic) effect [Inoue et al., 2004]. Functional studies of a mutant allele that resulted in disruption of the native stop codon (i.e. stop-loss) and extension of the native protein for an additional 82 amino acids, allowed for identification of a specific WR domain responsible for the deleterious properties of the mutant SOX10 and supported a toxic gain of function [Inoue et al., 2007]. Our findings suggest that overexpression of the wild-type allele

leads to toxicity. A similar illustration of genotype-phenotype correlation depending on the triggering or escape of NMD by a PTC has been observed in *MPZ* (MIM 159440), *ROR2* (MIM 602337), and *FBN1* (MIM 134797) [Graul-Neumann et al., 2010; Ben-Shachar et al., 2009; Inoue et al., 2004; Khajavi et al., 2006].

Myelin function depends on fine regulation and expression levels of specific genes (Table S1). Disease manifestation in about 70% of patients with different peripheral nervous system and central nervous system myelinopathies, specifically Charcot-Marie-Tooth and Pelizaeus Merzbacher disease (PMD; MIM 312080) respectively, can be attributed to altered gene dosage resulting from duplication of either *PMP22* or *PLP1* [Lupski, 2015]. Similarly, *SOX10* over-expression as the result of duplication seems to promote gain-of-function toxicity in the nervous system. A literature review did not point toward any association between PCWH and sex reversal. It is unclear why *SOX10* duplication may lead to sex-reversal and PCWH, whereas *SOX10* SNVs cause WS4 or PCWH. Limitations include misdiagnosis, limited genetic testing, and rarity of the syndrome.

In conclusion, we provide evidence suggesting that *SOX10* duplication is associated with both sex reversal and PCWH, although the underlying pathogenic mechanism is unknown. Further dosage studies using animals and confirmation of this association in additional humans will aid in interpretation of this association.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

A) Phenotypic facial features of the described individual at 19 years of age. Notable findings include microcephaly, telecanthus, hypertelorism, broad nasal bridge, thick lower vermillion, and short neck. B) Hypopigmentation can be observed in the individual's upper limbs and eyebrows. C) Distal muscle atrophy and foot abnormalities of the affected individual. D) Foot deformities (very high-arched feet), pes equinovarus, and loss of muscle bulk in the lower legs are evident. E) DNA microarray results. aCGH (right) and SNP array (left) results indicate a copy number gain of 22q12.1q13.31. The solid blue-green rectangle indicates the chromosomal position of the copy number gain. The approximate position of *SOX10* is noted.