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Biallelic *SOX8* Variants Associated With Novel Syndrome With Myopathy, Skeletal Deformities, Intellectual Disability, and Ovarian Dysfunction

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Abstract

Background and Objectives

The human genome contains ~20,000 genes, each of which has its own set of complex regulatory systems to govern precise expression in each developmental stage and cell type. Here, we report a female patient with congenital weakness, respiratory failure, skeletal dysplasia, contractures, short stature, intellectual delay, respiratory failure, and amenorrhea who presented to Medical Genetics service with no known cause for her condition.

Methods

Whole-exome and whole-genome sequencing were conducted, as well as investigational functional studies to assess the effect of *SOX8* variant.

Results

The patient was found to have biallelic *SOX8* variants (NM_014587.3:c.422+5G>C; c.583dup p.(His195ProfsTer11)). SOX8 is a transcriptional regulator, which is predicted to be imprinted (expressed from only one parental allele), but this has not yet been confirmed. We provide evidence that while *SOX8* was maternally expressed in adult-derived fibroblasts and lymphoblasts, it was biallelically expressed in other cell types and therefore suggest that biallelic variants are associated with this recessive condition. Functionally, we showed that the paternal variant had the capacity to affect mRNA splicing while the maternal variant resulted in low levels of a truncated protein, which showed decreased binding at and altered expression of SOX8 targets.

Discussion

Our findings associate *SOX8* variants with this novel condition, highlight how complex genome regulation can complicate novel disease-gene identification, and provide insight into the molecular pathogenesis of this disease.

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Glossary

CM = congenital myopathy; **EMG** = electromyogram; **POI** = primary ovarian insufficiency.

Introduction

Congenital myopathies (CMs) are genetically heterogeneous muscle disorders typically presenting with neonatal or childhood-onset weakness and hypotonia with a static or slowly progressive disease course.[1] Patients with CM may have dysmorphic characteristics secondary to the myopathy, including high-arched palate, elongated facies, scoliosis, joint contractures, and foot deformities.[2] Diagnoses of CM can be challenging because of a range of disease severity, and many patients may have nonspecific or normal biopsy findings.[3] Advances in molecular genetics have enabled broadening of the clinical phenotypes in known CM genes, while leading to the identification of novel genetic etiologies.

Our understanding of the human genome has significantly advanced in the past decade, but much is left to be discovered.[4] The human genome contains ~20,000 protein-coding genes, many of which are tightly regulated for precise expression in each cell type at all developmental stages. In addition, although most genes have both alleles regulated concurrently, some are only expressed from one parental allele (imprinted genes). The regulation of genes is highly relevant to human health; the combination of a gene's function and expression pattern dictates the phenotypic consequences of genetic aberrations. These factors also influence the inheritance pattern for genetic diseases. Finally, many genes are associated with multiple different phenotypes depending on the mutational mechanism and inheritance pattern.

SOX [SRY (sex-determining region on the Y chromosome)-related HMG (high mobility group)-box] proteins are a family of transcriptional regulators defined by the presence of a highly conserved HMG domain that is responsible for sequence-specific DNA binding and subsequent DNA bending.[5,6] The human genome contains 20 SOX family members, which have highly divergent developmental functions in a vast array of tissues (reviewed in reference 7). This SOX family is further subdivided into 8 groups on the basis of sequence similarity (Groups A-H).[8] One of the best characterized groups of SOX proteins is subgroup E, which includes SOX8, SOX9, and SOX10. *SOX9* is predominantly expressed in chondrocytes and sertoli cells,[9,10] and mutations cause campomelic dysplasia.[11,12] *SOX10* is expressed in both the peripheral and central nervous systems,[13] and mutations cause Waardenburg-Hirschsprung disease.[14-16] *SOX8* has been associated with a range of human reproductive anomalies including 46, XY disorders of (or differences in) sex development (DSD), as well as male infertility and primary ovarian insufficiency (POI) in women.[17,18] While *Sox8* is expressed in the mouse testis, it is also expressed in the CNS, neural crest derivatives, myotomes, skeletal muscles, satellite cells, cartilage, and kidney.[19] Given the broad expression, it remains to be seen if *SOX8* is responsible for additional phenotypes. In mice, loss of *Sox8* results is subtle skeletal deformities, weight reduction, and osteopenia.[20]

Here, we report a female patient with clinical features of a novel syndrome with severe facial weakness, micrognathia, high-arched palate, kyphoscoliosis/skeletal dysplasia, contractures, congenital nonprogressive myopathy with proximal and distal leg weakness, short stature, intellectual delay, and respiratory failure and amenorrhea. Exome and genome sequencing identified compound heterozygous variants in *SOX8*. We find that the regulation of *SOX8* is complex and that it displays imprinted expression in some cell types, but not in others, providing molecular evidence that both identified variants adversely affected gene function. Our findings expand the disease associations of *SOX8* while beginning to provide molecular insights into disease pathogenesis.

Methods

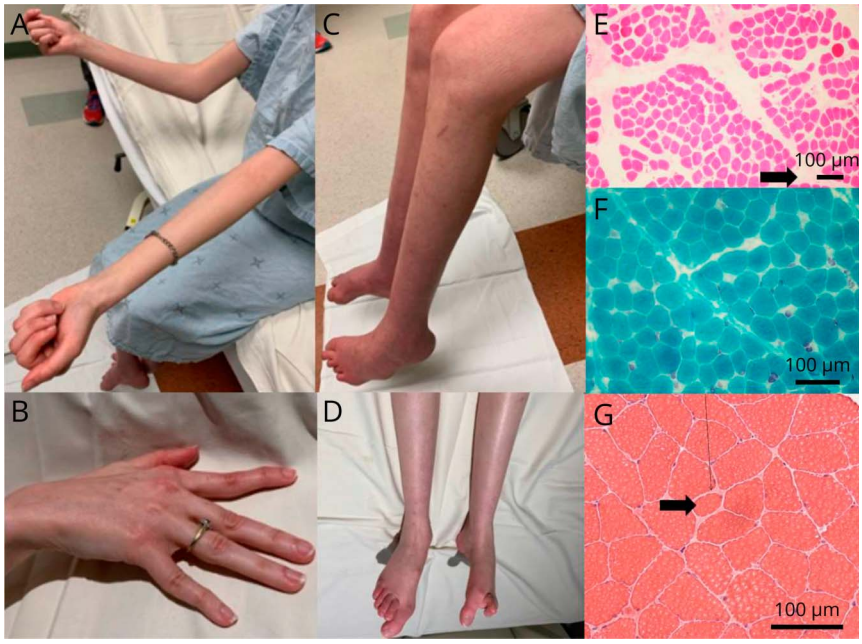
Standard Protocol Approvals, Registrations, and Patient Consents

The affected individual presented to the Medical Genetics service for evaluation. She and her family were enrolled in the Care4Rare Canada research study because of the lack of a molecular diagnosis. Approval of the study design was obtained from the institutional research ethics board (Children's Hospital of Eastern Ontario; #1104E and CTO1577), and free and informed consent was obtained before enrollment for all participants.

Muscle Biopsy

For muscle biopsy of the lateral quadriceps, immunohistochemical and histologic studies, including the electron microscopic examination, were performed. Some fragments of the muscle biopsy were flash frozen in liquid nitrogen at −80°C while others were immersed in 4% buffered paraformaldehyde before being embedded in paraffin. Sections of the flash-frozen muscle were cut at 5 μm on a cryotome and stained using hematoxylin and eosin, periodic acid Schiff, modified Gomori trichrome, Masson trichrome, vanGieson, and oil red O. Histochemical staining for lactate dehydrogenase, NADH, acid phosphatase, and myophosphorylase and myofibrillar ATPase (developed at pH 4.3, 4.6, and 9.4) was also performed on sections, as well as immunostaining using antibodies α-sarcoglycan, β-sarcoglycan, δ-sarcoglycan, and γ-sarcoglycan and C-terminal, N-terminal and rod domains of dystrophin, spectrin, emerin, dysferlin, slow and fast myosin, collagen VI, merosin, αB-crystallin, and β-dystroglycan. Electron microscopy was also performed.

Figure 1 Diffuse Decreased Muscle Bulk With Contractures and Mild Variation in Fiber Size on Muscle Biopsy



(A–D) Clinical images demonstrated marked decrease in muscle bulk with asymmetric contractures. Muscle biopsy at age 1 year (E, F) demonstrated mild variation in fiber size with scattered moderately small rounded polyhedral fibers of both types. The fiber size variation was most prominent just under the fascia. The endomysial connective tissue was mildly increased (arrow). (G) Repeat muscle biopsy at age 33 years revealed mild variation in fiber size attributable to rare, small regenerating fibers with rare angular, atrophic fibers (arrow).

MRI

Whole-body muscle MRI was performed (1.5T Siemens Magneton, 5 mm slice thickness). Coronal T1 sequences of the thorax and abdomen, and axial T1 and STIR-weighted sequences of the thorax, abdomen, and upper and lower limbs were obtained. Multiplanar multisequential MRI of the head without contrast was also performed.

Identification of Rare Variants by Exome and Genome Sequencing

For exome sequencing, exonic DNA was selected using the Agilent SureSelect 50 Mb (V5) All Exon Kit following manufacturer's instructions and sequenced on an Illumina HiSeq 2500. Read alignment, variant calling, and annotation were performed as previously described for FORGE and Care4-Rare Canada projects, with a pipeline based on Burrows-Wheeler Aligner, Picard, ANNOVAR, and custom annotation scripts.[21-24] Average coverage for the exomes was 141× for the affected individual and 114× and 129× for the mother and father, respectively, and 95% of the CCDS exons in all exomes were covered at >10×. Details of genome sequencing are available in the eMethods (links.lww.com/NXG/A629).

Genomic Imprinted Expression, Real-Time PCR, Minigene, Western Blot, Coimmunoprecipitation, and Chromatin Immunoprecipitation Analyses

Details of the laboratory assays used in this study are available in the eMethods (links.lww.com/NXG/A629).

Data Availability

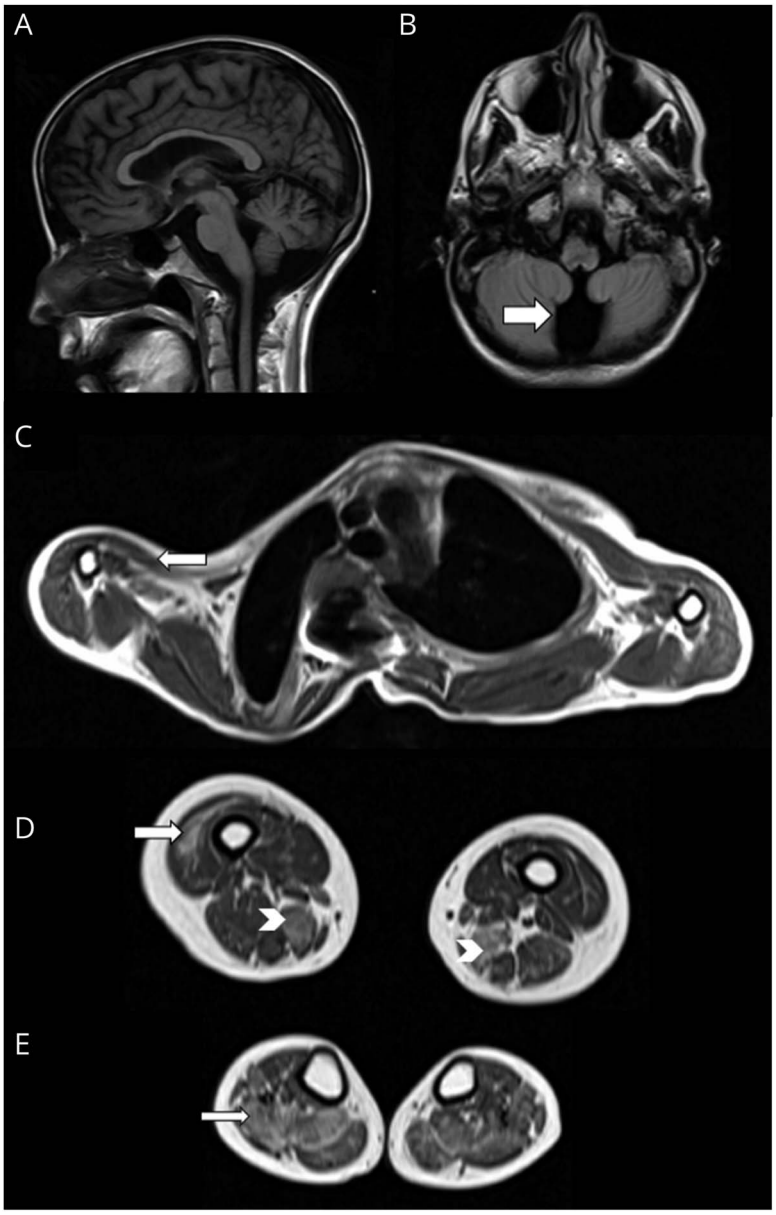
The data are not publicly available because of privacy and ethical restrictions. The data that support the findings of this study are available on request from the corresponding author. Specifically, the data from the family can be accessed by request through the Genomics4RD platform.

Results

Patient Description

The female proband was born to a 22-year-old, G3P2, French Canadian mother and a 30-year-old Caucasian father. The parents and 2 siblings (brother and sister) were healthy. There was no known consanguinity, and the family histories were noninformative. The proband had weakness at birth with poor suck, micrognathia, hypotonia, and talipes. She was documented to have significant motor delay as a child and required spinal fixation surgery at age 15 years. She attended regular school with documented learning difficulties. She represented to the Medical Genetics service at age 27 years with thin body habitus (weight 26.5 kg, −5.0 SD; BMI 14.1, −3.4 SD), short stature (144 cm, −2.7 SD), and a head circumference of 52 cm (−1.8 SD). She had mild intellectual delay. She had clinical features of a congenital, nonprogressive myopathy with moderate proximal and distal weakness (2- to 4+ diffusely) and scapular winging. She had prominent cranial weakness, with marked facial paresis with no frontalis contraction and incomplete eye closure, upper lid retraction, narrow mouth, tented upper lip vermilion, micrognathia/dental crowding, and prominent dysarthria and dysphonia. She had marked asymmetric contractures in elbows, knees, and ankles and long, tapered, finger hyperlaxity (Figure 1). She also had amenorrhea until age 17 years and has mild chronic oligomenorrhea (6–10/y). She has chronic respiratory failure

Figure 2 MRI Demonstrates Large Posterior Fossa CSF Spaces and Diffuse Decreased Muscle Volume, With Fatty Replacement of Most Prominent in the Right Pectoralis, Vastus Lateralis, Semimembranosus, and Soleus

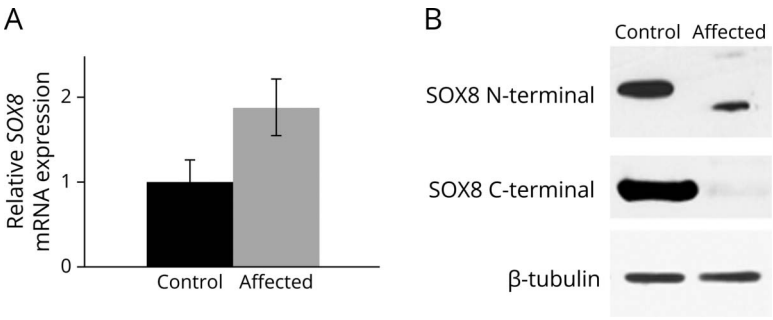


(A) Head MRI with sagittal T1 FLAIR and (B) axial T2 FLAIR demonstrate large posterior fossa CSF spaces, which appear in direct relationship with enlarged fourth ventricle (arrow). There is also slight deformity of the cerebellum. (C, D and E) Whole-body muscle MRI T1-weighted axial images demonstrate severe atrophy of the right pectoralis major and minor (C), with severe fatty infiltration (arrow). (D) Mild-to-moderate atrophy and fatty infiltration of the vastus lateralis on the right along its central and inferior thirds (arrow) and bilateral moderate-to-severe fatty infiltration and moderate atrophy of the semimembranosus (chevron). There were no signal changes in the STIR sequence (not shown). (E) Moderate-to-severe bilateral fatty infiltration of the soleus (arrow) and mild fatty infiltration of the medial head of the gastrocnemius (left more than right), with mild atrophy.

requiring nocturnal Bilevel Positive Airway Pressure at age 27 years, and pulmonary function testing demonstrated severe restrictive lung disease. Transthoracic echocardiogram was normal. Creatine kinase was normal. Electrodiagnostic motor and sensory nerve conduction studies were normal. Needle electromyogram (EMG) studies demonstrated short-duration, small-amplitude motor unit action potential, and early recruitment patterns were observed in the involved proximal muscles and distal muscles, suggesting myopathy, with no myotonic discharges. Repetitive stimulation studies at 3 Hz of the abductor digiti minimi and trapezius were normal. Single-fiber EMG studies of the forehead did not demonstrate any motor units.

Muscle biopsy of the right medial gastrocnemius at age 1 year (Figure 1) demonstrated mild variation in fiber size with scattered, moderately small, rounded polyhedral fibers of both types. The endomysial connective tissue was mildly increased. The muscle biopsy did not demonstrate fiber splitting, ring fibers, nuclear chains, or sarcoplasmic masses. There was no inflammation degeneration, necrosis, or regeneration identified. Intramuscular nerve fibers and twigs were normally myelinated. Repeat muscle biopsy of the lateral vastus medialis at age 33 years revealed mild variation in fiber size attributable to rare, small regenerating fibers in formalin-fixed, paraffin-embedded specimen with rare angular, atrophic fibers (Figure 1). There were no significant dystrophic features.

Figure 3 Expression of *SOX8* mRNA Was Elevated and Produced a Truncated Protein in Affected Cells



(A) Real-time PCR analysis on cDNA from fibroblast cells showing increased *SOX8* transcript abundance in affected cells (Aff) compared with control (Ctrl). Graphed data represent the mean of 3 biological replicates, and error bars depict standard error of the mean. Paired *t* test was performed; $p = 0.067$. (B) Western blot analysis on extracts from fibroblast cells using both an N-terminal and a C-terminal SOX8 antibody in control and affected samples. The N-terminal antibody detects a protein of lower molecular weight and lower abundance in lysate from affected cells, whereas the C-terminal antibody failed to detect any protein in affected lysates.

Histochemical staining for myofibrillar ATPase revealed a predominance of type II muscle fibers. On immunostaining, normal staining patterns were observed for the following antigens: α-sarcoglycan, β-sarcoglycan, δ-sarcoglycan, and γ-sarcoglycan and C-terminal, N-terminal, and rod domains of dystrophin, spectrin, emerin, dysferlin, slow and fast myosin, collagen VI, merosin, αB-crystallin, and β-dystroglycan. Ultrastructural analysis did not show significant findings.

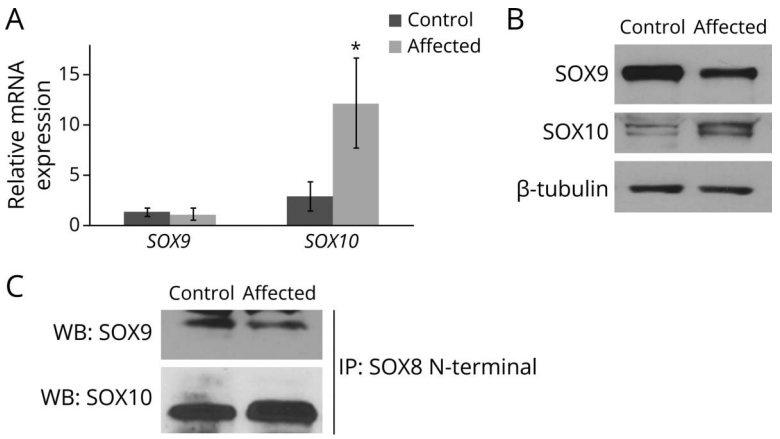
MRI of the brain demonstrated large posterior fossa CSF spaces, which appear in direct relationship with enlarged fourth ventricles, possibly consistent with a mild congenital, Dandy-Walker malformation (Figure 2, A and B). Muscle MRI demonstrated fatty infiltration of the paravertebral muscles, severe atrophy and fatty infiltration of the right pectoralis major and minor (normal on the left) and serratus anterior, moderate-to-severe fatty infiltration and moderate atrophy of the semimembranosus, mild-to-moderate atrophy and fatty infiltration of the vastus lateralis on the right along its central and inferior thirds, moderate-to-severe bilateral fatty infiltration of the soleus and mild fatty infiltration of the medial head of the gastrocnemius (left more than right), with mild atrophy. The shoulder girdle and visualized areas of the upper extremities were reported as presenting minimal fatty infiltration and mild-to-moderate symmetrical global loss of volume (Figure 2, C–E). There were no signal changes in the STIR sequence. X-rays showed skeletal dysplasia, including cervical thoracic scoliosis and lumbar scoliosis, overtubulated long bones with distal femoral condyles, which were not well formed, mild bowing for both tibias and fibulas, and positional abnormalities of the feet.

Clinical genetic testing was noninformative and included testing for myotonic dystrophy type 1 and type 2, FSHD1, and a microarray. The microarray did identify a 0.655 Mb duplication in chromosome 6q22.31 (1118,619,406-119,274,667 chr37) that involved 7 reference sequence genes (SLC35F1, CEP85L, BRD7P3, LOC100287632, ASF1A), but this had no clear clinical significance and was felt to be noncontributory because the genes involved either had no known associated phenotypes or were unrelated.

Exome and Genome Sequencing Identified Biallelic Variants in *SOX8*

Given the lack of family history of a similar condition, we hypothesized that this rare disease was caused by autosomal recessive or de novo dominant variant(s). Exome sequencing was performed on genomic DNA from the affected patient and her parents. Variants present in ≥0.1% minor allele frequency in gnomAD were excluded. No homozygous or de novo variants were identified. Three known disease genes were identified with compound heterozygous variants: *NEB* (NM_001164507.1: c.21797C>T; NM_001164507.1:c.402+4_402+27del) while both variants formally classify as a variant of unknown significance (VUS), the patient's phenotype did not fit because there were no nemaline rods in the muscle biopsy. Therefore, this gene was eliminated as a candidate. *SYNE1* (NM_033071.3: c.8381C>T, c.16646T>G) and *EYS* (NM_001142800.1: c.7796A>G; c.7033C>T) also contained 2 VUS each but were ruled out as causative because the associated conditions did not resemble our patient (SYNE1 causes spinocerebellar ataxia, autosomal recessive 8 and arthrogryposis multiplex congenital 3, myogenic type and EYS causes retinitis pigmentosa 25). Compound heterozygous variants were identified in 2 additional genes not yet linked to phenotypes: *PRUNE2* (NM_001308047.1: c.8677C>T; c.1132C>G) and SOX8 (NM_014587.3:c.422+5-G>C; c.583dup). *PRUNE2* is a loss-of-function tolerant gene with a pLi of 0 and has a significant number of individuals that are homozygous for loss-of-function variants in gnomAD; as such, it seemed unlikely that this gene caused a loss-of-function autosomal recessive condition. *SOX8* is a conserved gene, with only 5 heterozygous loss-of-function alleles in gnomAD (of >200,000 alleles, pLi 0.67), suggesting that this was not tolerant to recessive loss-of-function variants. *SOX8* expression coincides with essential phases of development in many organs, including the CNS, neural crest derivatives, myotomes, skeletal muscles, and cartilage,[19] many of which overlap with the phenotype presentations in our patient. The *SOX8* splice variant, c.422+5G>C, was predicted to affect the donor splice site, and the duplication variant, c.583dup p.(His195ProfsTer11), caused a frameshift and premature stop codon about half way through the protein. Neither variant had been seen in gnomAD or our in-house control database. Interestingly, *SOX8* was associated with autosomal

Figure 4 SOX8/SOX9/SOX10 Complex Was Maintained in Affected Cells, Although SOX10 Was Upregulated, Possibly Providing Some Compensation for SOX8 Deficiency



(A) Real-time PCR analysis on cDNA from fibroblast cells showed no change in *SOX9* levels and an increase in *SOX10* mRNA abundance in affected cells compared with control. Graphed data represent the mean of 3 biological replicates, and error bars depict standard error of the mean. Paired *t* test was performed; $*p < 0.05$. (B) Western blot analysis on extracts from fibroblast cells showed a decrease in SOX9 protein and an increase in SOX10 protein in affected compared with control cells. (C) Immunoprecipitation was performed using a SOX8 N-terminal antibody, which coimmunoprecipitated both SOX9 and SOX10, using lysates of control and affected fibroblast cells.

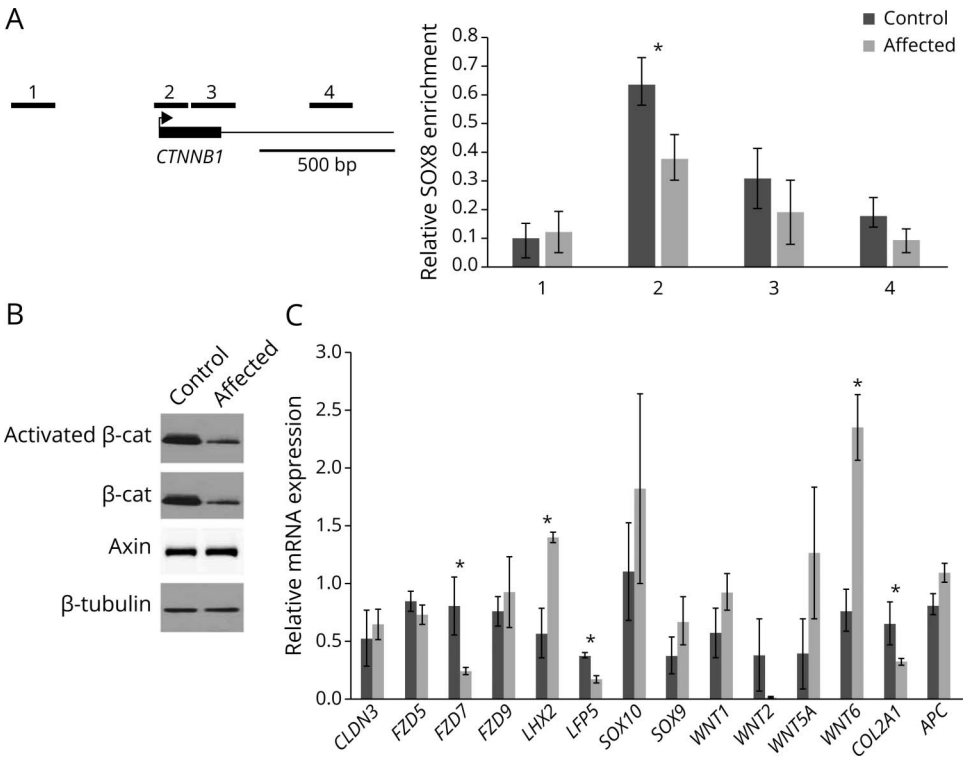
dominant 46, XY disorders of sex development, as well as infertility in men and POI in women.[17,18] However, this has not been validated in sufficient independent families to confirm it as a disease-gene association. Given the compound heterozygous, highly deleterious variants, we found *SOX8* to be the most compelling candidate. With the advances in genome sequencing, we then conducted trio whole-genome sequencing to ensure that there was no other genomic variation that might contribute to the patient's condition. SV, CNV, and STR analysis did not identify any variants of interest, and SNV analysis did not identify any further variants to consider. We entered this gene in the Match Maker Exchange via Phenome Central in July 2018. Unfortunately, no matches of interest have been identified. We conclude that *SOX8* remains the most compelling candidate for this patient's phenotype and set out to investigate the significance of the *SOX8* variants in our functional laboratory.

In the Affected Individual, the Maternal *SOX8* mRNA Produced a Truncated Protein While the Paternal Variant Potentially Affected mRNA Splicing

We began by assessing *SOX8* expression levels in cells from the affected individual compared with age-matched and sex-matched control fibroblast cells. Muscle tissue was not used for functional studies because, unfortunately, there was no material available from previous muscle biopsies available for this purpose. Real-time PCR revealed a modest increase in *SOX8* mRNA transcript abundance in fibroblast cells from the affected individual (Figure 3A). Western blot analysis using a SOX8 N-terminal antibody detected a protein of reduced molecular weight in affected cells, which also showed decreased protein abundance compared with the wild-type form of this protein in control fibroblast cells (Figure 3B). Immunodetection of SOX8 at the C-terminal domain revealed an absence of SOX8 protein in patient cells (Figure 3B). Given the presence of only one band on the western blot, we sequenced the cDNA and identified transcripts containing only the maternal frameshift variant, c.583dup p.(His195ProfsTer11), which corresponded appropriately to the protein size observed by western blot analysis. Therefore, we only detected the maternal allele in both the cDNA and protein assays. At this time, *SOX8* was computationally predicted to be imprinted, with paternal-specific expression, but this finding was not confirmed.[25] Given our findings, we pursued the hypothesis that the *SOX8* gene was in fact imprinted, with only the maternal allele expressed. We assessed allelic *SOX8* expression in 3 control fibroblast and 2 lymphoblast samples for whom we had available paternal and maternal SNPs within cell lines and confirmed that *SOX8* is maternally expressed in fibroblasts and lymphoblasts in all samples (data not shown). We conclude that *SOX8* is maternally expressed in fibroblasts and lymphoblasts and that the maternally inherited variant, c.583dup p.(His195ProfsTer11), produced a truncated protein in our patient. Using a lymphoblast cell line derived from the patient's father, we revealed that the c.422+5C>G variant allele was not expressed and therefore was likely grandpaternally inherited, supporting maternal-specific *SOX8* expression in these cells. We note that *SOX8* has a neighboring gene, *LMF1*, which also displayed imprinted expression in some tissues (data mined from[26]), and that there are maternal and paternal gametic differentially methylated regions nearby, suggesting that *SOX8* and *LMF1* may be part of an imprinted domain.

The finding that *SOX8* is imprinted was intriguing in light of the biallelic variants and the reports of autosomal dominant conditions in the literature. We hypothesized that if SOX8 was expressed from both alleles in other cell types or during particular developmental stages, it would produce a more extensive phenotype. We mined data in the literature and found evidence, where SNPs were informative, of tissue-specific *SOX8* imprinting. Namely, a study by Baran et al.[26] found some tissues with allelic *SOX8* expression while others exhibited biallelic expression. Given that the paternal allele

Figure 5 SOX8 Mutations in Affected Cells Lead to Misregulation of SOX8 Targets and Defective DNA Binding of SOX8 at Some Target Sites



(A) Quantitative SOX8 ChIP analysis was performed at the β-catenin gene, *CTNNB1*. A schematic of this gene is shown with numerically labeled black bars above the gene representing qPCR amplicons that were interrogated for ChIP-qPCR analysis. Graphed data on the right are the results of SOX8 quantitative ChIP analysis at the indicated *CTNNB1* locations from the schematic for affected and control cells. Enrichment at site 2 was found to be significantly decreased (*$p < 0.05$; paired *t* test). (B) Western blot analysis on extracts from fibroblast cells showing a decrease in total and activated β-catenin protein levels in affected cells compared with controls while axin protein levels remained unchanged. (C) Real-time PCR analysis on cDNA from fibroblast cells showed altered expression of many components of the Wnt/β-catenin pathway and various other SOX targets in affected compared with control cells. Graphed data represent the mean of 3 biological replicates, and error bars depict standard error of the mean (*$p < 0.05$; paired *t* test).

was expressed in some cell types and/or developmental windows,[26] we sought to assess if the paternally inherited c.422+5C>G variant had an effect on mRNA splicing, which was predicted to affect the donor splice site. Given the inability to assess the paternal c.422+5C>G in the available patient-derived cells because it was silenced, we cloned this variant into a minigene splicing construct and assessed for splicing. We observed that this variant produced 3 different products confirmed by sequencing, a normal length transcript and 2 other transcripts which were missing regions of the adjacent exon, indicating an adverse effect on mRNA splicing. Given the absence of significant coding portions, it is anticipated that these transcripts would lead to a protein with functional deficits.

Both of the proband's parents carry a variant in *SOX8* gene. If both parents inherited the *SOX8* variant from their fathers, the variant would have no effect on the parent's phenotype because the paternal allele is silenced. In tissue where there may be biallelic expression, the parents would have one wild-type and one variant allele. Because both parents had no phenotype, it suggests that these variants do not have an effect in a heterozygous state and that other alleles that have been associated with a dominant condition in the literature either have a different mechanism or that *SOX8* variation was not likely the explanation for their findings. We note these variants are missense variants and so a gain-of-function mechanism is possible. Taken together, we propose that *SOX8* has both biallelic and maternal expression, depending on the cell type, and that both variants observed in our patient could have an effect on the *SOX8* product.

SOX10 is Upregulated in SOX8-Deficient Cells

Reports from SOX8-deficient mice suggest that there may be functional compensation for loss of SOX8 by SOX9 and/or SOX10 in some tissues.[19,27] As such, we assessed the expression patterns of SOX9 and SOX10 in patient fibroblast cells to determine whether this effect is occurring in our patient. While *SOX9* mRNA levels were similar between affected and control cells, *SOX10* mRNA levels were increased in affected cells (Figure 4A). Congruently, western blot analysis showed no significant differences in SOX9 protein levels, whereas there was an increase in SOX10 in the affected cells (Figure 4B). In addition to potential functional redundancy between SOX8, SOX9, and SOX10 proteins, it has been shown that the SOXE transcription factors not only homodimerize but can also heterodimerize with each other.[27,28] Based on these findings, we investigated the interaction between SOX8-SOX9 and SOX8-SOX10 in control and affected fibroblast cells. Coimmunoprecipitation experiments using a SOX8 N-terminal antibody detected SOX8-SOX9 and SOX8-SOX10 protein-protein interactions in both control and patient cells were approximately equivalent (Figure 4C). Overall, SOX8 deficiency in patient cells results in potential compensatory SOX10 regulation, although the truncated

SOX8 protein can heterodimerize with the other SOXE members.

Downstream SOX8 Targets Are Misregulated

Given that the maternally expressed truncated protein can bind other SOXE members, we wondered whether downstream function was affected, especially considering that SOX8 is a transcription factor with a number of previously defined targets.[5,6] We began by using quantitative ChIP analysis to assess SOX8 binding near the β-catenin gene (*CTNNB1*). We found that the highest level of occupancy was close to the transcriptional start site and that this occupancy was decreased in patient cells (Figure 5A). We conclude that the truncated SOX8 protein has impaired binding capacity at this site, thereby potentially affecting transcription at other known SOX8 target sites. To test this, we assessed expression levels at SOX8 target genes. We found that the overall mRNA transcript abundance was altered for components of the Wnt/β-catenin pathway and various other SOX targets, with notable increases for *LHX2*, *SOX10*, *WNT5A*, *WNT6*, and *APC* and decreases for *FZD7*, *LRP5*, *WNT2*, and *COL2A1* expression levels. Furthermore, we found a decrease in both total and active β-catenin protein levels (Figure 5, B and C). We conclude that the maternal SOX8 truncated protein has impaired function.

Discussion

We have identified an autosomal recessive condition characterized by congenital myopathy with skeletal dysplasia, respiratory failure, intellectual delay, contractures, short stature, and amenorrhea. The affected female patient carries compound heterozygous variants in *SOX8*. Molecular profiling of affected cells showed both variants have a functional effect.

In the literature, there was a previously reported female patient with delayed growth and development and skeletal defects, with early failure of gonad development with a large duplication immediately upstream of *SOX8*.[29] The phenotype of this patient overlaps with the one reported here, including short stature, distinctive craniofacial abnormalities, developmental delay, and gonadal dysfunction. Heterozygous *SOX8* variants have been noted in a handful of individuals with reproductive anomalies, although the mechanism for these phenotypes remains unknown.[17,18] SOX8 is a transcription factor expressed in many tissues, including muscle, testis, nervous system, and cartilage, and involves evolutionary conserved regulatory elements.[30] *SOX8* appears to display tissue-specific imprinting. However, the presence of dual loss-of-function variants and lack of a phenotype in her parents suggests that our patient truly has an autosomal-recessive condition.

SOX8 is expressed in many organs, including skeletal muscles, cartilage, the CNS, kidneys, and gonads/testis,[19] and is a known regulator of differentiation of skeletal muscle, possibly by impairing myogenic basic helix-loop-helix protein function.[30] SOX proteins perform unique functions in different cell types through interactions with different binding partners (reviewed in reference 31). As a well-characterized SOX8 target, we showed that the WNT/β-catenin pathway was misregulated in fibroblasts. Notably, the WNT pathway plays important roles in bone and muscle development.[32] Although at the moment we cannot directly link *SOX8* and other genes implicated in congenital myopathies, these genes play critical roles in muscle structure and function,[33] which SOX8 is predicted to influence through the WNT/β-catenin pathway. Similarly, with respect to the brain MRI findings, SOX8 is expressed and plays a role in neuroepithelial and glial precursor cells[34]; of the more than 100 genes associated with malformations of cortical development, the biological pathways they are involved with include cell cycle regulation, cell fate specification, and neuronal migration and basement membrane function, among others.[35] In addition, it is anticipated that there are many more genes affected by SOX8 regulation and that this may vary by cell type, depending on the interacting cofactors.[36] In *Sox8*-deficient mice, major developmental defects do not occur because many SOX8 targets overlap with those for SOX9, SOX10, or both proteins, suggesting that a compensatory mechanism exists between these proteins.[19,30,37] Additional evidence of functional compensation between SOXE proteins comes from mouse models deficient for *Sox10* and *Sox9,* where additional loss of *Sox8* led to worsening phenotypes.[38,39] In the patient's fibroblasts, we observed upregulation of *SOX10*, possibly representing a compensatory mechanism. Increase *SOX10* expression, but not *SOX9*, may reflect a cell-specific effect in fibroblasts; we cannot exclude the possibility that other tissues or cell types may show different patterns of *SOX9* and *SOX10* expression on *SOX8* disruption because both proteins have the capacity to heterodimerize with SOX8.

In summary, we have used exome sequencing to identify a novel autosomal-recessive condition associated with *SOX8* loss-of-function. The existence of tissue-specific imprinted genes raises an interesting concern in the gene discovery field in which gene candidates are assessed based on their presumed inheritance pattern, and it is significantly more difficult to identify conditions with more complex underlying genetics. Better resources defining imprinted genes are required for optimal gene discovery research. The phenotype of this condition includes congenital, nonprogressive disorder of CNS, skeletal muscles/cartilage, skeletal and gonadal system with congenital myopathy with contractures, scoliosis, intellectual delay, and amenorrhea. This phenotype may represent a novel condition or phenotypic extension of the previously described Marden-Walker syndrome.[40] Marden-Walker syndrome is characterized by psychomotor retardation, kyphoscoliosis, contractures, and a facial paresis with blepharophimosis, micrognathia, and a high-arched palate. Further phenotypes may include Dandy-Walker malformation with hydrocephalus and vertebral malformation. The proband reported here has additional atypical features of amenorrhea and nocturnal respiratory failure not commonly reported in the Marden-Walker syndrome. The molecular

cause of Marden-Walker syndrome is currently unknown and may represent a genetically heterogeneous condition, and molecular assessment of additional patients will be informative. Additional patients with biallelic variants in *SOX8* are required to confirm this novel disease association and delineate the phenotypic spectrum. More broadly further studies looking at tissue-specific imprinted genes are also required to assess the relevance for rare diseases.

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Continued

Appendix *(continued)*

Name	Location	Contribution
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References

1. Gonorazky HD, Dowling JJ, Volpatti JR, Vajsar J. Signs and symptoms in congenital myopathies. *Semin Pediatr Neurol.* 2019;29:3-11. doi. 10.1016/J.SPEN.2019.01.002
2. Natera-de Benito D, Ortez C, Jou C, et al The phenotype and genotype of congenital myopathies based on a large pediatric cohort. *Pediatr Neurol.* 2021;115:50-65. doi. 10.1016/J.PEDIATRNEUROL.2020.11.002
3. Nicolau S, Liewluck T, Tracy JA, et al Congenital myopathies in the adult neuromuscular clinic: diagnostic challenges and pitfalls. *Neurol Genet.* 2019;5:e341. doi. 10.1212/NXG.0000000000000341
4. Bamshad MJ, Nickerson DA, Chong JX. Mendelian gene discovery: fast and furious with no end in sight. *Am J Hum Genet.* 2019;105:448-455. doi. 10.1016/J.AJHG.2019.07.011
5. Mertin S, McDowall SG, Harley VR. The DNA-binding specificity of SOX9 and other SOX proteins. *Nucleic Acids Res.* 1999;27:1359-1364. doi. 10.1093/nar/27.5.1359
6. Kamachi Y, Cheah KSE, Kondoh H. Mechanism of regulatory target selection by the SOX high-mobility-group domain proteins as revealed by comparison of SOX1/2/3 and SOX9. *Mol Cell Biol.* 1999;19:107-120. doi. 10.1128/mcb.19.1.107
7. Kamachi Y, Kondoh H. Sox proteins: regulators of cell fate specification and differentiation. *Development*. 2013;140:4129-4144. doi. 10.1242/dev.091793
8. Schepers GE, Teasdale RD, Koopman P. Twenty pairs of Sox: extent, homology, and nomenclature of the mouse and human Sox transcription factor gene families. *Dev Cell.* 2002;3:167-170.
9. Wright E, Hargrave MR, Christiansen J, et al The Sry-related gene Sox9 is expressed during chondrogenesis in mouse embryos. *Nat Genet.* 1995;9:15-20. doi. 10.1038/ng0195-15
10. Kent J, Wheatley SC, Andrews JE, et al A male-specific role for SOX9 in vertebrate sex determination. *Development.* 1996;122:2813-2822.
11. Wagner T, Wirth J, Meyer J, et al Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. *Cell.* 1994;79:1111-1120. doi. 10.1016/0092-8674(94)90041-8
12. Foster JW, Dominguez-Steglich MA, Guioli S, et al Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. *Nature*. 1994;372:525-530. doi. 10.1038/372525a0
13. Kuhlbrodt K, Herbarth B, Sock E, et al Sox10, a novel transcriptional modulator in glial cells. *J Neurosci.* 1998;18:237-250. doi. 10.1523/jneurosci.18-01-00237.1998
14. Herbarth B, Pingault V, Bondurand N, et al Mutation of the Sry-related Sox10 gene in dominant megacolon, a mouse model for human Hirschsprung disease. *Proc Natl Acad Sci USA*. 1998;95:5161-5165. doi. 10.1073/pnas.95.9.5161
15. Pingault V, Bondurand N, Kuhlbrodt K, et al SOX10 mutations in patients with Waardenburg-Hirschsprung disease. *Nat Genet.* 1998;18:171-173. doi. 10.1038/ng0298-171
16. Southard-Smith EM, Kos L, Pavan WJ. Sox10 mutation disrupts neural crest development in Dom Hirschsprung mouse model. *Nat Genet.* 1998;18:60-64. doi. 10.1038/ng0198-60
17. O'Bryan MK, Takada S, Kennedy CL, et al Sox8 is a critical regulator of adult Sertoli cell function and male fertility. *Dev Biol.* 2008;316:359-370. doi. 10.1016/j.ydbio.2008.01.042
18. Portnoi M-F, Dumargne M-C, Rojo S, et al Mutations involving the SRY-related gene SOX8 are associated with a spectrum of human reproductive anomalies. *Hum Mol Genet.* 2018;27:1228-1240. doi. 10.1093/hmg/ddy037
19. Sock E, Schmidt K, Hermanns-Borgmeyer I, et al Idiopathic weight reduction in mice deficient in the high-mobility-group transcription factor Sox8. *Mol Cell Biol.* 2001;21:6951-6959. doi. 10.1128/mcb.21.20.6951-6959.2001
20. Schmidt K, Schinke T, Haberland M, et al The high mobility group transcription factor Sox8 is a negative regulator of osteoblast differentiation. *J Cell Biol.* 2005;168:899-910. doi. 10.1083/jcb.200408013
21. Beaulieu CL, Majewski J, Schwartzentruber J, et al FORGE Canada Consortium: outcomes of a 2-year national rare-disease gene-discovery project. *Am J Hum Genet.* 2014;94:809-817. doi. 10.1016/j.ajhg.2014.05.003
22. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics.* 2009;25:1754-1760. doi. 10.1093/bioinformatics/btp324
23. McKenna A, Hanna M, Banks E, et al The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 2010;20:1297-1303. doi. 10.1101/gr.107524.110
24. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* 2010;38:e164. doi. 10.1093/nar/gkq603
25. Luedi PP, Dietrich FS, Weidman JR, et al Computational and experimental identification of novel human imprinted genes. *Genome Res.* 2007;17:1723-1730. doi. 10.1101/GR.6584707
26. Baran Y, Subramaniam M, Biton A, et al The landscape of genomic imprinting across diverse adult human tissues. *Genome Res.* 2015;25:927-936. doi. 10.1101/GR.192278.115
27. Stolt CC, Lommes P, Friedrich RP, Wegner M. Transcription factors Sox8 and Sox10 perform non-equivalent roles during oligodendrocyte development despite functional redundancy. *Development.* 2004;131:2349-2358. doi. 10.1242/dev.01114
28. Huang Y-H, Jankowski A, Cheah KSE, et al SOXE transcription factors form selective dimers on non-compact DNA motifs through multifaceted interactions between dimerization and high-mobility group domains. *Sci Rep.* 2015;5:10398. doi. 10.1038/srep10398
29. Erickson RP, Yatsenko SA, Larson K, Cheung SW. A case of agonadism, skeletal malformations, bicuspid aortic valve, and delayed development with a 16p13.3 duplication including GNG13 and SOX8 upstream enhancers: are either, both or neither involved in the phenotype? *Mol Syndromol.* 2011;1:185-191. doi. 10.1159/000321957
30. Schmidt K, Glaser G, Wernig A, et al Sox8 is a specific marker for muscle satellite cells and inhibits myogenesis. *J Biol Chem.* 2003;278:29769-29775. doi. 10.1074/jbc.M301539200
31. Bernard P, Harley VR. Acquisition of SOX transcription factor specificity through protein-protein interaction, modulation of Wnt signalling and post-translational modification. *Int J Biochem Cell Biol.* 2010;42:400-410. doi. 10.1016/j.biocel.2009.10.017
32. Rudnicki MA, Williams BO. Wnt signaling in bone and muscle. *Bone*. 2015;80:60-66.
33. Gineste C, Laporte J. Therapeutic approaches in different congenital myopathies. *Curr Opin Pharmacol.* 2023;68:102328. doi. 10.1016/J.COPH.2022.102328
34. Weider M, Wegner M. SoxE factors: transcriptional regulators of neural differentiation and nervous system development. *Semin Cell Dev. Biol.* 2017;63:35-42.
35. Parrini E, Conti V, Dobyns WB, Guerrini R. Genetic basis of brain malformations. *Mol Syndromol.* 2016;7:220. doi. 10.1159/000448639
36. Wissmüller S, Kosian T, Wolf M, et al The high-mobility-group domain of Sox proteins interacts with DNA-binding domains of many transcription factors. *Nucleic Acids Res.* 2006;34:1735-1744. doi. 10.1093/nar/gkl105
37. Schepers G, Wilson M, Wilhelm D, Koopman P. SOX8 is expressed during testis differentiation in mice and synergizes with SF1 to activate the Amh promoter in vitro. *J Biol Chem.* 2003;278:28101-28108. doi. 10.1074/jbc.M304067200
38. Maka M, Claus Stolt C, Wegner M. Identification of Sox8 as a modifier gene in a mouse model of Hirschsprung disease reveals underlying molecular defect. *Dev Biol.* 2005;277:155-169. doi. 10.1016/j.ydbio.2004.09.014
39. Chaboissier MC, Kobayashi A, Vidal VIP, et al Functional analysis of Sox8 and Sox9 during sex determination in the mouse. *Development*. 2004;131:1891-1901. doi. 10.1242/dev.01087
40. McMillin MJ, Beck AE, Chong JX, et al Mutations in PIEZO2 cause Gordon syndrome, Marden-Walker syndrome, and distal arthrogryposis type 5. *Am J Hum Genet.* 2014;94:734-744. doi. 10.1016/J.AJHG.2014.03.015