INTRODUCTION

Pierre Robin sequence (PRS) is a triad of micrognathia, glossoptosis (frequently leading to upper airway obstruction and feeding problems at birth), and cleft palate. The literature does not offer any review of the pathogenesis of the clinical features of syndromes with Pierre Robin sequence (PRS). The senior author (MMA) proposed a hypothesis that SOX9 and its interactions may play a key role in this pathogenesis. The current review aims to test this hypothesis.

METHODS

Three literature searches were made. The first aimed to document the main syndromes associated with PRS; and the second was to document the main functions of SOX9 in development; and the third was to investigate if SOX9 and its interactions may play a role in the pathogenesis.

Results: SOX9 is the main positive regulator in the development of the mandibular cartilage and it also enhances collagen type II (the main collagen type in cartilage) expression in the mandibular cartilage. Furthermore, SOX9 participates in neural crest development, binds to the exon junction complex, and participates in sex determination. The interactions of SOX9 could explain the pathogenesis of the clinical features of syndromic PRS. These included interactions with collagen type II (in Strickler syndrome), exon junction complex (in Richier-Costa–Periera syndrome), glucose (in Catel–Manzke syndrome), RNA-binding proteins (in TARP syndrome), and the spliceosome (in cerebra-costo-mandibular syndrome). Finally, SOX9 mutations cause campomelic dysplasia.

Conclusions: The review supports the hypothesis of the participation of SOX9 in the pathogenesis of the clinical features of syndromic and nonsyndromic PRS. This should guide future research on the topic. (Plast Reconstr Surg Glob Open 2022;10:e4241; doi: 10.1097/GOX.0000000000004241; Published online 8 April 2022.)
features of the syndrome though SOX9 interactions were included in the current communication.

RESULTS

The First Literature Search: Genes Responsible for Syndromic and Nonsyndromic PRS

A summary of the common syndromes (with known gene mutations) that are associated with PRS is shown in Table 1. It was interesting to note that SOX9 mutations were the most encountered gene mutations in nonsyndromic PRS.

The Second Literature Search: Functions of SOX9 in Development

A. SOX9 is the main positive regulator in the development of the fetal mandibular cartilages:

Mandibular (Meckel’s) cartilage is derived from the first pharyngeal arch. Immuno-histochemical studies have clearly shown that the SOX9 protein is the main positive regulator in the hypertrophic differentiation process of mandibular cartilages.1 The major cartilage matrix protein is collagen type II (encoded by the gene COL2A1). At the time of mandibular development, SOX9 is coexpressed with the collagen type II in the mandibular cartilages. Although RUNX2 and OSX proteins are also coexpressed with the collagen type II in the mandibular cartilages, they do not play the main role in the development of the mandible.1

B. SOX9 is an enhancer of collagen type II expression:

As mentioned above, collagen type II is the main cartilage matrix protein. Tsuda et al2 have shown that SOX9 binds to two transcriptional co-activators (CBP and P300) leading to enhanced SOX9-dependent COL2A1 promoter activity. Disruption of this binding complex resulted in a decrease of collagen type II expression.2

C. SOX9 is upstream of micro RNA-140 in palatal cartilage development:

Table 1. Genetics of Syndromic and Nonsyndromic PRS

| Clinical Presentation | Genetics (Gene Mutations) |
|-----------------------|---------------------------|
| A. Nonsyndromic PRS   | SOX9 is the most frequently encountered gene mutation |
| B. Syndromic PRS*     | COL2A1 mutations           |
| 1. Stickler syndrome type I (16% of cases) | E1F4A3 biallelic expansion |
| 2. Richieri-Costa–Pereira syndrome (8% of cases) | TGDS mutations |
| 3. Catel–Manzke syndrome (5.5% of cases) | SOX9 mutations |
| 4. Acampomelic and acampomelic dysplasia; with or without sex reversal (4.7% of cases) | RBM10 mutations |
| 5. TARP syndrome (3% of cases) | SNRIP FB mutations |
| 6. Cerebro-costo-mandibular syndrome (2.7% of cases) | SNRPB mutations |

*The syndrome is clinically diagnosed and genetically related in less than 50% of cases of syndromic PRS. In the remaining cases, there are either chromosomal abnormalities not related to a specific syndrome or no gene mutations could be identified.

Micro RNA-140 enhances PDGF-alpha in the palatal cartilage during development. Nakamura et al3 found that micro RNA-140 is regulated by SOX9.

D. SOX9 is required for neural crest development:

Neural crest cells migrate and differentiate into various craniofacial structures, including the mandible. Liu et al4 have shown that phosphorylation of SOX9 is required for neural crest delamination.

E. SOX9 has distinct regulator roles in alternative splicing and transcription:

Girardot et al5 have shown that SOX9 binds to RNA and associates with the several RNA-binding proteins. They also demonstrated that SOX9 binds to the exon junction complex (EJC). The EJC is a protein complex (mainly composed of E1F4A3, Magoh, and Y14). During RNA splicing, exons are joined together. The EJC forms on the premessenger RNA strand at the junction of every two exons and plays a major role in translation. SOX9 binds to the EJC. Hence, SOX9 is known to alter the splicing of hundreds of genes.6 Relevant to the current review is the Y14 protein (which binds to SOX9). The Y14 protein is encoded by the RBM8A gene, which plays a major role in the pathogenesis of radial ray deficiency.6 This is relevant because syndromic patients with PRS may also have radial ray deficiency.

F. SOX9 and sex determination

Determination of the male sex starts when the Y chromosome SRY gene is expressed in the gonad. This upregulates SOX9 gene expression in the gonad. SRY–SOX9 interactions result in the differentiation of the gonad into a testis and the result is a male phenotype.7 Hence, a defective SOX9 protein may result in the inability of the gonad to differentiate into a testis in fetuses with XY karyotype. This results in a female phenotype and is known as “sex reversal.” This is relate because sex reversal is a feature of syndromic PRS. This fact may also relate to the fact that isolated cleft palate is mostly seen clinically in the female phenotype.8

The Third Literature Search: The Pathogenesis of the Clinical Features of Syndromic and Nonsyndromic PRS through SOX9 and Its Interactions

Once the functions of SOX9 are studied, the understanding of the pathogenesis of the clinical features of syndromic and nonsyndromic PRS falls into place. As seen in
Table 1, it is of no surprise that SOX9 is the most frequent gene mutation seen in nonsyndromic PRS. The following part of the review will document our findings regarding the pathogenesis of the phenotype in syndromic patients through SOX9 interactions.

A. Strickler syndrome type I (OMIM 108300)

Strickler syndrome type I is an autosomal dominant syndrome caused by mutations in the COL2A1 gene which encodes collagen type II. It is the most frequently encountered syndrome in syndromic PRS. The clinical features of the syndrome include PRS, high myopia, cataract, hearing loss, spondyloepiphyseal dysplasia, and early-onset osteoarthritis. The pathogenesis of PRS is explained by the fact that the normal development of the mandible requires the coexpression and interaction of SOX9 and collagen type II in the Meckel’s cartilage, as discussed earlier.

B. Richieri-Costa–Pereira syndrome (OMIM 268305)

About 8% of patients with syndromic PRS have this syndrome. The syndrome is inherited as autosomal recessive and is caused by biallelic expansion of a complex repeated motif in the 5’ untranslated region of the ELF4A3 gene. Clinical features include PRS, abnormal fusion of the mandible in the midline (frequently with absent lower central incisors), and preaxial ray deficiency (hypoplastic thumbs and halluces). The PRS and preaxial ray deficiency are explained by SOX9 interactions with the EJC (including EIF4A3 and the Y14 proteins) as discussed earlier.

C. Catel–Manzke syndrome (OMIM 616145)

About 5% of patients with syndromic PRS have this syndrome (see Table 1). It is inherited as autosomal recessive and is caused by TGDS gene mutations. This syndrome is also known as the micrognathia-digital syndrome and is characterized by PRS and clinodactyly of the index fingers with hyperphalangism (the finger has four phalanges). The most proximal phalanx is triangular or trapezoidal in shape, causing clinodactyly. The TGDS gene encodes the TGDS protein, which is an enzyme involved in glucose metabolism. Sun et al. have shown that glucose regulates chondrogenic differentiation via O-GlcNAcylation of SOX9.

D. Acampomelic/campomelic dysplasia with or without sex reversal (OMIM 114290)

This autosomal dominant syndrome is caused by SOX9 mutations. It is characterized by PRS, shortness of the lower limbs, hypoplastic scapulae, small chest size with abnormal thoracic cage mineralization, and tracheobronchial hypoplasia. Some patients with 46, XY have sex reversal. The syndrome is usually fatal in infancy due to respiratory failure. The role of SOX9 in mandibular development, chondrogenic differentiation, and sex determination has been previously discussed.

E. TARP syndrome (OMIM 311900)

About 3% of patients with syndromic PRS have the TARP syndrome. It is inherited as X-linked recessive and is caused by RBM10 gene mutations. As the name implies (TARP), it is characterized by talipes equinovarus, Atrial septal defect, Robin sequence, and persistence of the left superior vena cava. Some patients show limb defect such as hypoplastic radii and syndactyly. RBM10 is one of the RNA-binding proteins that participate in neural crest and craniofacial development. Specifically, RBM10 is highly expressed in the first bronchial arch which gives rise to the mandible. This mimics the high expression of SOX9 and collagen II in the developing mandible. Furthermore, SOX9 is known to associate with several RNA-binding proteins, including RBM10.

F. Cerebro-costo-mandibular syndrome (OMIM 117650)

Only 2.7% of syndromic patients with PRS have this autosomal dominant syndrome. Different mutations of the SNRPB gene cause this syndrome. The phenotype includes PRS posterior rib defects, microcephaly, and mental retardation. RNA is first synthesized in the nucleus and is known as the primary transcript and contains both exons (which will code for the final amino-acid sequence) and introns (noncoding segments). The spliceosome (a large ribonucleoprotein complex made up of several small nuclear RNAs bound to specific proteins) will then remove introns, allowing exons to join together. The SNRPB gene encodes components of the core spliceosomal machinery. It is interesting to note that SOX9 also participates in the splicing process.

DISCUSSION

The “SOX9 hypothesis” proposed by the senior author (MMA) was based on prior knowledge of two well-known facts. The primary defect in PRS is micrognathia, and SOX9 is the main player and modulator of chondrogenesis in the mandibular cartilage. Prior knowledge of these two facts brought the idea of the hypothesis of the participation of SOX9 in the pathogenesis even in syndromes caused by other gene mutations.

Our review supports the hypothesis and demonstrates that SOX9 is a possible key factor in the pathogenesis of the clinical features of nonsyndromic and syndromic PRS. The most common gene mutation in nonsyndromic cases is SOX9, and campomelic dysplasia is also caused by SOX9 mutations. In the remaining syndromes, the pathogenesis of PRS could be attributed to the interactions of SOX9 and the proteins encoded by causative genes of these syndromes, as shown in Table 2. SOX9 also have other actions during development such as modulation of cartilage precursors of various skeletal elements as well as sex determination. Hence, SOX9 mutations are also expected to cause skeletal defects in all syndromic patients with PRS (Table 3 summarizes these skeletal defects). Three syndromes (Richieri-Costa–Pereira, Catel–Manzke, and TARP syndromes) have preaxial ray defects, and two syndromes (acampanlemic-campamelic
cavity. The "relatively" large tongue will hinder the nor-
discrepancy between the volumes of the tongue and oral
mandibles.
This is interesting and requires further research in the
publications aimed to demonstrate that these interac-
tions lead to the development of the clinical features of
the syndrome. Further focused experimental research
are needed regarding this issue, and the findings of our
review may be used as a guide.

Table 2. The Pathogenesis of PRS in Syndromic Patients with PRS

| The Syndrome and Its Gene Mutation | The Pathogenesis through SOX9 Interactions |
|------------------------------------|------------------------------------------|
| Stricker syndrome type I (Col2A1 which encodes collagen II) | SOX9 interacts and modulates collagen type II (the main collagen in cartilage) in the development of the mandibular cartilage |
| Richieri-Costa-Periera syndrome (EIF4A3, part of the EJC) | SOX9 interacts with EJC (which includes EIF4A3) |
| Catel-Manzke syndrome (TGDS) | TGDS is involved in glucose metabolism. Glucose regulates chondrogenic differentiation of the mandibular cartilage via O-GlcNAcylation of SOX9 |
| Acampomelic/campomelic dysplasia (SOX9) | SOX9 is the key player in mandibular chondrogenesis |
| TARP syndrome (RBM110) | Both RBM110 and SOX9 are highly expressed and interact with each other in the developing mandible |
| Cerebro-costo-mandibular syndrome (SNRPB) | Both SNRPB and SOX9 participate in the splanchnosomal machinery |

Table 3. Skeletal Defects in Syndromic Patients with PRS

| The Syndrome | The Skeletal Defect |
|--------------|---------------------|
| Stricker syndrome type I | Spondyloepiphysial dysplasia |
| Richieri-Costa-Periera syndrome | Preaxial ray deficiency |
| Catel-Manzke Syndrome | Hyperphalangism with clinodactyl of both index fingers |
| Acampomelic-campomelic dysplasia | Abnormal hypoplastic thoracic cage, short lower limbs, hypoplastic scapulae |
| TARP syndrome | Talipes equino varus, hypoplastic radii |
| Cerebro-costo-mandibular syndrome | Posterior rib defects |

dysplasia and cerebro-costo-mandibular syndromes) have
rib defects. This indicates the participation of SOX9 in
the development of the preaxial ray and the thoracic
cage. Furthermore, the participation of SOX9 in sex
determination explains the sex reversal in acompmomelic-
campomelic dysplasia. Isolated cleft palate (without PRS)
is much more prevalent in women than in men.8
This is interesting and requires further research in the
genetics of patients with isolated cleft palate and normal
mandibles.

The pathogenesis of PRS starts with micrognathia
regardless of the etiology. The small mandible results in
discrepancy between the volumes of the tongue and oral
cavity. The “relatively” large tongue will hinder the nor-
mal vertical-to-horizontal movement of the developing
palatal shelves, resulting in cleft palate. After birth, the
relatively large tongue also leads to breathing and feed-
ing problems. The current review shows that SOX9 and
its interactions probably contribute to the pathogenesis
of the clinical features of syndromic PRS. However, sev-
eral points should be taken in consideration. The first
is related to the fact that no gene mutations could be iden-
tified in patients with nonsyndromic PRS. Hence, other
environmental (intrauterine) and intrinsic factors are
probably involved in nonsyndromic cases. The second
point is related to syndromic cases with PRS. SOX9 is the
primary factor in only one syndrome (campomelic dys-
plasia). In the remaining syndromes, other genes/pro-
teins are the primary factors, although SOX9 interactions
may explain a link to the pathogenesis. Finally, this link
was concluded from a “basic science” literature search,
demonstrating the molecular interactions of SOX9 and
the causative genes in development but none of these
publications aimed to demonstrate that these interac-
tions lead to the development of the clinical features of
the syndrome. Further focused experimental research
are needed regarding this issue, and the findings of our
review may be used as a guide.

ACKNOWLEDGMENTS
A list of abbreviations used in the article is available as
Supplemental Digital Content: http://links.lww.com/PRSGO/
C14.

REFERENCES
1. Zhang H, Zhao X, Zhang Z, et al. An immunohistochemistry
study of Sox9, Runx2, and Osterix expression in the mandibular
cartilages of newborn mouse. Biomed Res Int. 2013;2013:265380.
2. Tsuda M, Takahashi S, Takahashi Y, et al. Transcriptional co-acti-
vators CREB-binding protein and p300 regulate chondrocyte-
specific gene expression via association with Sox9. J Biol Chem.
2003;278:27224–27229.
3. Nakamura Y, He X, Kato H, et al. Sox9 is upstream of microRNA-140
in cartilage. Appl Biochem Biotechnol. 2012;166:64–71.
4. Liu JA, Wu MH, Yan CH, et al. Phosphorylation of Sox9 is
required for neural crest delamination and is regulated down-
stream of BMP and canonical Wnt signaling. Proc Natl Acad Sci
USA. 2013;110:2882–2887.
5. Girardot M, Bayet E, Maurin J, et al. SOX9 has distinct regulatory
roles in alternative splicing and transcription. Nucleic Acids Res.
2018;46:9106–9118.
6. Al-Qattan MM. The pathogenesis of radial ray deficiency in
Thrombocytopenia-Absent Radius (TAR) syndrome. J Coll
Physicians Surg Pak. 2016;26:912–916.
7. Morais da Silva S, Hacker A, Harley V, et al. Sox9 expression
during gonadal development implies a conserved role for the
genome in testis differentiation in mammals and birds. Nature Genet.
1996,14:62–68.
8. Shapira Y, Lubit E, Kufinec MM, et al. The distribution of clefts
during gonadal development implies a conserved role for the
genome in testis differentiation in mammals and birds. Nature Genet.
1996,14:62–68.
9. Varadarajan S, Balaji TM, Raj AT, et al. Genetic mutations associ-
ated with Pierre Robin syndrome/sequence: a systematic review. Med Syndromol. 2021;12:69–86.
10. Ahmad NN, Ala-Kokko L, Knowlton RG, et al. Stop codon in the procollagen II gene (Col2A1) in a family with the Stickler syndrome (arthro-opthalmopathy). Proc Nat Acad Sci. 1991;88:6624–6627.

11. Favaro FP, Alvizi L, Zechi-Ceide RM, et al. A noncoding expansion in EIF4A3 causes Richieri-Costa-Pereira syndrome, a craniofacial disorder associated with limb defects. Am J Hum Genet. 2014;94:120–128.

12. Ehmke N, Caliebe A, Koenig R, et al. Homozygous and compound-heterozygous mutations in TGDS cause Catel-Manzke syndrome. Am J Hum Genet. 2014;95:763–770.

13. Sun C, Lan W, Li B, et al. Glucose regulates tissue-specific chondro-osteogenic differentiation of human cartilage endplate stem cells via O-GlcNAcylation of Sox9 and Runx2. Stem Cell Res Therapy. 2019;10:357.

14. Kwok C, Weller PA, Guioli S, et al. Mutations in SOX9, the gene responsible for Campomelic dysplasia and autosomal sex reversal. Am J Hum Genet. 1995;57:1028–1036.

15. Johnston JJ, Sapp JC, Curry C, et al. Expansion of the TARP syndrome phenotype associated with de novo mutations and mosaicism. Am J Med Genet A. 2014;164A:120–128.

16. Forman TE, et al. The role of RNA-binding proteins in vertebrate neural crest and craniofacial development. J Dev Biol. 2021;9:34.

17. Johnston JJ, Teer JK, Cherukuri PF, et al.; NIH Intramural Sequencing Center (NISC). Massively parallel sequencing of exons on the X chromosome identifies RBM10 as the gene that causes a syndromic form of cleft palate. Am J Hum Genet. 2010;86:743–748.

18. Lynch DC, Revil T, Schwartzentruber J, et al.; Care4Rare Canada. Disrupted auto-regulation of the spliceosomal gene SNRPB causes cerebro-costo-mandibular syndrome. Nat Commun. 2014;5:4485.

19. Bacrot S, Doyard M, Huber C, et al. Mutations in SNRPB, encoding components of the core splicing machinery, cause cerebro-costo-mandibular syndrome. Hum Mutat. 2015;36:187–190.

20. Ohe K, Lalli E, Sassone-Corsi P, et al. A direct role of SRY and SOX proteins in pre-m RNA splicing. PNAS. 2002;99:1146–1151.