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SPECIAL TOPIC

Craniofacial/Pediatric

The Pathogenesis of Pierre Robin Sequence through a Review of SOX9 and Its Interactions

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Background: 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.)*

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 syndromic 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 different literature searches were made. The first identified common syndromes (with known gene mutations) that are associated with PRS; and this was done by searching Google/PubMed for the combination of two terms: "gene mutations" and "Pierre Robin sequence." The syndromes were tabulated and their clinical features were documented. In the second literature search, the terms "SOX9" and "development" were put in Google/PubMed and related publications were reviewed to summarize the main functions of SOX9 in development. Finally, the gene/protein responsible for each of these syndromes was put into Google/PubMed along with the term "SOX9." All publications that included both terms were reviewed, and only relevant ones that could explain a possible pathogenesis of the clinical

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Received for publication December 21, 2021; accepted February 10, 2022.

DOI: 10.1097/GOX.0000000000004241

Disclosure: *The authors have no financial interest to declare in relation to the content of this article.*

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. Immunohistochemical 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 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 al[2] have shown that SOX9 binds to two transcriptional co-activators (CBP and P300) leading to enhanced SOX9-dependent *COL2A1* promotor 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*	
1.Stickler syndrome type I (16% of cases)	*COL2A1* mutations
2.Richieri-Costa–Pereira syndrome (8% of cases)	*E1F4A3* biallelic expansion
3.Catel–Manzke syndrome (5.5% of cases)	*TGDS* mutations
4.Acampomelic and campomelic dysplasia; with or without sex reversal (4.7% of cases)	*SOX9* mutations
5.TARP syndrome (3% of cases)	*RBM10* 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.

Takeaways

Question: The pathogenesis of the clinical features of syndromic and nonsyndromic cases with Pierre Robin sequence has not been studied.

Findings: The pathogenesis is explored and documented through a study of related genetics.

Meaning: SOX9 is the key factor in the pathogenesis of the clinical features of syndromes with Pierre Robin sequence.

Micro RNA-140 enhances PDGF-alpha in the palatal cartilage during development. Nakamura et al[3] 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 al[4] 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 al[5] 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.[5] 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 radical ray deficiency.[6] This is relevant because syndromic patients with PRS may also have radical 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 phonotype.[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 phonotype 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 phonotype.[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.[9] The clinical features of the syndrome include PRS, high myopia, cataract, hearing loss, spondyloepiphyseal dysplasia, and early-onset osteoarthritis.[10] 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 *EIF4A3* 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).[11] 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.[12]

The *TGDS* gene encodes the TGDS protein, which is an enzyme involved in glucose metabolism. Sun et al[13] 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.[14] 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.[15]

RBM10 is one of the RNA-binding proteins that participates in neural crest and craniofacial development.[16] Specifically, RBM10 is highly expressed in the first bronchial arch which gives rise to the mandible.[17] 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.[5]

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.[18] 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.[19] It is interesting to note that SOX9 also participates in the splicing process.[20]

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.[1] 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*,[9] and campomelic dysplasia is also caused by *SOX9* mutations.[14] 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[2] as well as sex determination.[7] 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–Periera, Catel–Manzke, and TARP syndromes) have preaxial ray defects, and two syndromes (acampomelic-campamelic

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

The Syndrome and Its Gene Mutation	The Pathogenesis through SOX9 Interactions
Strickler 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 (*RBM10*)	Both RBM10 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 spliceosomal machinery

Table 3. Skeletal Defects in Syndromic Patients with PRS

The Syndrome	The Skeletal Defect
Strickler syndrome type I	Spondyloepiphyseal dysplasia
Richieri-Costa–Periera syndrome	Preaxial ray deficiency
Catel–Manzke Syndrome	Hyperphalangism with clinodactyly 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 acompomelic-campamelic 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 normal vertical-to-horizontal movement of the developing palatal shelves, resulting in cleft palate. After birth, the relatively large tongue also leads to breathing and feeding problems. The current review shows that SOX9 and its interactions probably contribute to the pathogenesis of the clinical features of syndromic PRS. However, several points should be taken in consideration. The first is related to the fact that no gene mutations could be identified 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 dysplasia). In the remaining syndromes, other genes/proteins 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 interactions 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.

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ACKNOWLEDGMENTS

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

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