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Understanding the Role of *Tbx1* as a Candidate Gene for 22q11.2 Deletion Syndrome

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Abstract

22q11.2 deletion syndrome (22q11.2DS) is caused by a commonly occurring microdeletion on chromosome 22. Clinical findings include cardiac malformations, thymic and parathyroid hypoplasia, craniofacial dysmorphisms, and dental defects. These phenotypes are due mainly to abnormal development of the pharyngeal apparatus. Targeted deletion studies in mice and analysis of naturally occurring mutations in humans have implicated *Tbx1* as a candidate gene for 22q11.2DS. Tbx1 belongs to an evolutionarily conserved T-box family of transcription factors, whose expression is precisely regulated during embryogenesis, and it appears to regulate the proliferation and differentiation of various progenitor cells during organogenesis. In this review, we discuss the mechanisms of *Tbx1* during development of the heart, thymus and parathyroid glands, as well as during formation of the palate, teeth, and other craniofacial features.

Keywords

22q11.2 Deletion syndrome; DiGeorge syndrome; Velocardiofacial syndrome; Tbx1; Pharyngeal apparatus; Cardiovascular; Pharyngeal arch artery; Odontogenesis; Palatogenesis; Proliferation; Craniofacial; Thymus; Parathyroid; Differentiation; Candidate gene

Introduction

22q11.2DS is the unifying term for patients with a microdeletion within the proximal long arm of chromosome 22 and manifests as DiGeorge syndrome (DGS), velocardiofacial syndrome (VCFS), and conotruncal anomaly face syndrome. With an incidence of 1 in every 4,000 newborns, 22q11.2DS is the most frequent microdeletion syndrome and occurs sporadically in most patients [1]. Clinical features are highly variable with respect to penetrance and expressivity. Characteristic features include congenital heart defects (CHD), thymic and parathyroid hypoplasia and aplasia, craniofacial dysmorphisms, cleft palate, and

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Compliance with Ethics Guidelines

Conflict of Interest

Shan Gao, Xiao Li, and Brad A. Amendt declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human subjects performed by any of the authors. With regard to the author's research cited in this paper, all institutional and national guidelines for the care and use of laboratory animals were followed.

learning disabilities [2, 3]. Less commonly tooth defects, (including enamel hypoplasia, hypomineralization, small teeth and missing teeth) are observed [4].

Approximately 90 % of 22q11.2DS patients are hemizygous for a 3-Mb (mega-base) deletion that, encompasses over 40 genes, and most of the remaining patients are hemizygous for a smaller (1.5-Mb) deletion that encompasses 30 of these genes. The uniformity in size of the deletions has been attributed to the four large, complex, and highly homologous regions known as low-copy repeat (LCR) units [5]; their mispairing and facilitation of unequal meiotic exchange and non-random asynchronous replication within the 3-Mb region is thought to generate chromosomal rearrangements with little variety [5, 6].

Tbx1 as a Candidate Gene for 22q11.2DS

The deleted region in 22q11.2 is highly conserved on mouse chromosome 16. A 1999 study revealed that mice with a hemizygous deletion of this portion of chromosome 16 (*Df1/+*) exhibit mild defects in the cardiac outflow tract (OFT) [7]. Subsequently, three research groups identified *Tbx1* as the candidate gene for 22q11.2DS using segmental deletions and single-gene knockouts in mice [8-10]. Specifically, the cardiac anomalies observed in $Tbx1^{+/-}$ mice were identical to those found in *Df1/+* mice [8], and $Tbx1^{-/-}$ mice display cardiac, craniofacial, thymic, and parathyroid defects, reminiscent of those seen in patients with severe forms of DGS/VCFS [8–10]. In light of these findings from mouse genetic studies, *TBX1* became the focus of mutation analysis in patients with DGS/VCFS-like characteristics and Yagi et al. identified *TBX1* point mutations in several individuals with characteristic 22q11.2DS phenotypes [11]. The extensive evidence gathered from these studies strongly supports the notion that *TBX1* drives 22q11.2DS.

Tbx1 belongs to a large family of transcription factors whose members share a conserved DNA binding domain known as the T-box [12]. *Tbx1* expression varies both spatially and temporally across tissues, and gene dosage studies in mice have shown that *Tbx1* expression during embryogenesis requires precise regulation [13]; expression analyses have dynamic *Tbx1* expression in tissues that form the pharyngeal apparatus (including the surface ectoderm, pharyngeal endoderm, and non-neural crest-derived mesoderm), which gives rise to the heart and face [14–16]. A dose-response study revealed that as *Tbx1* transcript levels decrease phenotype severity increases [17]; and overexpression analyses showed that increases in *Tbx1* transcript levels also result in malformations similar to those observed in 22q11.2DS patients [18, 19]. This review focuses on mechanisms whereby *Tbx1* is regulated during development of the heart, thymus and parathyroid glands, and craniofacial features including the palate and teeth.

Tbx1 in Development of the Pharyngeal Arch Arteries

The main cause of death in 22q11.2DS patients is congenital heart defects (CHDs), which affect the following tissues: the cardiac OFT (resulting in Tetralogy of Fallot and persistent truncus arteriosus); the fourth pharyngeal arch artery (PAA; resulting in interrupted aortic arch type B, IAA-B); and the right subclavian artery (resulting in aberrant origin of the right subclavian artery, ARSA) (reviewed in [20]). Moreover, most 22q11.2DS phenotypes, including CHDs, are caused by defects in development of the pharyngeal apparatus during early embryogenesis. The pharyngeal apparatus consists of the vertebrate-specific transient pharyngeal arches (PAs), which are lined externally with ectoderm (clefts) and internally with endoderm (pouches). These mesoderm-derived arches are visible as bilaterally symmetric bulges that form in a segmental pattern along the cranial-caudal axis, and each consists of a PAA.

Tbx1$^{+/-}$ mice display defective fourth PAAs, whereas *Tbx1*$^{-/-}$ mice lack PAs II-VI, PAAs 2-6, and pharyngeal pouches III-IV (Fig. 1) [8–10]. During normal development, the fourth PAA undergoes extensive remodeling to produce the asymmetric aortic arch and great vessels. Using cell-fate mapping and tissue-specific gene deletion driven by a variety of Cre lines, Zhang et al. determined that the development of the fourth PAA requires early cell-autonomous *Tbx1* expression in both the surface ectoderm and the pharyngeal endoderm [21]. Notably, although all *Tbx1*$^{+/-}$ mice all have hypoplastic fourth PAAs at E10.5, only 30–50 % have associated defects at birth, and even those phenotypes are less severe than those observed in 22q11.2DS patients [8]. The extensive phenotypic variability in both humans and mice supports the existence of modifier genes on chromosome 22 and other non-deleted regions.

One *Tbx1* modifier in the context of PAA development is retinoic acid (RA), the active derivative of vitamin A (reviewed in [22]). RA is synthesized by retinaldehyde dehydrogenases (RALDHs) and is degraded by the 26s class of cytochrome P450 proteins (CYP26s). In the developing PAs of the chick embryo, both the application of exogenous RA and reduction of RA levels repress *Tbx1* expression [23]. Consistent with these findings, *Raldh2*-null mice, which express very little RA during embryogenesis, were found to exhibit DGS-like aortic arch defects [24]. Tbx1 has also been implicated in the repression of RA signaling in experiments involving the down-regulation of Raldh2 [25] and the up-regulation of Cyp26 enzymes [26]. Interestingly, in *Tbx1*$^{+/-}$ mice with a decrease in RA levels, the penetrance of defects in the fourth PAA was reduced [25]. Thus, it appears that a precise balance between *Tbx1* expression and RA signaling is crucial for PAA development, and a reduction in RA levels contributes to early recovery of DGS-related aortic arch defects.

In addition to acting on *Tbx1* directly, RA also affects interactions between *Tbx1* and other genes during embryogenesis. CRK-like (CRKL), an adaptor protein that functions downstream of several classes of receptor tyrosine kinases, is also located within the 3-Mb deleted region of 22q11.2DS. *Crkl*-null mice exhibit defects in both the PAAs and the cardiac OFT [27]. Moreover, neonatal *Tbx1*$^{+/-}$*X Crkl*$^{+/-}$ mice have a higher incidence of aortic arch malformation and exhibit more extreme phenotypes than either *Tbx1*$^{+/-}$ or *Crkl*$^{+/-}$ mice alone, and the phenotype of the compound heterozygote is partially rescued by a decrease in RA production [28]. Thus, dosage-sensitive interactions between *Tbx1, Crkl*, and locally expressed RA affect development of the PAs.

Chromatin modifiers also contribute to *Tbx1* regulation during PAA development. In mice, a lack of *Moz,* a member of the Myst family of histone acetyltransferases, recapitulates many 22q11.2DS phenotypes. Interestingly, the severity of these phenotypes increases with an increase in RA. Also, a *Tbx1* transgene is able to rescue the *Moz*-null cardiac defects [29]. This study also showed that the Moz complex is required for transcription of the *Tbx1* locus in vivo. A second chromatin remodeling protein to which *Tbx1* expression has been linked is chromodomain helicase DNA-binding protein 7 (*Chd7*), which is mutated in CHARGE syndrome. *Tbx1* and *Chd7* are both required in the pharyngeal ectoderm for the growth and remodeling of the fourth PAA. Both act cell-non-autonomously in this context, signaling to the mesenchymal cells that populate the PAs [30].

Neuroepithelium-derived cardiac neural crest cells (cNCCs) are needed for proper septation of the OFT into the aorta and pulmonary trunk, as well as for development of the cardiac valves, and the connective tissues that surround the thymus and parathyroid glands. cNCCs migrate through the PAs and differentiate into the vascular smooth muscle (VSM) layer through which the PAAs pass (reviewed in [31]). Various signaling pathways play roles in the migration, proliferation, and differentiation of the cNCCs. Although, *Tbx1* is not expressed in these cNCCs, it regulates their migration in a non-cell-autonomous manner, by

activating expression of the homeobox-containing gene *Gbx2* in the pharyngeal surface epithelium [32]. *Gbx2* is important for both development of the fourth PAA and proper cNCC migration into the PAs; cNCC patterning defects in mouse embryos lacking this gene lead to aortic arch defects such as IAA-B [33]. *Tbx1/Gbx2* signaling is thought to regulate cNCC migration during PAA formation via the *Slit/Robo* signaling pathway [32].

Tbx1 has also been shown to influence cNCCs by interacting with bone morphogenetic proteins (Bmps) to affect differentiation of these cells. Bmps constitute a large proportion of the secreted factors that comprise the transforming growth factor β (Tgfβ) family. *Tbx1* interacts genetically with *Smad7,* an inhibitor of the Tgfβ/Bmp pathway, to trigger cNCCs to differentiate into VSM cells, and to subsequently produce extracellular matrix (ECM) around the fourth PAA. Heterozygosity for *Tbx1* and *Smad7* is associated with a ecrease in formation of both VSM and ECM, resulting in higher proportion of fourth PAA deformities [34]. Together with Gbx2 and Smad7, Tbx1 regulates the migration and differentiation, respectively, of cNCC during development of the fourth PAA.

Tbx1 in Cardiac Outflow Tract Development

Tetralogy of Fallot and persistent truncus arteriosus are defects of the cardiac OFT that occur in 22q11.2DS patients. Formation of the OFT is a complex process that requires precisely regulated spatiotemporal expression of various genes in several cell types. *Tbx1* is required in the pharyngeal mesoderm [35]; the OFT phenotypes observed in $Tbx1^{-/-}$ mice are associated with decreased cell proliferation and premature differentiation [36]. Recently, a reservoir of cardiac progenitor cells that gradually migrates and populates the OFT, right ventricle, and parts of the atria was identified in the pharyngeal mesoderm (reviewed in [37]). Known as the “second heart field” (SHF), this structure is dependent on Tbx1 expression for its development [13, 38]. Tbx1 cell-autonomously maintains the cardiac progenitor population within the SHF by enhancing cell proliferation and inhibiting cardiomyocyte differentiation [39, 40]. Tbx1 networks with other signaling factors to keep the SHF cells in a progenitor state and to promote the progressive addition of these cells to the heart [41, 42].

Maximal cell proliferation in the SHF also requires Sonic hedgehog (Shh), which is expressed in the ventral pharyngeal endoderm [42]. *Shh*-null mice lack *Tbx1* expression, and. in the chick embryo exposure of the pharyngeal epithelium to Shh-soaked beads. Up-regulates *Tbx1* [43]. Shh acts on Tbx1 via a conserved element upstream of *Tbx1* that can be bound by winged helix/forkhead (Fox) transcription factors such as *Foxc1, Foxc2,* and *Foxa2* [16]. Interestingly, in the pharyngeal mesoderm of $Tbx1^{-/-}$ mice *Foxa2* is down-regulated, suggesting that the Fox and Tbx1 proteins are part of an auto-regulatory loop [44]. However, a recent study showed that the conserved *Fox* binding element is not required for endogenous *Tbx1* expression in the pharyngeal mesoderm [45]. Nevertheless, these findings indicate that Tbx1 positively regulates SHF cell proliferation by interacting with the Shh pathway.

Tbx1 also positively regulates cell proliferation within the SHF through transcriptional regulation of *fibroblast growth factor 8 (Fgf8), Fgf10,* and *Fgf receptor 1 (Fgfr1)*. Fgf8 and Fgf10 both drive SHF proliferation [46,47], and Tbx1 positively modulates *Fgf8* and *Fgf10* expression in the SHF mesoderm [40, 44, 48]. Studies have shown that Tbx1 binds to the first intron of *Fgf10* to regulate its expression in the SHF [47]. The loss of *Tbx1* disrupts the expression of *Fgfr1*[46], and Crkl interacts with Fgfr1 to mediate Fgf8 signaling [49]. Another study shows that decreased *Fgf8* function enhances the DGS/VCSF phenotypes observed in $Tbx1^{+/-}X\ Crkl^{+/-}$ mice [28]. Collectively, these data demonstrate that Tbx1 interacts with Shh and Fgf signaling pathways to positively regulate SHF proliferation.

Recent research has shown that Bmp signaling promotes OFT myocardial differentiation and that it does so by regulating the activity of microRNAs [50], which are essential regulators of embryogenesis. Specifically, Bmp signals through a conserved Smad-binding element to regulate miR-17-92, which results in down-regulation of *Tbx1* expression. Smad1 is a critical negative regulator of SHF proliferation in vivo, and its ablation in the SHF enhances cell proliferation [51]. Tbx1 binds to Smad1 and negatively modulates the Bmp–Smad signaling pathway by interfering with the Smad1–Smad4 interaction [52]. A bidirectional negative feedback loop links Bmp, Smad, miR-17-92, and Tbx1. Tbx1 inhibits Bmp signaling to maintain the cardiac progenitor population in the SHF, and to prevent immature myocardial differentiation.

Tbx1 inhibits SHF differentiation by antagonizing not only the Bmp pathway but also the activities of Mef2c and serum response factor (Srf). Mef2c is key transcription factor in OFT myocardial differentiation [53], and Srf is a myogenic transcription factor that regulates muscle differentiation. Tbx1 interacts with Srf proteins, decreasing protein stability and increasing proteasome-mediated degradation of Srf protein levels [40]. Together, these findings indicate that Tbx1 is precisely regulated in the SHF, and that it maintains the balance between the cardiac progenitor population and differentiating cardiac cells by interacting with proproliferation and pro-differentiation factors during development of the OFT.

Within the SHF, *Tbx1* also plays a role in left-right (L-R) asymmetry. *Pitx2,* a *bicoid/*paired-related homeobox transcription factor, regulates L-R asymmetry during organ morphogenesis and is expressed in the left but not right portion of the SHF [54]. In $Tbx1^{-/-}$ mice, Pitx2 was down-regulated in the left SHF [55]. Tbx1, along with transcription factor Nkx2.5, maintains the asymmetric expression of *Pitx2c* in cardiac progenitor cells, and is thus implicated in L-R asymmetry [55].

Finally, studies of *Wnt5a* mutations which lead to defects in the outflow tract in both mice and humans have implicated chromatin modifiers in development of the OFT. *Wnt5a* encodes a ligand of the non-canonical Wnt pathway and is a transcriptional target of *Tbx1* [56, 57]. Notably, Tbx1 recruits both Baf60a, (a subunit of the BAF chromatin-remodeling complex), and histone modifier Setd7 to Wnt5a, and this results in the monomethylation of histone 3 lysine 4 in the T-box binding element of the Wnt5a gene, which leads to enhanced Wnt5a transcription [56]. Thus, Tbx1 interacts with chromatin modifiers during the development of both the fourth PAA and the OFT.a

Tbx1 in Development of the Thymus and the Parathyroid Glands

$Tbx1^{-/-}$ mice have severe pathologies in tissues derived from the third pharyngeal pouch, including hypoplasia of the thymus and parathyroid glands [8–10]. Thymic epithelial cells derived from the endodermal lining of the pharyngeal pouch differentiate into various subpopulations of cells of the thymus [58]. NCC also migrate into the third pharyngeal pouch during the patterning of thymic and parathyroid regions. Epithelial–mesenchymal crosstalk between thymic epithelial cells and neural crest-derived mesenchymal cells is essential for correct pharyngeal segmentation and the development of the thymus. However, the specific molecular signals necessary remain unclear.

The critical time for *Tbx1* expression during pharyngeal segmentation of the thymus is E9–E9.5 [13]. If *Tbx1* is deleted at E10.5, a hypoplastic thymus forms and deletion after E11.5 does not impact thymus morphogenesis [13]. The homeobox gene *Six1* is expressed in the third pharyngeal pouch, and the *eye absent* gene *Eya1* is expressed in both the third and fourth pouches. *Eya1*-null mice lack both the thymus and parathyroid glands, whereas *Six1*-null mice lack only the thymus. The Eya1 protein is required for early initiation of thymus/

parathyroid organogenesis, whereas Six proteins are required for an early differentiation step [59]. The expression of both *Tbx1* and *Fgf8* are down-regulated in *Eya1*-null mice and slightly down-regulated in heterozygous *Six1;Six4* mice [59]. Recently, one group suggested that Tbx1 acts upstream and *Fgf8* downstream of the *Six1-Eya1* pathway [60]. Overall, the existing data indicate that Tbx1 interacts with signaling factors such as *Fgf8* and additional transcription factors during development of both the thymus and the parathyroid glands.

Tbx1 in Palatogenesis

Craniofacial malformations occur in more than half of 22q11DS patients, and various degrees of cleft palate (complete, submucosal, and soft) are among the most frequent features [61]. In mice, *Tbx1* is expressed in the developing facial regions during late embryogenesis specifically within the epithelial regions of the tooth germs, palatal shelves, facial processes, and hair follicles. Palatogenesis involves a sequence of critical events including the growth, elevation, elongation, and fusion of the palatal shelves. [62], specifically fusion of the primary palate (frontonasal process) and of the secondary palatal shelves (maxillary processes). Timed deletion studies show that secondary palate formation requires *Tbx1* expression around E11.5–E12.5, which coincides with the initial development of the secondary shelves [13]. $Tbx1^{-/-}$ mice exhibit abnormal epithelial adhesion between the palate and the mandible, leading to clefts similar to those observed in 22q11DS and inhibited elongation and elevation of the palatal shelves. *Tbx1* regulates the balance between proliferation and differentiation in the epithelium of the palatal primordium and is essential for palatal fusion [63, 64]. *Cyp26b1*-null mice, which produce excess RA also exhibit cleft palate in association with decreased expression of Tbx1 in the palatal primordium. Thus, a precise balance between RA and Tbx1 is necessary during palatogenesis [65].

Tbx1 in Odontogenesis

Dental anomalies such as enamel hypoplasia and hypomineralization, hypodontia, and aberrant tooth shapes have been documented in 22q11DS patients [4]. Traditionally, enamel disturbances in 22q11DS patients were thought to be a secondary effect of the hypocalcemia caused by hypoparathyroidism. However, a recent study concluded that a diagnosis of hypoparathyroidism did not correlate with the prevalence of enamel anomalies [66]. Tooth morphogenesis involves a series of sequential and reciprocal epithelial–mesenchymal interactions. Four layers form the dental epithelium: the stratum intermedium, the stellate reticulum (SR), the outer enamel epithelium (OEE), and the inner enamel epithelium (IEE). The IEE cells mature into enamel-secreting ameloblasts, leading to matrix deposition and subsequent mineralization of the enamel [67]. Each incisor has two cervical loops (CLs) at its proximal end; these are known as the labial and lingual CLs (Fig. 2).

Tbx1 interacts with transcription factors and signaling molecules to regulate the proliferation, differentiation, and maturation of ameloblasts. *Tbx1* expression is restricted to the IEE at E12.5, and its expression is maintained by mesenchymal Fgf signaling [68]. In $Tbx1^{-/-}$ mice, the molars, incisors, and labial CLs are significantly smaller than in wild-type animals due to a decrease in cell proliferation (Fig. 2). Moreover, the dental cells are unable to differentiate into ameloblasts, and amelogenin, a major protein component of the enamel matrix secreted by ameloblasts, has significantly reduced expression [69, 70]. Axenfeld–Rieger syndrome is an autosomal dominant disorder associated with *PITX2* mutations, and is characterized by dental, eye, and umbilical anomalies. *Pitx2* is the earliest transcription factor detected during tooth development, and its expression is restricted to the dental epithelium [71]. Cao et al. showed that Tbx1 can bind to the C-terminal tail of Pitx2, and this attenuates Pitx2 transcriptional activation of itself and p21, a cyclin-dependent kinase

inhibitor involved in the differentiation of dental cells [69]. Thus, Tbx1 promotes dental cell proliferation through the Pitx2–p21 pathway.

Unlike human teeth, the mouse incisor has the unique ability to regenerate throughout its lifetime. It thus represents an excellent model for examining the epithelial stem cell niche that resides in the labial and lingual CLs. On the labial side, the self-renewing stem cells give rise to transit-amplifying cells in the IEE region that differentiate into mature ameloblasts [72]. The expression of *Tbx1* in the CL suggests that it may play a role in regulating the stem cell niche. Consistent with such a role, Tbx1 acts as an on–off switch for Bmp signaling in the hair follicle, another ectoderm appendage, regulating the transition of follicular stem cells between the quiescent and proliferative states [73]. Studies using conditional knockout and conditional overexpression of *Tbx1* in the dental epithelium are underway, to elucidate its role in the proliferation and maintenance of the dental stem cell niche of the mouse incisor. *Sox2*, a transcription factor that plays a critical role in stem cell maintenance in other organs, is expressed at high levels in the labial CL from E16.5 to PN20 [74]. Recently, *Sox2* was shown to specifically mark the epithelial stem cells in labial CLs, and *Sox2*-positive progenitor cells were found to contribute to all cell lineages of the dental epithelium [75]. It is likely that *Tbx1* plays a role in maintaining epithelial stem cells by regulating *Sox2* (unpublished data).

Recent research has focused on the role of microRNAs in regulating the expression of factors key to tooth development. The ~70-nucleotide stem loop precursor of microRNAs (pre-microRNA) is cleaved by the RNase Dicer1, within the cytoplasm, to generate a ~21- to 25-nucleotide mature microRNA strand [76]. The deletion of epithelial *Dicer-1* in mice leads to aberrations in molar cusping, expansion of the CLs, and defects in ameloblast differentiation [77, 78]. In addition, different subsets of microRNAs are expressed in the CLs, dental mesenchyme, and differentiated ameloblasts [78, 79]. MicroRNAs may play a role in ameloblast proliferation and differentiation by regulating the expression of *Tbx1*, and it is possible that microRNAs and Tbx1 function in an auto-regulatory manner. Studies are currently underway to identify the specific microRNAs that regulate *Tbx1* expression during tooth development as well as any downstream microRNAs that might be regulated by Tbx1 during tooth development.

Tbx1 in Other Aspects of Craniofacial Development

Other 22q11DS craniofacial malformations include ocular hypertelorism, blunted nose, ear abnormalities, and micrognathia [4,11]. *Chordin* (*Chrd*) is a Bmp antagonist, and *Chrd*-null mice display phenotypes similar to those observed in $Tbx1^{-/-}$ embryos [80]. In the pharyngeal region of *Chrd*-null mice, levels of the *Tbx1* and *Fgf8* mRNAs are reduced, and in *Xenopus* embryos, overexpression of the *Chrd* mRNA leads to increased expression of both *Tbx1* and *Fgf8* [80]. One study found a linked mutation on the *Tbx1* locus ($Tbx1^{G>T}$) in a $Chrd^{+/-}$ mouse strain, which causes mis-splicing. The phenotypes of the $Tbx1^{G>T/G>T}$ mice are similar to those of the $Tbx1^{-/-}$ mice, except that they do not include major craniofacial defects unless *Chrd* is also absent [81]. Thus, *Chrd* appears to be a second-site modifier of the craniofacial anomalies observed in $Tbx1^{G>T/G>T}$ mutants, and *Chrd* loss of function enhances the craniofacial phenotype observed in $Tbx1^{-/-}$ mice. This finding is consistent with other studies showing that *Tbx1* mutant phenotypes can be enhanced by excessive Bmp signaling.

Given that the pharyngeal mesoderm gives rise to the progenitor cells of both SHF and branchiomeric muscles (reviewed in [37]), it is not surprising that the heart and craniofacial skeletal muscles share transcriptional programs during organogenesis. A recent study identified a hierarchical network of transcription factors expressed in the pharyngeal

mesoderm, and showed that it coordinates both normal heart and craniofacial development, including genetic interactions between *Tbx1, Pitx2, Lhx2*, and *Tcf21* [82]. This study further concluded that *Tbx1* levels can be fine-tuned by interactions with other transcription factors, and suggested that those transcription factors may be modifiers of 22q11.2DS [82].

Conclusions

Over the last decade, much progress has been made in defining the genetic, molecular, and cellular roles of *Tbx1,* and toward establishing how this gene contributes to the cardiovascular, thymic, parathyroid, craniofacial, palatal, and dental phenotypes observed in 22q11.2DS. The findings from these studies have underscored the importance of precisely orchestrated spatiotemporal control of the signaling and transcription factors that regulate embryogenesis. The key functions of *Tbx1* in regulating cell fate, proliferation, and differentiation during organogenesis in various tissues have been found to depend on interactions with a variety of transcription factors, microRNAs, and signaling pathways. *Tbx1* regulates and maintains a balance between the proliferation and differentiation of progenitor cells. The genetic basis for the high variability and penetrance of symptoms in 22q11.2DS patients remains unknown. However, it seems likely that the influence of unknown modifier genes and epigenetic factors that affect *Tbx1* expression during development accounts for at least some of this. The challenge continues to be to translate current molecular knowledge about *Tbx1* into clinical applications that will aid 22q11.2DS patients. Current clinical care strategies focuses on optimizing the alleviation of the symptoms observed in 22q11.2DS patients after birth. However, future research on the genetic and cellular mechanisms in which *Tbx1* participates is expected to make it possible to prevent these symptoms from developing in the fetus leading to enhanced quality of life of the adult.

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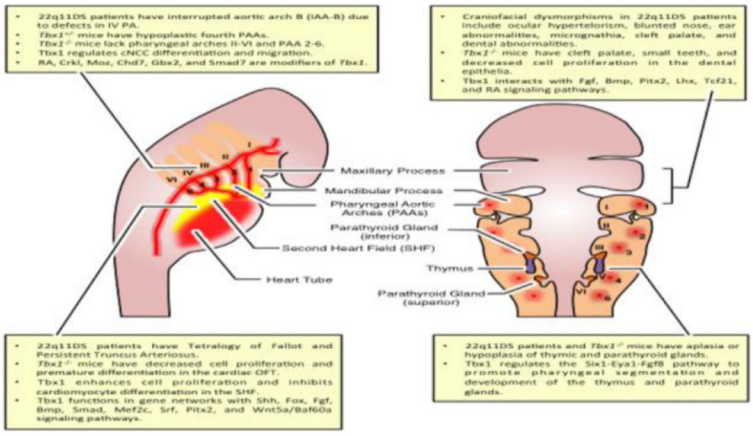

Fig. 1.

Clinical manifestations of 22q11.2DS and the corresponding Tbx1 function during organogenesis. Lateral (*left panel*) and frontal views (*right panel*) of a human embryo. *I–VI* indicate the pharyngeal arches (*PA*), numbers *1–5* indicate the pharyngeal arch arteries (*PAA*)

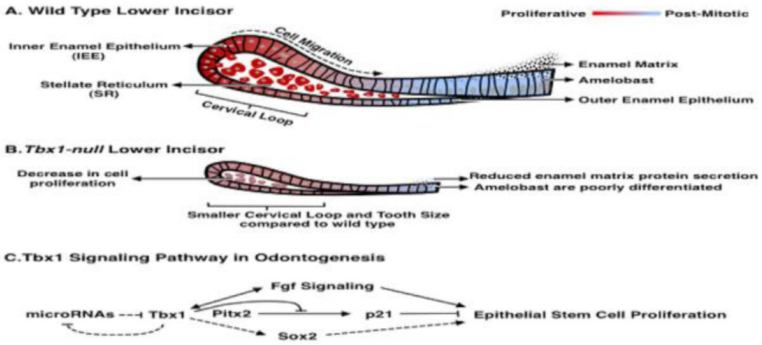

Fig. 2.

Mouse odontogenesis and Tbx1 function. **a** In the mouse lower incisor, the epithelial stem cells proliferate in the stellate reticulum and are continually inserted into the basal layer of the epithelium that encompasses this structure. The epithelial cells migrate towards the anterior, differentiate into ameloblasts and exit the mitotic cycle. The mature ameloblasts deposit enamel proteins. **b** *Tbx1*$^{-/-}$ mice develop significantly smaller teeth and labial CLs. Both cell proliferation and differentiation in the epithelium of the *Tbx1*$^{-/-}$ teeth are dramatically decreased. Enamel protein expression and secretion are severely reduced. **c** The molecular signaling network whereby Tbx1 regulates proliferation and differentiation in the dental epithelium. Tbx1 act as an upstream activator of the Fgf signaling pathway and Sox2 to initiate and maintain epithelial stem cell proliferation. Tbx1 negatively regulates p21 expression by inhibiting its activator, Pitx2, to promote proliferation. Unpublished data suggests that Tbx1 is also regulated by microRNAs and that it is involved in a feedback mechanism