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Review Article

22q11.2 Deletion Syndrome: Unmasking the Role of Tbx1 in Craniofacial Development

Funato N*

Human Gene Sciences Center, Tokyo Medical and Dental University, Japan

***Corresponding author:** Funato N, Research Center for Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan

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Abstract

T-box transcription factor gene (*TBX1*) is thought to be responsible for chromosome 22q11.2 deletion syndrome (DiGeorge/velocardiofacial syndrome), which is characterized by craniofacial defects, cardiac malformations, thymic and parathyroid hypoplasia, and cleft palates. Tbx1 regulates the cell fate of progenitor cells in cranial and pharyngeal tissues during embryogenesis. In this review, I discuss the mechanisms of Tbx1 during craniofacial development of tissues including the palate, bones, teeth, and muscle.

Keywords: DiGeorge syndrome; Velocardiofacial syndrome; Pharyngeal arch; Palatogenesis; Bone anomalies; Cleidocranial dysplasia

Abbreviations

22q11.2DS: 22q11.2 deletion syndrome; CP: cleft palate; SNP: single nucleotide polymorphism; CCD: cleidocranial dysplasia; CL: cervical loop; FGF: fibroblast growth factor

Introduction

TBX1, a member of the T-box transcription factor gene family, is considered to be a candidate gene for chromosome 22q11.2 deletion syndrome (22q11.2DS). 22q11.2DSmanifests as DiGeorge syndrome (OMIM 188400), velocardiofacial syndrome (OMIM 192430), and conotruncal anomaly face syndrome (OMIM 217095).22q11.2DS is the most frequent micro-deletion syndrome, affecting approximately 1 in 4000 live births, and is characterized by a series of phenotypic abnormalities, including craniofacial anomalies, cardiovascular defects, thymic and parathyroid hypoplasia, velopharyngeal insufficiency, and skeletal muscle hypotonia [1-4]. Structures primarily affected in 22q11.2DS are derivatives of the pharyngeal arches and head mesenchyme [4,5].

Tbx1 is expressed in the pharyngeal tissues, including mesoderm, ectoderm, and endoderm, and throughout the head mesenchyme in mice [6-8] (Figure 1A). *Tbx1*knockout (*Tbx1*$^{-/-}$) mice exhibit most features of 22q11.2DS, including cardiac, craniofacial, thymic, and parathyroid defects, and skeletal muscle hypotonia [1-3,8,9]. Craniofacial anomalies occur in ~60% of 22q11.2DS patients [5]; the most frequent craniofacial defects include micrognathia, ear abnormalities, hypertelorism, blunted nose, various degrees of Cleft Palate (CP), and tooth defects [5,10,11]. This review focuses on the functions of Tbx1incraniofacial development.

Tbx1 and cleft palate

CP is the most frequent craniofacial birth defects in humans, occurring in 1 in 500 to 1000 live births worldwide [6]. The craniofacial malformations observed in 22q11DS patients include various subtypes of CP (complete CP, incomplete CP, sub mucosal CP, and bifid uvula). *TBX1* mutations have been identified in patients with characteristic phenotypes of 22q11.2DS, conotruncal anomaly face syndrome (OMIM 217095), and nonsyndromic CP [12,13]. These findings suggest that *TBX1* is a potential candidate gene for various degrees of nonsyndromic CP. Indeed, two adjacent Single Nucleotide Polymorphisms (SNPs) upstream of *TBX1* suggest a potential association with the CP phenotype, although they are not significant after correcting for multiple testing [14]. Regulatory elements for *TBX1* expression on 22q11.2 may also be involved in the CP phenotype.

Tbx1 is expressed in both the anterior and posterior edges of the paired palatal shelves in mice, highlighting the intrinsic function of Tbx1 in regulating palatogenesis. Deletion of *Tbx1* results in abnormal epithelial fusion between the palatal shelves and the mandible, which induces CP by inhibiting elevation of the palatal shelves [15]. *Tbx1*$^{-/-}$ mice present various degrees of CP phenotype, including complete CP, incomplete CP, submucosal CP, and anterior

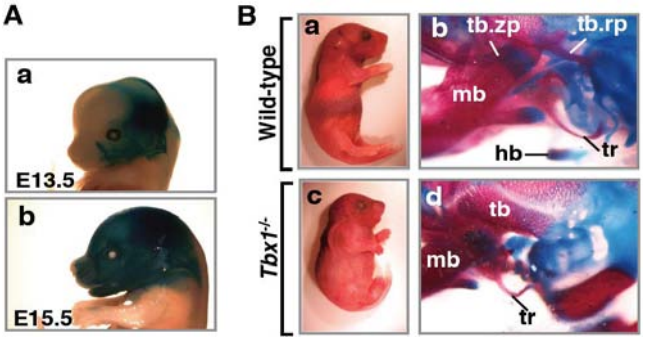

Figure 1: Craniofacial malformations in *Tbx1*mutants.
(A) Lateral view of the head of whole-mount lacZ-stained *Tbx1-Cre; R26R* mice at E13.5 (a) and E15.5 (b). LacZ-positive cells are widely detectable in the head. **(B)** Craniofacial phenotype of *Tbx1*$^{-/-}$ mice. (a,c) Appearance of P1 wild-type (a) and *Tbx1*$^{-/-}$ (c) mice. *Tbx1*$^{-/-}$ mutants (c) are small compared to wild-type mice (a).(b,d) Alizarin red (for mineralized bone) and alcian blue (for cartilage) staining of wild-type (b) and *Tbx1*$^{-/-}$ (d) bones of the neck. In *Tbx1*$^{-/-}$ mice, the hyoid bone is a plastic, and the tympanic ring and the processes of the temporal bone are hypoplastic (d). hb, hyoid bone; tr, tympanic ring; tb, temporal bone; tb.rp, retroarticular process of temporal bone; tb.zp, zygomatic process of temporal bone; mb, mandibular bone.
All animal experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Tokyo Medical and Dental University and University of Texas Southwestern Medical Center.

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Table 1: Skeletal and palatal phenotypes of human genetic disease and mouse mutants.

Gene	Genetic Disease/Mice	Palatal phenotype	Skeletal phenotype
TBX1	22q11.2DS (OMIM 188400, 192430, 217095, 217095)	CP, submucosal CP, bifid uvula	Short stature, microcephaly, retrognathia, micrognathia, blunted nose, hypoplastic nasal alae
RUNX2	CCD syndrome (OMIM 119600)	Submucosal CP	CCD syndrome*
Tbx1	*Tbx1*$^{+/-}$mice	Normal	Normal
	Tbx1$^{-/-}$ mice	CP, incomplete CP, submucosal CP [15]	Normal
	Tbx1$^{LoxP/KO}$*; KRT14-Cre* mice (epithelium)	Anterior CP [15]	Normal [8]
	Tbx1$^{LoxP/KO}$*; Mesp1-Cre* mice (mesoderm)	Normal	CCD syndrome-like*, hypomorphic HB [8]
	Tbx1$^{LoxP/KO}$*; Twist2-Cre* mice (bone primordium)	Normal	CCD syndrome-like*, no mineralized HB [8]
	Tbx1$^{LoxP/KO}$*; Wnt1-Cre* mice (neural crest)	Normal	No mineralized HB[8]
Runx2	*Runx2*$^{+/-}$mice	Not reported	CCD syndrome*, HB hypoplasia [25]
	Runx2$^{-/-}$ mice	CP [37]	No mineralized bone [23,25]

22q11.2DS: 22q11.2 Deletion Syndrome; CCD: Cleidocranial Dysplasia; CP: Cleft Palate; HB: Hyoid Bone *hypoplastic clavicle, abnormal neurocranium morphology, and a nasal bone defect

CP, whereas *Tbx1*$^{+/-}$mice are phenotypically normal [15] (Table 1). Ablation of *Tbx1* specifically in the epithelial cells (*Tbx1*$^{LoxP/KO}$*; KRT14-Cre*) results in anterior CP [15]. The variations in palatal phenotypes across different *Tbx1* mutants strongly suggest that Tbx1 is involved in stochastic factors and/or modifier genes. Expression of *Pax9*, mutations of which lead to CP and adontogenesis [16], is down-regulated in the pharyngeal arch and palatal shelves of *Tbx1*$^{-/-}$ embryos [15,17]. *Tbx1*$^{-/-}$ hyperproliferative epithelium displays incomplete differentiation, suggesting that Tbx1 controls the balance between proliferation and differentiation of epithelial cells. CP phenotypes of *Tbx1*$^{-/-}$ mice suggest that the various degrees of CP phenotypes could be induced by the pathogenic adhesion-separation of the oral epithelium, together with compromised growth of palatal mesenchyme [15,18].

Tbx1 and bone abnormalities

22q11.2DS patients manifest with craniofacial malformations including short stature, brachycephaly, micrognathia, blunted nose, hypertelorism, and small low-set ears [3,19]. *Tbx1*$^{-/-}$mice also display skeletal abnormalities, such as short stature, persistently open fontanelles, micrognathia, hypoplasia of clavicle and zygomatic arch, small low-set ears, and absence of hyoid bone [8] (Figure 1B). A cell type-specific deletion of *Tbx1* in the mesoderm (*Tbx1*$^{LoxP/KO}$*; Mesp1-Cre*) or osteochondral progenitors (*Tbx1*$^{LoxP/KO}$*; Twist2-Cre*) partially recapitulates the *Tbx1*$^{-/-}$bone phenotypes (Table 1). Tbx1 is involved in the following aspects of osteogenesis. First, Tbx1 is expressed in mesoderm-derived calvarial bone primordium and is directly involved in calvarial bone development. Loss of *Tbx1* in the cranial mesoderm (*Tbx1*$^{LoxP/KO}$*; Mesp1-Cre*) or osteochondral progenitors (*Tbx1*$^{LoxP/KO}$*; Twist2-Cre*) impairs development of *Tbx1*-progeny calvarial bones [8]. Second, *Tbx1* expression in neural crest cells or in osteochondral progenitors is necessary for the morphogenesis and ossification of the hyoid bone. Interestingly, 22q11.2DSpatients exhibit delayed development of the hyoid bone [20], ordain visible hyoid ossification center [21]. Abnormalities in other neural crest-derived bones, such as frontal bones, mandibular bones, and temporal bones are secondary defects induced by non-neural crest cells in *Tbx1*$^{-/-}$mice [8,22]. It is likely that *Tbx1* expression in the cranial endoderm and mesoderm affects development of adjacent neural crest derivatives. *Tbx1*$^{-/-}$mice also have bone abnormalities in the endochondral bones, including the atlas, axis, xiphoid process, and the cranial base. The synchondroses in the *Tbx1*$^{-/-}$cranial base are completely ossified [3,8,9]. These results indicate that Tbx1 is required for mesoderm- and neural crest-derived bone morphogenesis and ossification.

Skeletal abnormalities observed in *Tbx1*$^{-/-}$mice are similar to those of cleidocranial dysplasia (CCD, OMIM 119600) in humans, which is caused by heterozygous mutation in the *RUNX2* gene (Table 1). Runx2, a member of the Runt-related transcription factors, is essential for osteoblast differentiation, and *Runx2*$^{-/-}$mice display no mineralized bones [23-25]. Similar to CCD in humans, *Runx2*$^{+/-}$ mice exhibit short stature, abnormal neurocranium morphology, persistently open fontanel's, nasal bone defects, and hypoplasia of the clavicle, hyoid bone, and zygomatic arch [23,25]. Deletion of *Tbx1*affects *Runx2* expression in parietal bones, suggesting that Tbx1 may be involved in the maintenance of cell populations expressing *Runx2*at the onset of bone development. Since *Tbx1*overexpression induces *Runx2* expression *in vitro*, it is also possible that Tbx1 may act upstream of *Runx2*. These results suggest that *TBX1* mutations could lead to CCD-like skeletal phenotypes in humans and *TBX1* may be the candidate gene for recessive inheritance of CCD (OMIM 216330).

Tbx1 and dental anomalies

In 22q11DS patients, dental anomalies (enamel hypoplasia, hypomineralization, single central incisor, and small teeth) have been reported [10,11]. Similar to the human phenotype, the upper incisors are absent in 30% of *Tbx1*$^{-/-}$mice [15]. In developing teeth, *Tbx1* expression is controlled by Fibroblast Growth Factor (FGF) signaling [26]. Mouse incisors have the dental stem cell niche in the labial and lingual Cervical Loops (CLs).Since*Tbx1* is expressed in the CLs, Tbx1 may regulate the stem cell niche. Inactivation of *Tbx1* specifically in the keratinocyte lineage results in a slightly smaller tooth compared to wild-type [27]. The CL region of the incisoris either severely reduced or completely missing in *Tbx1*$^{-/-}$incisors [28]. Tbx1 binds to Pitx2 (paired-like homeodomain transcription factor 2) and activates promoters of *Pitx2*and *Cdkn1a* (a cyclin-dependent kinase inhibitor 1A, also known as p21), suggesting that Tbx1 regulates cell

proliferation in the dental epithelium through the *Pitx2-Cdkn1a* axis [29]. Tbx1 regulates the transition between stem cell quiescence and proliferation in hair follicles [30]. *Tbx1* in the CLsmay also regulate the proliferation, differentiation, and/or maintenance of the stem cell niche.

Tbx1 and Muscle Hypotonia

The branchiomeric muscles are derived from the mesodermal core of the pharyngeal arches [31]. In *Tbx1*$^{-/-}$embryos, branchiomeric muscles, including the masseter, pterygoid, and temporalis muscles, are intermittently absent [32], suggesting that Tbx1 is required for determining cell fate and survival of the myogenic cells in branchiomeric muscles. In branchiomeric muscle formation, the basic helix–loop–helix transcriptionfactorsTcf21 (transcription factor 21, also known as capsulin), Msc (musculin, also known as MyoR), Myf5 (myogenic factor 5), and Myod1 (myogenic differentiation 1), and other transcription factors such as Pitx2 and Isl1 (ISL1 transcription factor, LIM/homeodomain) play critical roles [33-35]. Tbx1 functions downstream of Tcf21, Pitx2, and Isl1, and upstream of Lhx2 (LIM homeobox protein 2), Myf5, Myod1, Tlx1 (T cell leukemia, homeobox 1), and Fgf10 in cardiogenesis or myogenesis [32-34,36]. Therefore, Tbx1 controls the onset and the development of branchiomeric muscles through transcriptional regulation of myogenic determination genes.

Conclusion

Progress has been made to establish how Tbx1 contributes to the palatal, craniofacial, dental, and muscle phenotypes observed in 22q11.2DS patients. Epigenetics and the microRNA regulation could change *Tbx1* expression [27,38]. Further research is needed to elucidate the genetic, cellular, and molecular roles of Tbx1 and apply this knowledge to the management of 22q11.2DS patients in the future.

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