Craniofacial Phenotypes and Genetics of DiGeorge Syndrome

Subjects: Genetics & Heredity

Contributor: NORIKO FUNATO

TBX1, located on chromosome 22q11.21, encodes a T-box transcription factor and is a candidate gene for DiGeorge syndrome (DGS) and velocardiofacial syndrome (VCFS). Studies of *Tbx1*-mutant mice have provided insights into the underlying pathogenesis of DGS/VCFS and the knowledge to diagnose patients with DGS/VCFS. Genes, miRNAs, and epigenetics could change *Tbx1* expression. Polymorphisms, variations, and mutations in *TBX1* may induce the penetrance and severity of DGS/VCFS-like craniofacial phenotypes. The molecular basis of the variant sequence of *TBX1* will further define how *TBX1* contributes to the craniofacial and other phenotypes of DGS/VCFS. Since interactions with TBX1 and other molecules in transcriptional complexes or chromatin remodeling are crucial for TBX1 function, identifying and understanding these genetic and epigenetic modifiers individually for each patient may direct therapeutics to minimize the severity.

22q11.2 deletion syndrome | DiGeorge syndrome | velocardiofacial syndrome | cleft palate | skull base

1. Introduction

The 22q11.2 deletion syndrome is one of the most common chromosomal microdeletions, affecting approximately 1 in 4000 live births in humans [1]. A 1.5 to 2.5 Mb hemizygous deletion of chromosome 22q11.2 causes DiGeorge syndrome (DGS; OMIM #188400) and velocardiofacial syndrome (VCFS or Shprintzen VCF syndrome; OMIM #192430) [2]. DGS/VCFS appears to be a genomic disorder distinct from 22q11.2 distal deletion syndrome (OMIM #611867). The clinical phenotype of DGS/VCFS is a complex and variable congenital disability, including cardiovascular defects, thymic hypoplasia, parathyroid hypoplasia, and craniofacial malformations [3]. Craniofacial malformations occur in approximately 60% of patients with DGS/VCFS [4].

TBX1, located on chromosome 22q11.21, encodes a T-box transcription factor and is considered a candidate gene for DGS/VCFS since mutations in *TBX1* have been found in patients with DGS/VCFS [5]. Heterozygous *Tbx1*-mutant (*Tbx1*$^{+/-}$) mice exhibit DGS/VCFS-related cardiovascular, parathyroid, and thymic phenotypes, suggesting that *TBX1* dosage is critical for cardiovascular, parathyroid and thymic development [6][7][8][9]. *Tbx1*-null mice exhibit the most clinical features of DGS/VCFS, including craniofacial phenotypes, while *Tbx1*$^{+/-}$ mice exhibit no significant craniofacial phenotypes [6][7][8][9][10].

There have been some excellent reviews on genetics and cardiovascular anomalies of DGS/VCFS [3][11][12][13]. However, information on the craniofacial anomalies of DGS/VCFS is limited. This research focuses on these phenotypes and summarizes the current understanding of the genetic factors that impact DGS/VCFS-related phenotypes. The researchers also review DGS/VCFS mouse models that have been designed to better understand the pathogenic processes of DGS/VCFS.

2. Craniofacial Phenotypes of Patients with DGS/VCFS

Patients with DGS/VCFS manifest craniofacial anomalies involving the cranium, cranial base, jaws, pharyngeal muscles, ear-nose-throat, palate, teeth, and cervical spine (**Table 1** and **Table 2**). Frequently observed craniofacial phenotypes include velopharyngeal insufficiency (27–92%), enamel hypomineralization (39–41%), hearing loss (33–39%), platybasia (50–91%), and cervical spine anomalies (75%) (**Table 1**). Delayed development of the hyoid bone has also been reported [14][15].

Table 1. Craniofacial anomalies in patients with DGS/VCFS.

Phenotypes	Features	Frequency
Palatal anomalies	Overt cleft palate	7–11%
	Submucous cleft palate	5–23%
	Bifid uvula	5–10%
	Velopharyngeal insufficiency	27–92%
Dental anomalies	Tooth agenesis	15%
	Hypoplasia of primary teeth	32%
	Hypoplasia of permanent teeth	10%
	Enamel hypomineralization of primary teeth	39%
	Enamel hypomineralization of permanent teeth	41%
Ear-nose-throat abnormalities	Hearing loss	33–39%
	Otitis media with effusion	2%
	Tracheomalacia/laryngomalacia	2%
	Laryngeal web	1%
Ocular abnormalities	Hooding of the upper lid	41%
	Ptosis	9%

Phenotypes	Features	Frequency
	Hooding of the lower lid	6%
	Epicanthal folds	3%
	Distichiasis	3%
Cranial base anomalies	Platybasia	50–91%
	Basilar impression	3%
Cervical spine anomalies	Atlas (C1) anomalies	75%
	Axis (C2) anomalies	59%

	DGS/VCFS	*Tbx1*-Null Mice
Cranium	Dolichocephaly	Small cranium
	Abnormal skull morphology	Hypoplastic parietal bone
	Malar flattening	Hypoplastic interparietal bone
	Long face	Unfused cranial sutures between frontal and parietal bones
		Temporal bone hypoplasia
		Absent zygomatic arch
		Abnormal zygomatic arch morphology
Cranial Base	Platybasia	Abnormal fusion of the basioccipital and basisphenoid bones
	Basilar impression	Abnormal presphenoid bone morphology
		Abnormal basioccipital bone morphology
Palate	Cleft palate	Cleft palate
	Submucous cleft palate	Submucous cleft palate
	Bifid uvula	Bifid uvula
	Highly arched palate	
	Velopharyngeal insufficiency	
Mandible	Retrognathia	Absent mandibular coronoid process
	Short mandible	Short mandible

	DGS/VCFS	*Tbx1*-Null Mice
	Micrognathia	Micrognathia
Teeth	Enamel hypoplasia	Abnormal upper incisor morphology
	Single central incisor	Absent upper incisors
	Small teeth	
	Abnormality of the dentition	
	Carious teeth	
Muscles	Pharyngeal hypotonia	Absent masseter muscle
		Absent pterygoid muscle
		Absent temporalis muscle
Eyes	Hypertelorism/telecanthus	Hypertelorism
	Downslanted palpebral fissures	
	Proptosis	
	Strabismus	
	Abnormal eyelid morphology	
	Epicanthus	
	Microphthalmia	
External Ears	Small earlobe	Ear lobe hypoplasia
	Low-set ears	Lowered ear position
	Abnormally folded pinna	Abnormal ear shape
	Preauricular pit	Absent outer ear
		Anotia
Middle and Inner Ears	Chronic otitis media	Abnormal middle ear ossicle morphology
	Conductive hearing loss	Absent middle ear ossicles
	Sensorineural hearing loss	Abnormal stapes morphology
	Auditory canal stenosis	Abnormal incus morphology

	DGS/VCFS	*Tbx1*-Null Mice
	Pulsatile tympanic membrane	Abnormal malleus morphology
	Thickened tympanic membrane	Absent stapes
	Tympanic membrane retraction	Abnormal external auditory canal morphology
		Decreased tympanic ring size
Nose	Prominent nasal bridge	Short snout
	Abnormal nasal morphology	
	Underdeveloped nasal alae	
	Choanal atresia	
Throat	Abnormal thorax morphology	Small thyroid cartilage
	Abnormality of the pharynx	Small cricoid cartilage
		Abnormal thyroid cartilage morphology
		Pharynx hypoplasia
Hyoid bones	Delayed development of the hyoid bone	Hyoid bone hypoplasia
	Invisible hyoid ossification center	Abnormal hyoid bone morphology
Cervical spine	Dysmorphic C1	Abnormal cervical atlas (C1) morphology
	Anterior arch cleft of C1	Absent arcus anterior of C1
	Open posterior arch C1	
	Fusion of C1–C2	
	Fusion of C2–C3 [35]	
	Upswept C2 lamina	
	Platyspondyly [35]	
Others		Short clavicle
References	[14][15][16][17][18][19][20][21][22][23][24][25][26]	[6][7][8][9][10][27][28][29][30][31][32][33][34]

DGS/VCFS is caused by a 1.5 to 2.5 Mb hemizygous deletion of chromosome 22q11.2 (**Figure 1**). Chromosomal microdeletions at 10p14-p13 (the *DGS2* locus) in patients with DGS/VCFS phenotypes are defined as the DGS/VCFS complex 2. The researchers here focus on the 22q11.2 locus, its associated genes, and miRNAs.

Data were summarized from the following references [6][7][8][9][10][14][15][16][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34], OMIM (https://www.omim.org accessed on 3 August 2021) and the Monarch Initiative

(https

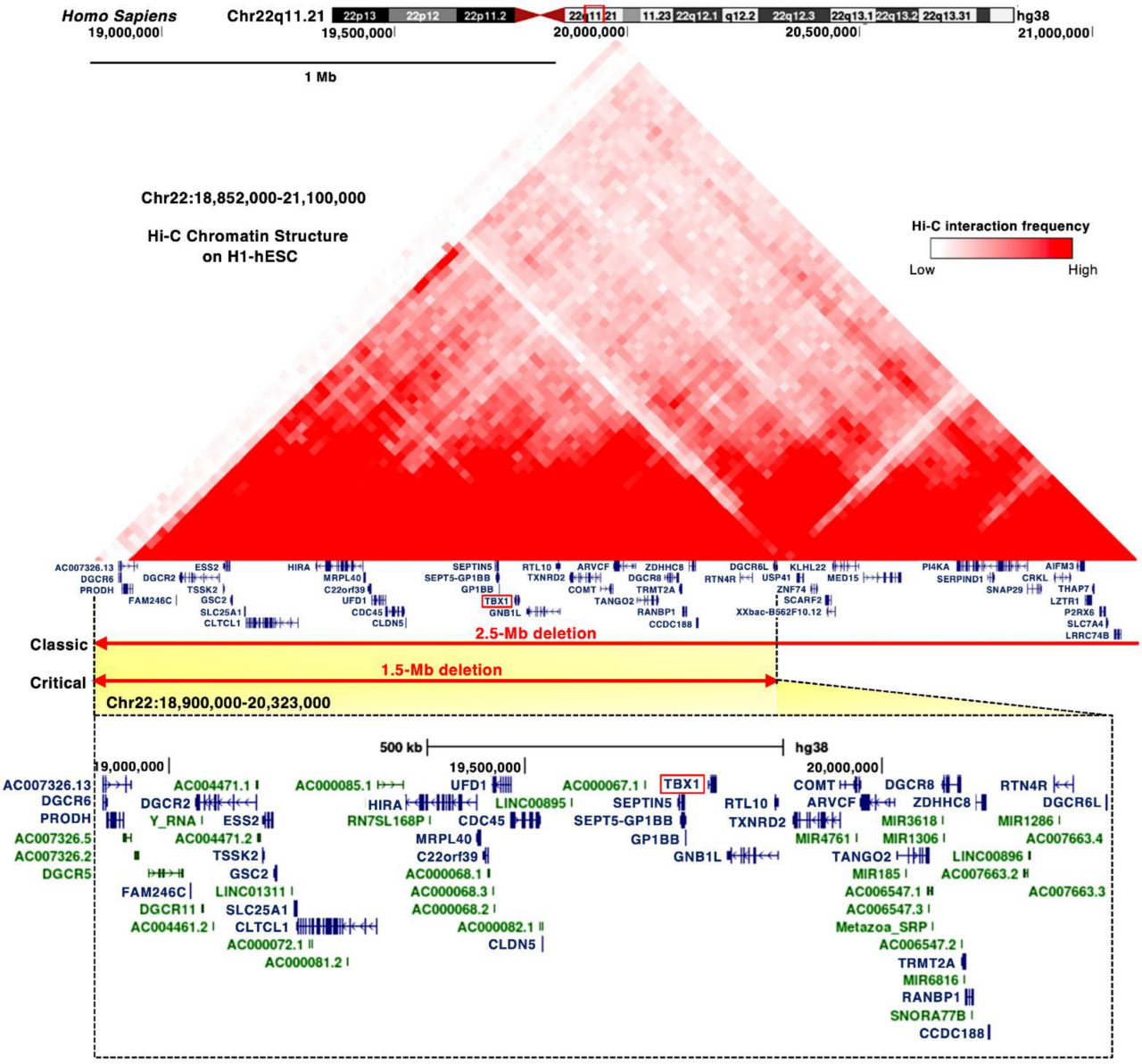

Figure 1. Proximal deletions of chromosome 22q11.2 are responsible for the clinical features of DGS/VCFS. Snapshot of the UCSC Genome Browser (http://genome.ucsc.edu accessed on 3 August 2021) in the hg38 assembly showing the genomic context in the proximal deletions of chromosome 22q11.2. Top, the 25 kb resolution Hi-C data in H1 human embryonic stem cell line (H1-hESC). Bottom, the coding (blue) and noncoding RNAs (green), including miRNAs and long noncoding RNAs, are shown.

Most of the chromosomal deletions of the 22q11.2 locus are de novo, but inherited deletions of the 22q11.2 locus have been reported in 6–28% of patients as autosomal dominant . The majority of clinical phenotypes of DGS/VCFS are caused by proximal 1.5 Mb microdeletions [3][20], resulting in a hemizygosity of approximately 30 coding genes, including *DGCR6, PRODH, DGCR2, ESS2, TSSK2, GSC2, FAM246C, SLC25A1, CLTCL1, UFD1, HIRA, CDC45, MRPL40, C22orf39, CLDN5, TBX1, SEPTIN5, SEPT5-GP1BB, GP1BB, GNB1L, RTL10, TXNRD2, COMT, ARVCF, TANGO2, TRMT2A, RANBP1, CCDC188, DGCR8, ZDHHC8, RTN4R, DGCR6L,* and *C007326*, as well as microRNAs (miRNAs) and long noncoding RNAs (**Figure 1**). The Hi-C chromatin structure of the 1.5 Mb region indicates interactions between these loci and their neighboring regions (**Figure 1**).

3.1. TBX1 Gene

The proximal deletion of 1.5 Mb on the 22q11.2 locus includes *TBX1* (**Figure 1**). *TBX1* is considered a candidate gene of DGS/VCFS because haploinsufficiency of *TBX1* leads to the typical phenotypes of DGS/VCFS, conotruncal anomaly face syndrome (OMIM #217095), and tetralogy of Fallot (OMIM #187500) (**Table 3**). Identical mutations in *TBX1* present among patients resulted in distinct phenotypes, suggesting that genetic and epigenetic changes or environmental factors are involved in the clinical phenotypes [5]. Further investigation is required to confirm that the variants cause DGS/VCFS and how they impact the phenotypes.

Table 3. DGS/VCFS-associated variants of *TBX1.*

Mutation	Domain	Condition	Craniofacial Anomalies	References
c.89_284del	N-terminal	DiGeorge syndrome	Yes	ClinVar Variant: 971780
c.199_224del	N-terminal	DiGeorge syndrome	Yes	ClinVar Variant: 949172
c.292A>T	N-terminal	DiGeorge syndrome	Yes	ClinVar Variant: 526036
c.385G>A	T-box	Tetralogy of Fallot	No	ClinVar Variant: 488618
c.443T>A (F148Y)	T-box	Conotruncal anomaly face syndrome	Yes	[5]
c.503T>C	T-box	DiGeorge syndrome Velocardiofacial syndrome (Shprintzen syndrome) Tetralogy of Fallot	Yes	ClinVar Variant: 973222
c.569C > A (P190Q)	T-box	Congenital heart defects	No	[36]
c.582C>G (H194Q)	T-box	Velocardiofacial syndrome	Yes	[37]
c.928G>A (G310S)	C-terminal	DiGeorge syndrome	Yes	[5]
c.967_977dup AACCCCGTGGC	C-terminal	Thymic hypoplasia Postaxial polydactyly of the right fifth toe	No	[38]
c.1158_1159delinsT	C-terminal	Hypoparathyroidism and hypocalcemia Facial asymmetry Deafness	Yes	[39]

Mutation	Domain	Condition	Craniofacial Anomalies	References
c.1223delC	C-terminal	Conotruncal anomaly face syndrome Velocardiofacial syndrome	Yes	[5]
c.1253delA	C-terminal	DiGeorge syndrome	Yes	[40]
c.1320-1342del23bp	C-terminal	Velocardiofacial syndrome	Yes/No	[41]
c.1399-1428dup30	C-terminal	Tetralogy of Fallot Scoliosis Facial asymmetry Upslanting palpebral fissures Absent pulmonary valve Isolated left pulmonary artery	Yes	[42]

ClinVar (https://www.ncbi.nlm.nih.gov/clinvar accessed on 3 August 2021).

DGCR8 is duces the expression of miRNAs in the brain [43]. DGCR6 and DGCR6L genes encode a protein with a sequence similar to the Drosophila gonadal [44]. In a chicken model, targeting DGCR6 function resulted in a vascular phenotype [45]. Attenuation of DGCR6 affects the expression of three genes localized within the 1.5 Mb region, upregulating the expression of TBX1 and UFD1 and reducing the expression of HIRA in the heart and pharyngeal arches of the chicken embryos [45]. Thus, the haploinsufficiency of DGCR8 or DGCR6 may be linked to DGS/VCFS phenotypes when targeting DGS/VCFS-related genes and miRNAs.

3.3. MicroRNAs

The deleted 1.5 Mb on the 22q11.2 locus includes several miRNAs, such as miR-185, miR-4716, miR-3618, miR-1286, miR-1306, and miR-6816 (**Figure 1**). The TargetScan miRNA target prediction program (http://www.targetscan.org accessed on 3 August 2021) identified that the 3′ UTR of *TBX1* includes conserved sites for miR-183-5p, miR-96-5p, miR-1271-5p, miR-182-5p, miR-144-3p, miR-139-5p, miR-101-3p, and miR-451. Two miRNAs were confirmed to target the 3′ UTR of *TBX1*. miR-96-5p represses *Tbx1* expression and, in turn, TBX1 suppresses the promoter activity and expression of miR-96 [46]. miR-451a, a tumor suppressor, also directly targets *TBX1* [47]. The expression of this gene is upregulated in cutaneous basal cell carcinoma, inversely to miR-451a [47]. miR-17-92 fine-tunes the expression of *Tbx1* in craniofacial development, suggesting miR-17-92 as a candidate genetic modifier for *Tbx1* [48]. Thus, miRNAs both inside and outside the 22q11.2 locus may influence the severity of the clinical phenotypes of DGS/VCFS.

4. Craniofacial Phenotypes of DGS/VCFS Mouse Models

Mouse models with DGS/VCFS help identify additional candidate genes or modifier genes that influence the penetrance and/or severity of DGS/VCFS-related phenotypes. According to the mouse genome informatics (MGI) database (http://www.informatics.jax.org accessed on 3 August 2021), DGS/VCFS-related anomalies concerning Tbx1, Chrd, Tgfbr2, Vegfa, Fgf8, Crkl, Aldh1a2/Raldh2, Hoxa3, Kat6a/Moz/Myst3, Dicer1, Plxnd1, Dock1, Ndst1, Prickle1, Trappc10, Zfp366, and Foxn1 have been reported in genetically altered mice (**Table 4**). When these

genes were analyzed according to biological process, “heart morphogenesis” and “cranial skeletal system development” were enriched. Our enrichment analysis using ToppCluster [49] indicated that genes associated with DGS/VCFS phenotypes in mice are specifically enriched in the morphogenesis of craniofacial tissues and heart (**Figure 2**A). Interestingly, among these genes, only *Tbx1* and *Chrd* were specifically enriched in the morphogenesis of cricoid and thyroid cartilages (**Figure 2**A). Genes associated with DGS/VCFS phenotypes in mice also indicated that DGS/VCFS-related phenotypes involve the interaction of several signaling pathways, including bone morphogenetic protein (BMP), transforming growth factor (TGF)β, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and retinoic acid signaling pathways (**Figure 2**B). Genes involved in the genetic pathway of *Tbx1* are likely to induce phenotypes similar to *Tbx1*-null mice (**Figure 2**B, **Table 4**). These are described below.

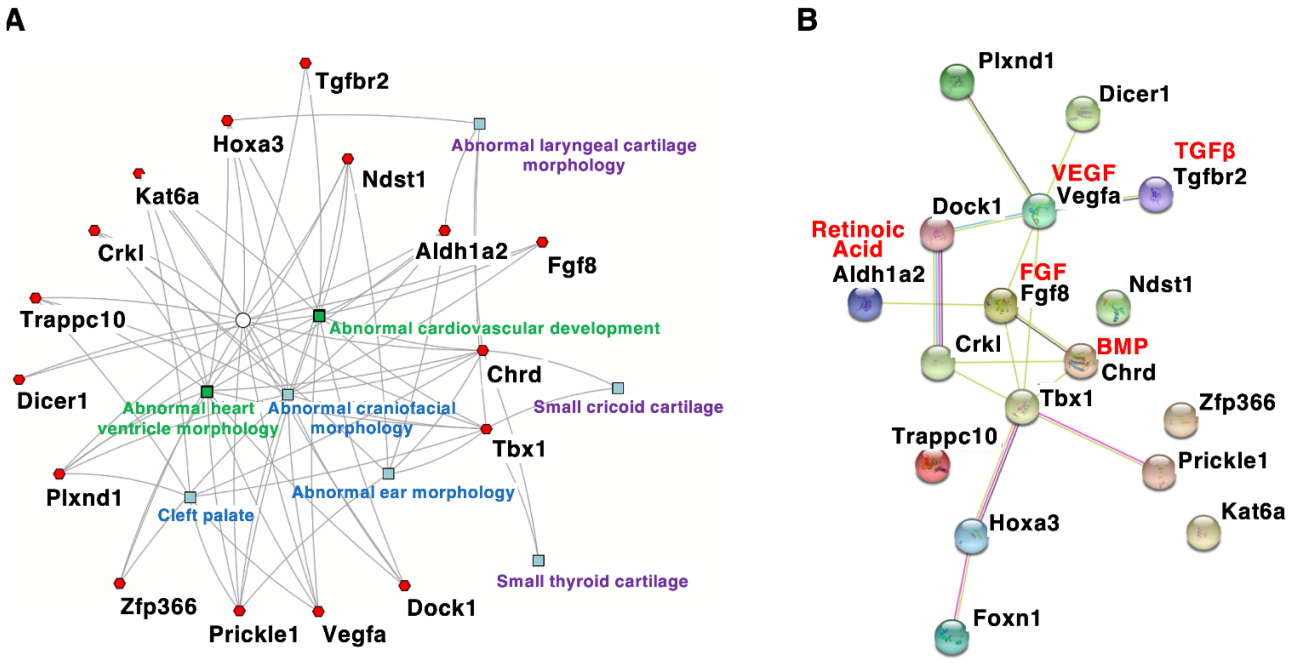

Figure 2. Interaction network of genes associated with DGS/VCFS phenotypes in mice. (**A**) A gene-based network where each gene connects to a feature. The network was constructed using ToppCluster (https://toppcluster.cchmc.org/ accessed on 6 May 2022). Mouse phenotypes are shown in the network. (**B**) The protein–protein interaction network was constructed using the STRING tool (https://string-db.org/ accessed on 6 May 2022). Genes associated with DGS/VCFS phenotypes in mice (**Table 4**) were the input. Different colors represent different types of evidence of a connection between proteins.

Table 4. Craniofacial phenotypes of DGS/VCFS mouse model.

Gene Symbol	Induced Mutation Type	Cranium	Palate	Teeth	Muscles	Ear-Nose-Throat	Hyoid Bones	Cardio-Vascular
Tbx1	Null	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Chrd	Null	Yes	Yes	nr	nr	Yes	Yes	Yes

Gene Symbol	Induced Mutation Type	Cranium	Palate	Teeth	Muscles	Ear-Nose-Throat	Hyoid Bones	Cardio-Vascular
Tgfbr2	Deletion (*Wnt1-Cre*)	Yes	Yes	nr	nr	nr	nr	Yes
Vegfa	Null	Yes	Yes	Yes	nr	nr	nr	Yes
Fgf8	Hypomorphic allele	Yes	Yes	Yes	nr	Yes	Yes	Yes
Crkl	Null	Yes	nr	nr	nr	Yes	nr	Yes
Aldh1a2	Hypomorphic allele	nr	nr	nr	nr	Yes	Yes	Yes
Hoxa3	Null	nr	Yes	nr	Yes	Yes	Yes	Yes
Kat6a	Null	nr	Yes	nr	nr	Yes	nr	Yes
Dicer1	Deletion (*Wnt1-Cre*)	Yes	nr	nr	nr	nr	nr	Yes
Plxnd1	Single point mutation	nr	Yes	nr	nr	Yes	nr	Yes
Dock1	Undefined	nr	nr	nr	nr	Yes	nr	Yes
Ndst1	Single point mutation	nr	nr	nr	nr	Yes	nr	Yes
Prickle1	Single point mutation	Yes	Yes	nr	nr	Yes	nr	Yes
Trappc10	Undefined	Yes	Yes	nr	nr	nr	nr	Yes
Zfp366	Single point mutation	nr	nr	nr	nr	Yes	nr	Yes
Foxn1	Intragenic deletion	nr	nr	nr	nr	Yes	nr	Yes

Mouse models of DiGeorge syndrome with phenotypic similarity to human diseases can be found in the Mouse Genome Informatics (MGI) database (http://www.informatics.jax.org, accessed on 3 August 2021). Data were summarized from the following references [6][7][8][9][10][29][30][31][32][33][34][35][36][50][51][52][53][54][55][56][57][58][59]. nr, not reported.

1. Kaimal, V.; Bardes, E.E.; Tabar, S.C.; Jegga, A.G.; Aronow, B.J. ToppCluster: A multiple gene list feature analyzer for comparative enrichment clustering and network-based dissection of biological systems. Nucleic Acids Res. 2010, 38, W96–102.
2. Jerome, L.A.; Papaioannou, V.E. DiGeorge syndrome phenotype in mice mutant for the T-box gene, Tbx1. Nat. Genet. 2001, 27, 286–291.
3. Lindsay, E.A.; Vitelli, F.; Su, H.; Morishima, M.; Huynh, T.; Pramparo, T.; Jurecic, V.; Ogunrinu, G.; Sutherland, H.F.; Scambler, P.J.; et al. Tbx1 haploinsufficieny in the DiGeorge syndrome region causes aortic arch defects in mice. Nature 2001, 410, 97–101.
4. Merscher, S.; Funke, B.; Epstein, J.A.; Heyer, J.; Puech, A.; Lu, M.M.; Xavier, R.J.; Demay, M.B.; Russell, R.G.; Factor, S.; et al. TBX1 is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. Cell 2001, 104, 619–629.

5. Hu, T.; Yamagishi, H.; Maeda, J.; McAnally, J.; Yamagishi, C.; Srivastava, D. Tbx1 regulates fibroblast growth factors in the anterior heart field through a reinforcing autoregulatory loop involving forkhead transcription factors. Development 2004, 131, 5491–5502.
6. Liao, J.; Kochilas, L.; Nowotschin, S.; Arnold, J.S.; Aggarwal, V.S.; Epstein, J.A.; Brown, M.C.; Adams, J.; Morrow, B.E. Full spectrum of malformations in velo-cardio-facial syndrome/DiGeorge syndrome mouse models by altering Tbx1 dosage. Hum. Mol. Genet. 2004, 13, 1577–1585.
7. Zhang, Z.; Huynh, T.; Baldini, A. Mesodermal expression of Tbx1 is necessary and sufficient for pharyngeal arch and cardiac outflow tract development. Development 2006, 133, 3587–3595.
8. Arnold, J.S.; Werling, U.; Braunstein, E.M.; Liao, J.; Nowotschin, S.; Edelmann, W.; Hebert, J.M.; Morrow, B.E. Inactivation of Tbx1 in the pharyngeal endoderm results in 22q11DS malformations. Development 2006, 133, 977–987.
9. Arnold, J.S.; Braunstein, E.M.; Ohyama, T.; Groves, A.K.; Adams, J.C.; Brown, M.C.; Morrow, B.E. Tissue-specific roles of Tbx1 in the development of the outer, middle and inner ear, defective in 22q11DS patients. Hum. Mol. Genet. 2006, 15, 1629–1639.
10. Choi, M.; Klingensmith, J. Chordin is a modifier of tbx1 for the craniofacial malformations of 22q11 deletion syndrome phenotypes in mouse. PLoS Genet. 2009, 5, e1000395.
11. Moraes, F.; Novoa, A.; Jerome-Majewska, L.A.; Papaioannou, V.E.; Mallo, M. Tbx1 is required for proper neural crest migration and to stabilize spatial patterns during middle and inner ear development. Mech. Dev. 2005, 122, 199–212.
12. Funato, N.; Nakamura, M.; Richardson, J.A.; Srivastava, D.; Yanagisawa, H. Tbx1 regulates oral epithelial adhesion and palatal development. Hum. Mol. Genet. 2012, 21, 2524–2537.
13. Funato, N.; Nakamura, M.; Richardson, J.A.; Srivastava, D.; Yanagisawa, H. Loss of Tbx1 induces bone phenotypes similar to cleidocranial dysplasia. Hum. Mol. Genet. 2015, 24, 424–435.
14. Funato, N.; Srivastava, D.; Shibata, S.; Yanagisawa, H. TBX1 Regulates Chondrocyte Maturation in the Spheno-occipital Synchondrosis. J. Dent. Res. 2020, 99, 1182–1191.
15. Bachiller, D.; Klingensmith, J.; Shneyder, N.; Tran, U.; Anderson, R.; Rossant, J.; De Robertis, E.M. The role of chordin/Bmp signals in mammalian pharyngeal development and DiGeorge syndrome. Development 2003, 130, 3567–3578.
16. Wurdak, H.; Ittner, L.M.; Lang, K.S.; Leveen, P.; Suter, U.; Fischer, J.A.; Karlsson, S.; Born, W.; Sommer, L. Inactivation of TGFβ signaling in neural crest stem cells leads to multiple defects reminiscent of DiGeorge syndrome. Genes Dev. 2005, 19, 530–535.
17. Stalmans, I.; Lambrechts, D.; De Smet, F.; Jansen, S.; Wang, J.; Maity, S.; Kneer, P.; von der Ohe, M.; Swillen, A.; Maes, C.; et al. VEGF: A modifier of the del22q11 (DiGeorge) syndrome? Nat. Med. 2003, 9, 173–182.

18. Abu-Issa, R.; Smyth, G.; Smoak, I.; Yamamura, K.; Meyers, E.N. Fgf8 is required for pharyngeal arch and cardiovascular development in the mouse. Development 2002, 129, 4613–4625.
19. Guris, D.L.; Fantes, J.; Tara, D.; Druker, B.J.; Imamoto, A. Mice lacking the homologue of the human 22q11.2 gene CRLK phenocopy neurocristopathies of DiGeorge syndrome. Nat. Genet. 2001, 27, 293–298.
20. Vermot, J.; Niederreither, K.; Garnier, J.M.; Chambon, P.; Dollé, P. Decreased embryonic retinoic acid synthesis results in a DiGeorge syndrome phenotype in newborn mice. Proc. Natl. Acad. Sci. USA 2003, 100, 1763–1768.
21. Chisaka, O.; Capecchi, M.R. Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene hox-1.5. Nature 1991, 350, 473–479.
22. Voss, A.K.; Vanyai, H.K.; Collin, C.; Dixon, M.P.; McLennan, T.J.; Sheikh, B.N.; Scambler, P.; Thomas, T. MOZ Regulates the Tbx1 Locus, and Moz Mutation Partially Phenocopies DiGeorge Syndrome. Dev. Cell 2012, 23, 652–663.
23. Sheehy, N.T.; Cordes, K.R.; White, M.P.; Ivey, K.N.; Srivastava, D. The neural crest-enriched microRNA miR-452 regulates epithelial-mesenchymal signaling in the first pharyngeal arch. Development 2010, 137, 4307–4316.
24. Gershwin, M.E. DiGeorge syndrome: Congenital thymic hypoplasia. Animal model: Congenitally athymic (nude) mouse. Am. J. Pathol. 1977, 89, 809–812.
25. Kobrynski, L.J.; Sullivan, K.E. Velocardiofacial syndrome, DiGeorge syndrome: The chromosome 22q11.2 deletion syndromes. Lancet 2007, 370, 1443–1452.
26. Oberoi, S.; Vargervik, K. Velocardiofacial syndrome with single central incisor. Am. J. Med. Genet. A 2005, 132A, 194–197.
27. Zhang, Z.; Huynh, T.; Baldini, A. Mesodermal expression of Tbx1 is necessary and sufficient for pharyngeal arch and cardiac outflow tract development. Development 2006, 133, 3587–3595.
28. Arnold, J.S.; Werling, U.; Braunstein, E.M.; Liao, J.; Nowotschin, S.; Edelmann, W.; Hebert, J.M.; Morrow, B.E. Inactivation of Tbx1 in the pharyngeal endoderm results in 22q11DS malformations. Development 2006, 133, 977–987.
29. Arnold, J.S.; Braunstein, E.M.; Ohyama, T.; Groves, A.K.; Adams, J.C.; Brown, M.C.; Morrow, B.E. Tissue-specific roles of Tbx1 in the development of the outer, middle and inner ear, defective in 22q11DS patients. Hum. Mol. Genet. 2006, 15, 1629–1639.
30. Choi, M.; Klingensmith, J. Chordin is a modifier of tbx1 for the craniofacial malformations of 22q11 deletion syndrome phenotypes in mouse. PLoS Genet. 2009, 5, e1000395.
31. Moraes, F.; Novoa, A.; Jerome-Majewska, L.A.; Papaioannou, V.E.; Mallo, M. Tbx1 is required for proper neural crest migration and to stabilize spatial patterns during middle and inner ear

development. Mech. Dev. 2005, 122, 199–212.

32. Funato, N.; Nakamura, M.; Richardson, J.A.; Srivastava, D.; Yanagisawa, H. Tbx1 regulates oral epithelial adhesion and palatal development. Hum. Mol. Genet. 2012, 21, 2524–2537.
33. Funato, N.; Nakamura, M.; Richardson, J.A.; Srivastava, D.; Yanagisawa, H. Loss of Tbx1 induces bone phenotypes similar to cleidocranial dysplasia. Hum. Mol. Genet. 2015, 24, 424–435.
34. Funato, N.; Srivastava, D.; Shibata, S.; Yanagisawa, H. TBX1 Regulates Chondrocyte Maturation in the Spheno-occipital Synchondrosis. J. Dent. Res. 2020, 99, 1182–1191.
35. Solot, C.B.; Sell, D.; Mayne, A.; Baylis, A.L.; Persson, C.; Jackson, O.; McDonald-McGinn, D.M. Speech-Language Disorders in 22q11.2 Deletion Syndrome: Best Practices for Diagnosis and Management. Am. J. Speech-Lang. Pathol. 2019, 28, 984–999.
36. Jaouadi, A.; Tabebi, M.; Abdelhedi, F.; Abid, D.; Kamoun, F.; Chabchoub, I.; Maatoug, S.; Doukali, H.; Belghuith, N.; Ksentini, M.A.; et al. A novel TBX1 missense mutation in patients with syndromic congenital heart defects. Biochem. Biophys. Res. Commun. 2018, 499, 563–569.
37. Zweier, C.; Sticht, H.; Aydin-Yaylagül, I.; Campbell, C.E.; Rauch, A. Human TBX1 Missense Mutations Cause Gain of Function Resulting in the Same Phenotype as 22q11.2 Deletions. Am. J. Hum. Genet. 2007, 80, 510–517.
38. Hasegawa, K.; Tanaka, H.; Higuchi, Y.; Hayashi, Y.; Kobayashi, K.; Tsukahara, H. Novel heterozygous mutation in TBX1 in an infant with hypocalcemic seizures. Clin. Pediatr. Endocrinol. 2018, 27, 159–164.
39. Alghamdi, M.; Al Khalifah, R.; Al Homyani, D.K.; Alkhamis, W.H.; Arold, S.T.; Ekhzaimy, A.; El-Wetidy, M.; Kashour, T.; Halwani, R. A novel TBX1 variant causing hypoparathyroidism and deafness. J. Endocr. Soc. 2020, 4, bvz028.
40. Ogata, T.; Niihori, T.; Tanaka, N.; Kawai, M.; Nagashima, T.; Funayama, R.; Nakayama, K.; Nakashima, S.; Kato, F.; Fukami, M.; et al. TBX1 mutation identified by exome sequencing in a Japanese family with 22q11.2 deletion syndrome-like craniofacial features and hypocalcemia. PLoS ONE 2014, 9, e91598.
41. Paylor, R.; Glaser, B.; Mupo, A.; Ataliotis, P.; Spencer, C.; Sobotka, A.; Sparks, C.; Choi, C.H.; Oghalai, J.; Curran, S.; et al. Tbx1 haploinsufficiency is linked to behavioral disorders in mice and humans: Implications for 22q11 deletion syndrome. Proc. Natl. Acad. Sci. USA 2006, 103, 7729–7734.
42. Rauch, R.; Hofbeck, M.; Zweier, C.; Koch, A.; Zink, S.; Trautmann, U.; Hoyer, J.; Kaulitz, R.; Singer, H.; Rauch, A. Comprehensive genotype-phenotype analysis in 230 patients with tetralogy of Fallot. J. Med. Genet. 2010, 47, 321–331.

43. Stark, K.L.; Xu, B.; Bagchi, A.; Lai, W.-S.; Liu, H.; Hsu, R.; Wan, X.; Pavlidis, P.; Mills, A.A.; Karayiorgou, M.; et al. Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. Nat. Genet. 2008, 40, 751–760.
44. Edelmann, L.; Stankiewicz, P.; Spiteri, E.; Pandita, R.K.; Shaffer, L.; Lupski, J.R.; Morrow, B.E.; Lupski, J. Two functional copies of the DGCR6 gene are present on human chromosome 22q11 due to a duplication of an ancestral locus. Genome Res. 2001, 11, 208–217.
45. Hierck, B.P.; Molin, D.G.M.; Boot, M.J.; Poelmann, R.E.; Gittenberger-De Groot, A.C. A chicken model for DGCR6 as a modifier gene in the DiGeorge critical region. Pediatr. Res. 2004, 56, 440–448.
46. Gao, S.; Moreno, M.; Eliason, S.; Cao, H.; Li, X.; Yu, W.; Bidlack, F.B.; Margolis, H.C.; Baldini, A.; Amendt, B.A. TBX1 protein interactions and microRNA-96-5p regulation controls cell proliferation during craniofacial and dental development: Implications for 22q11.2 deletion syndrome. Hum. Mol. Genet. 2015, 24, 2330–2348.
47. Sun, H.; Jiang, P. MicroRNA-451a acts as tumor suppressor in cutaneous basal cell carcinoma. Mol. Genet. Genom. Med. 2018, 6, 1001–1009.
48. Wang, J.; Bai, Y.; Li, H.; Greene, S.B.; Klysik, E.; Yu, W.; Schwartz, R.J.; Williams, T.J.; Martin, J.F. MicroRNA-17-92, a direct Ap-2alpha transcriptional target, modulates T-box factor activity in orofacial clefting. PLoS Genet. 2013, 9, e1003785.