Understanding velocardiofacial syndrome: how recent discoveries can help you improve your patient outcomes

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Purpose of review
Improved recognition of velocardiofacial syndrome (VCFS) has led to increasing awareness of VCFS by otolaryngologists. Understanding the developmental biologic processes affected in VCFS patients will help improve treatment and outcomes. Advanced application of molecular labeling techniques has better outlined the role of T-Box transcription factor 1 (*TBX*1) as the primary genetic anomaly leading to VCFS. *TBX*1 plays multiple roles during branchial, cardiac, and craniofacial development and increased understanding of how these systems are affected by *TBX*1 mutations will improve patient outcomes. Furthermore, additional modifiers of *TBX*1 expression have been identified that may explain the variability of VCFS phenotypes. The phenotypic spectrum of VCFS may include cardiac anomalies, velopharyngeal insufficiency, aberrant calcium metabolism, and immune dysfunction. Recent interest has focused on the cognitive and neuropsychiatric manifestations of VCFS. Improved understanding of the biology of VCFS associated mutations has the potential to improve therapeutic outcomes.

Recent findings
This article will discuss recent developmental biologic understanding of the role of *TBX*1 and genetic modifiers generating the phenotypic variability seen in VCFS patients. Special attention is given to advances in the realms of immunodeficiency, hypocalcemia, cardiac and arterial patterning anomalies, velopharyngeal insufficiency, as well as cognitive and psychiatric problems.

Summary
Enhanced understanding of the multiple systems affected by *TBX*1 mutations will result in improved patient outcomes and improved family education. Future research will lead to improved detection of potential targets for gene therapy and change the way physicians counsel families and treat patients.

Keywords
22q11.2, Digeorge, T-Box transcription factor 1, velocardiofacial, velopharyngeal insufficiency

INTRODUCTION

Since the initial description of syndromic hypoparathyroidism, athymia with immune compromise, and cardiovascular anomalies by Digeorge in the 1960s and the introduction of the term velocardiofacial syndrome (VCFS) by Robert Sphrintzen in 1978, much has been learned about this syndrome with diverse phenotypic presentation [1,2]. As DNA analysis became more sophisticated, genetic testing revealed that these syndromes, in addition to several others, share a common genetic basis: a deletion on the short arm of chromosome 22 [3]. Multiple eponymous syndromes are now commonly united under the names 22q11.2 deletion syndrome or VCFS. This article will focus on understanding the phenotypic variation of patients with VCFS and how the multiple systems affected from a developmental perspective are relevant to otolaryngologists.

EMBRYOLOGY

Branchial arch formation begins very early in human development during the 3rd and 4th week of gestation. The paired six arches are composed of

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KEY POINTS

- Multiple genes contribute to the phenotypic variability associated with VCFS, including TBX1, TBX2, TBX3, and VEGF.
- The genes contributing to VCFS guide pharyngeal arch development; therefore, patients with VCFS must be screened for defects in immunity, vascular development, and calcium homeostasis to ensure good surgical outcomes.
- Unrecognized otologic and cognitive dysfunction may affect speech and language rehabilitation of VCFS patients and must be treated to obtain optimal patient rehabilitation.

underlying endoderm, mesenchyme, and overlying ectoderm. As the cranial neural crest invades the paired branchial arches, the endoderm forms pouches and the ectoderm forms clefts. The first arch derivatives are responsible for auricular, mandibular, and maxillary formation. T-box transcription factor 1 (*TBX*1), EYA1, and EYA4 are transcription factors required for normal first arch formation [4]. Patients with VCFS have aberrant palate formation leading to the characteristic hypernasality. *Tbx*1 mutations in mice lead to aberrant palate formation, suggesting that intrinsic differences exist in VCFS patients, whether it is manifested by an overt cleft palate or not [5].

The 2nd, 3rd, and 4th arch derivatives include the tonsil and muscles of facial animation (2nd), thymus and inferior parathyroid (3rd), and superior parathyroid (4th). The 5th arch forms briefly and gives rise to the thyroid cartilage, whereas the 6th arch derivatives include aortic arch and pulmonary artery formation, alterations which lead to the cardiac malformations seen in VCFS patients [6]. Endoderm from each pouch dictates the formation of the overlying arch [4]. Fibroblastic growth factors (Fgfs), bone morphogenetic proteins (Bmps), and Sonic Hedgehog (Shh) are all growth factors required for pouch endodermal formation. *Tbx*1 mutant mice have altered expression patterns of Fgf, Bmp, and Shh in the pharyngeal endoderm, suggesting that *Tbx*1 is required for normal pharyngeal endoderm formation.

T-BOX TRANSCRIPTION FACTOR 1'S ROLE IN PHARYNGEAL DEVELOPMENT

The last decade of molecular genetics research has implicated impaired expression of *TBX*1 as the prime culprit of the VCFS phenotype, owning to its involvement in normal embryologic development of 3rd and 4th pharyngeal arch derivatives. Several authors have observed abnormal development of these structures in *TBX*1 haplo-insufficient individuals [5,7,8▪,9–12]. This has been borne out prospectively in mouse models and seen in genetic analysis of affected humans. Within the last year, it has been suggested that the genetic pathway of the VCFS phenotype is more complicated and relies on appropriate interaction of multiple transcription factors. *Tbx1* is known to be heavily present in pharyngeal endoderm and cells destined for the cardiac outflow tract. Mesbah *et al.* [8▪] have illustrated the interplay between TBX1 and additional transcription box factors, Tbx2 and Tbx3.

Tbx2 and Tbx3 concentrate in the caudal aspect of the pharyngeal endoderm and throughout the developing pericardial wall. Engineering of loss of function mice without normal Tbx1 function resulted in a severe diminution of Tbx2 and Tbx3, resulting in a specific, reproducible variety of abnormal pharyngeal and cardiac phenotypes. Furthermore, there appears to be a downregulatory function inherent in Tbx2 and Tbx3 crucial to limiting the action of Tbx1. In those mice with Tbx2/3 loss of function, Tbx1 function, particularly in the ventral foregut continued unabated, far beyond the normal, resulting in a variety of abnormal phenotypes. These data indicate that in addition to directing 3rd and 4th arch development, Tbx1 signals the production of Tbx2 and Tbx3, which in turn regulates the function of Tbx1.

Tbx1 function may also be influenced by retinoic acid synthesis (promoted by Raldh2). Mice with Tbx1 loss of function mutants demonstrate increased Raldh2 expression and an increase in circulating retinoic acid [10]. Double heterozygous Tbx1 +/-; Raldh2 +/- mice have decreased embryonic retinoic acid, and despite having Tbx1 mutations, demonstrate less severe cardiac abnormalities. This suggests that decreased circulating retinoic acid can lead to 'rescue' of the TBX1 cardiac phenotype. Retinoic acid supplementation has also led to cleft palate in mice. It has been previously described that retinoic acid exerts a complex influence on muscular development, suggesting that pharyngeal musculature in VCFS patients is intrinsically different [13]. Vascular endothelial growth factor (VEGF) was also identified as a modifier of Tbx1 function. VEGF deficient mice had decreased Tbx1 expression, which was associated with similar palate and cardiac phenotype [11].

IMMUNODEFICIENCY

VCFS patients are known to have disordered development of third arch structures, and thymic abnormalities are common. Hypothymia and athymia are

thought to affect 80% of patients with del22q11.2 [14]. Lymphoid progenitors in the bone marrow migrate to the thymic anlage, and the development of a large naive T-cell compartment is dependent on the presence of a normally functioning and architecturally sound thymus. Immature, undifferentiated T-cells normally migrate to the thymus where they undergo differentiation into CD4 and CD8 cells, as well as undergo negative selection to eliminate self-reactive cells. Maturation of T-cells is a crucial step in ongoing thymic maturation – one that is impaired by the decreased number of mature T-cells in 22q11 patients. This places VCFS patients at increased risk for routine infections (e.g., acute otitis media, pneumonia) and more severe infections (viral or fungal sepsis).

Thymic transplant has had early success in the treatment of immune compromise in this setting [3]. Transplantation has been shown to establish a reasonable naive T-cell population in VCFS patients. Furthermore, it is well tolerated, in that major histocompatibility (MHC) matching (crucial in other types of solid organ transplantation) is not required to achieve host tolerance [15]. A review of 60 VCFS patients with thymic transplantation showed excellent immune recovery; however, in this same group, nearly half of the patients developed some form of autoimmune disease (discussed in the following section) [16▪▪,17,18▪]. An increased understanding of immune maturation explains the increased survival and constitution of functional T-cell compartment following thymic transplantation for severely immunocompromised VCFS patients. Unrecognized T-cell dysfunction in VCFS patients will affect wound healing and surgical outcomes, highlighting the importance of understanding the full phenotypic expression of VCFS.

AUTOIMMUNITY

Autoimmunity in the VCFS population occurs as the thymus is not only involved in positive selection of naive T-cells to respond to nonself antigens, but also in the negative selection of self-reactive T-cells [18▪]. It follows that T-cells in VCFS patients with total athymia do not mature into normal or self-reactive T-cells; however, in patients with hypothymia/partial athymia, disordered T-cell selection allows development and release of self-reactive T-cells. A recent study of thymic transplant VCFS patients showed that 13 of 38 develop autoimmune thyroid disease [16▪▪]. This raises the question whether MHC matching can achieve similar immune reconstitution with lower rates of autoimmunity. This also has implications for parathyroid transplantation and calcium homeostasis.

CALCIUM HOMEOSTASIS

A frequent manifestation of 22q11 syndrome is primary hypocalcemia resulting from decreased parathyroid function, which is usually detected early in the postnatal period and manifests as low serum calcium. The numerous dangerous effects of hypocalcemia have led to many diverse efforts to treat hypoparathyroidism, with varying degrees of success including oral and intravenous calcium replacement, and parathyroid transplantation. Whereas thymic allograft transplants in VCFS patients have led to tolerance of donor thymus without MHC matching, parathyroid transplantation has been less successful due to graft rejection [17]. Transplantation of parathyroid tissue and thymus from the same donor in three participants initially resulted in good parathyroid function in all of them. However, two went on to develop autoimmune rejection of the parathyroid graft. The two unmatched MHC II transplants failed, whereas the third MHC II matched participant had excellent parathyroid function without signs of rejection for the length of the study (5 years) [16▪▪]. As knowledge about parathyroid and thymus transplantation increases, this suggests a role for MHC II complex matching.

ARTERIAL PATTERNING

VCFS patients constitute a leading population for cardiac anomalies from a genetic cause due to patterning defects in the 5th and 6th pharyngeal arches. Apart from being at risk for tetralogy of Fallot, pulmonary atresia, and ventricular septal defects (VSDs), they make up 30–35% of patients with a common arterial trunk [19]. Recent work has indicated that *Tbx*1 is the major regulator of a group of cardiac progenitor cells collectively known as the second heart field (SHF). Cell labeling in the mouse and zebra fish models has previously shown that SHF cells develop into the arterial outflow tract of the heart, specifically contributing to the formation of subpulmonary myocardium [9]. *Tbx*1 loss of function mutants demonstrate a narrow and shortened outflow tract and a common arterial trunk. The spatial relationships derived from this anomalous patterning also contribute to the characteristic coronary artery anomalies of VCFS, by spatially directing their growth [12]. VCFS patients are also known to have medialized internal carotid arteries, which likely occur from aberrant pharyngeal patterning of the 3rd pharyngeal arch, which may affect surgical correction of velopharyngeal insufficiency. Altered retinoic acid metabolism also contributes to VCFS cardiophenotypes. It was shown that decreased levels of embryonic retinoic acid lead to increased

muscle differentiation in the development of the cardiac outflow tract leading to accelerated conotruncal normalization in *Tbx*1-deficient mice [10].

COGNITIVE/LANGUAGE

VCFS patients demonstrate a number of psychiatric and neurocognitive problems. They suffer from high rates of depression, phobias, attention deficit-hyperactivity disorder, and difficulty with social interaction [20,21]. Furthermore, nearly one third of patients go on to develop some form of psychotic illness; VCFS is the strongest predictor for future development of schizophrenia [22]. Frontoparietal abnormalities seen in these patients are associated with attention deficits and decline in higher level cognitive function [21]. Further, Kates *et al.* [23] showed decreased recruitment of frontal lobe areas during executive function tasks with functional MRI. These same areas are involved in the modulation of mood and social interaction, offering an explanation for deficits in these arenas.

The gyrification index is an MRI-based measure of cerebral cortex development, and an evaluation of 91 VCFS patients and controls demonstrated that VCFS patients demonstrated lower gyrification index. The VCFS patients matured from this baseline at a rate similar to the control groups. The gyrification index pattern was most abnormal in the frontal and parietal regions that are implicated in cognitive and social functions. Differences in the occipital region of VCFS patients contribute to visual-spatial processing defects that are strongly associated with schizophrenia [24▪▪].

The combination of emotional, cognitive, and social impairments can come to dominate the later childhood of VCFS patients. Integration of VCFS children into peer groups exposes these non-anatomic issues and can be measured by consistently low Quality of Life scores by standardized measures. Functioning in school setting and mental fatigue are the greatest areas of difficulty for children with VCFS, with both of these deficiencies being

Table 1. Suspected genetic anomalies in velocardiofacial syndrome

Affected gene	Relevant areas of embryologic expression	Phenotypic findings	Clinical manifestation
TBX1	3rd Branchial arch	Parathyroid hypoplasia/aplasia	Hypocalcemia
		Neuromotor function of palatal and pharyngeal musculature	Velopharyngeal insufficiency
	4th Branchial arch	Thymic hyoplasia/aplasia	Immunodeficiency and increased autoimmunity
	Second heart field	Cardiac outflow tract anomalies	Conotruncal abnormalities
		Aberrant vascular patterns	Pulmonary hypertension
			Medial course of carotid arteries
	Palate	Bony and muscular palatal abnormalities	Cleft palate/VPI
	Prefrontal cerebral cortex (possible)	Abnormal gyrification	Poor cognitive function
			Impaired social interaction
TBX2/3	Second heart field	Cardiac outflow tract anomalies	Conotruncal abnormalities
			Pulmonary hypertension
			Medial course of carotid arteries
	Pharyngeal endoderm	Neuromotor function of pharyngeal muscle	Velopharyngeal insufficiency
Raldh2	Second heart field	Cardiac outflow tract anomalies (impaired retinoic acid metabolism prevents recovery from TBX1-related mutations)	Conotruncal abnormalities; pulmonary hypertension; medial course of carotid arteries
	Facial skeleton	Abnormal palatal development	Cleft palate
COMT	Prefrontal cortex	Abnormal dopamine metabolism	Poor cognitive function
			Predilection for psychiatric disease
Unknown/other	Occipital lobe	Abnormal gyrification	Impaired visual-spatial perception/learning
			High risk factor for psychiatric disease

significantly more pronounced in boys [20]. This has implications for their ability to adapt and rate of progress in conventional speech therapy when compared to peers without VCFS.

HEARING/EAR DEVELOPMENT

Chronic otitis media is a frequent manifestation of VCFS, resulting in significant conductive hearing loss. In addition, sporadic cases of sensorineural loss have been reported. Although the chronic otitis media may be attributed, in part, to the Eustachian tube dysfunction common to patients with cleft palate, a recent study has shown that *Tbx*1 is required for inner ear development. In addition, this same population of mice demonstrated a Mondini type malformation with sensorineural hearing loss [7]. Unrecognized hearing loss may contribute to delayed speech and cognitive development in VCFS patients.

CONCLUSION

Achieving optimal outcomes for patients with VCFS necessitates a thorough understanding of the physical and mental manifestations of the disease as outlined in Table 1. Early morbidity can be due to an array of conotruncal anomalies, infections, or severe hypocalcemia. Understanding the disordered embryogenesis that leads to these anomalies allows physicians to quickly recognize and correct these problems. Newer treatments that hold great potential include thymus and parathyroid transplantation, but more research is needed to understand the immunologic considerations surrounding these procedures. Better outcomes for surgical correction of ear disease and velopharyngeal insufficiency by otolaryngologists will occur by increased understanding of the phenotypic variability in VCFS patients. Emerging data show that VCFS patients will also demand special attention in speech therapy and in school to maximize their ability to integrate with unaffected children. Additionally, parents need to be counseled on the potential for future psychiatric concerns.

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Conflicts of interest

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. DiGeorge A. Congenital absence of the thymus and its immunologic consequences: concurrence with congenital hypoparathyroidism. March of Dimes-Birth Defect Foundation 1968; (IV):116–121.
2. Shprintzen RJ, Goldberg RB, Lewin ML, *et al.* A new syndrome involving cleft palate, cardiac anomalies, typical facies, and learning disabilities: velo-cardio-facial syndrome. Cleft Palate J 1978; 15:56–62.
3. Markert ML, Devlin BH, Alexieff MJ, *et al.* Review of 54 patients with complete DiGeorge anomaly enrolled in protocols for thymus transplantation: outcome of 44 consecutive transplants. Blood 2007; 109:4539–4547.
4. Grevellec A, Tucker AS. The pharyngeal pouches and clefts: development, evolution, structure and derivatives. Semin Cell Dev Biol 2010; 21:325–332.
5. Goudy S, Law A, Sanchez G, *et al.* Tbx1 is necessary for palatal elongation and elevation. Mech Dev 2010; 127:292–300.
6. Mirilas P. Lateral congenital anomalies of the pharyngeal apparatus: part I. Normal developmental anatomy (embryogenesis) for the surgeon. Am Surg 2011; 77:1230–1242.
7. Funke B, Epstein JA, Kochilas LK, *et al.* Mice overexpressing genes from the 22q11 region deleted in velo-cardio-facial syndrome/DiGeorge syndrome have middle and inner ear defects. Hum Mol Genet 2001; 10:2549–2556.
8. Mesbah K, Rana MS, Francou A, *et al.* Identification of a Tbx1/Tbx2/Tbx3
■ genetic pathway governing pharyngeal and arterial pole morphogenesis. Hum Mol Genet 2012; 21:1217–1229.

This article offers an excellent review of Tbx1's role in VCFS and outlines the genetic interplay between several genetic anomalies.

9. Parisot P, Mesbah K, Theveniau-Ruissy M, Kelly RG. Tbx1, subpulmonary myocardium and conotruncal congenital heart defects. Birth Defects Res A Clin Mol Teratol 2011; 91:477–484.
10. Ryckebusch L, Bertrand N, Mesbah K, *et al.* Decreased levels of embryonic retinoic acid synthesis accelerate recovery from arterial growth delay in a mouse model of DiGeorge syndrome. Circ Res 2010; 106:686–694.
11. Stalmans I, Lambrechts D, De Smet F, *et al.* VEGF: a modifier of the del22q11 (DiGeorge) syndrome? Nat Med 2003; 9:173–182.
12. Theveniau-Ruissy M, Dandonneau M, Mesbah K, *et al.* The del22q11.2 candidate gene Tbx1 controls regional outflow tract identity and coronary artery patterning. Circ Res 2008; 103:142–148.
13. Niederreither K, Dolle P. Retinoic acid in development: towards an integrated view. Nat Rev Genet 2008; 9:541–553.
14. Sauce D, Appay V. Altered thymic activity in early life: how does it affect the immune system in young adults? Curr Opin Immunol 2011; 23:543–548.
15. Markert ML, Devlin BH, McCarthy EA. Thymus transplantation. Clin Immunol 2010; 135:236–246.
16. Chinn IK, Markert ML. Induction of tolerance to parental parathyroid grafts
■■ using allogeneic thymus tissue in patients with DiGeorge anomaly. J Allerg Clin Immunol 2011; 127:1351–1355.

This study offers an excellent overview of hypocalcemia in VCFS, historical treatment options, and the exciting potential for parathyroid transplantation.

17. Chinn IK, Olson JA, Skinner MA, *et al.* Mechanisms of tolerance to parental parathyroid tissue when combined with human allogeneic thymus transplantation. J Allerg Clin Immunol 2010; 126:814–820; e818.
18. Gennery AR. Immunological aspects of 22q11.2 deletion syndrome. Cell Mol
■ Life Sci: CMLS 2012; 69:17–27.

This study contributes to the understanding of immune system abnormalities in VCFS, including autoimmunity.

19. Momma K. Cardiovascular anomalies associated with chromosome 22q11.2 deletion syndrome. Am J Cardiol 2010; 105:1617–1624.
20. Looman WS, Thurmes AK, O'Conner-Von SK. Quality of life among children with velocardiofacial syndrome. Cleft Palate Craniofac J 2010; 47:273–283.
21. Antshel KM, Fremont W, Kates WR. The neurocognitive phenotype in velo-cardio-facial syndrome: a developmental perspective. Dev Disabil Res Rev 2008; 14:43–51.
22. Shprintzen RJ, Goldberg R, Golding-Kushner KJ, Marion RW. Late-onset psychosis in the velo-cardio-facial syndrome. Am J Med Genet 1992; 42:141–142.
23. Kates WR, Krauss BR, Abdulsabur N, *et al.* The neural correlates of nonspatial working memory in velocardiofacial syndrome (22q11.2 deletion syndrome). Neuropsychologia 2007; 45:2863–2873.
24. Kunwar A, Ramanathan S, Nelson J, *et al.* Cortical gyrification in velo-cardio-
■■ facial (22q11.2 deletion) syndrome: a longitudinal study. Schizophr Res 2012; 137:20–25.

This study demonstrates specific brain abnormalities in VCFS populations, which can contribute cognitive and neuropsychiatric manifestations of the disease.