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Genetic insights into the Tetralogy of Fallot

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Abstract

Tetralogy of Fallot (TOF), (OMIM #187500) one of the first known congenital heart disease (CHDs) with a rising frequency of adult patients, is a suitable paradigm for our analysis given the expanding abundance of genetic data available and these clinical consequences. Given the complexity of cardiac development, it has been associated with untreated maternal diabetes, maternal intake of retinoic acid, phenylketonuria, chromosomal anomalies (trisomies 21, 18, 13), microdeletions of chromosome 22q11.2, and it is not unexpected that a variety of transcription factors and signaling molecules involved in 9 cardiogenesis have been linked to TOF, with the literature consistently reporting the existence of new, previously unrecognized genes. The well studied genes GATA4, NKX2.5, JAG1, FOXC2, TBX5, and TBX1, which have previously been linked to TOF, are the focus of this review.

Keywords: Cardiac; Genetic; Cardiogenesis; Tetralogy; Transcription

1. Introduction

Tetralogy of Fallot (TOF), the most prevalent cyanotic heart defect, remains poorly understood in terms of its underlying genetic mechanisms. Originally known as Steno-Fallot tetralogy, this condition was initially documented by Niels Stensen, a Danish physician and anatomist, also known as Nicoulas Steno in Latin, who made significant contributions to the fields of anatomy and geology. In 1671, Stensen published a brief paper titled “Dissection of a Monstrous Foetus in Paris,” where he described the unique characteristics of TOF, including the abnormal positioning of the arteries, pulmonary artery narrowing, absence of the ductus arteriosus, a defect in the interventricular septum beneath the aorta, and fetal cardiac circulation redirecting blood directly to the aorta rather than the pulmonary artery. Despite its historical significance, the precise genetic mechanisms underlying TOF remain unclear. ⁽¹⁾

Tetralogy of Fallot (TOF) is a common congenital heart disease that typically develops during the early stages of embryonic development. It is characterized by abnormal development of the conus arteriosus. Among infants with congenital heart disease, approximately 3.5% are diagnosed with TOF, which corresponds to a prevalence of 0.28 cases per 1000 live births or 1 in 3600 births. ⁽²⁾ The condition is distinguished by four main features: a ventricular septal defect, obstruction in the right ventricular outflow tract (often dynamic), an overriding aorta, and right ventricular hypertrophy. The severity and presentation of TOF depend on factors such as the extent of the obstruction in the right ventricular outflow tract, the relative pressures in the right and left ventricles, and the proportion of the aorta that overrides the ventricular septal defect. Early detection and prevention of fetal TOF are crucial, and there is significant interest in exploring the causes of this condition. ⁽³⁾

2. Pathophysiology of Tetralogy of Fallot

The pathophysiology of Tetralogy of Fallot (TOF) involves several factors contributing to the right ventricular outflow obstruction and the presence of ventricular septal defects (VSDs). Typically, the VSDs observed in TOF patients are

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perimembranous and may extend into the muscular septum. The obstruction in the right ventricular outflow tract can be attributed to various factors, including a stenotic and often bicuspid pulmonary valve, a hypoplastic pulmonary valve annulus, deviation of the infundibular septum leading to subvalvular obstruction, and hypertrophy of the muscular bands in this region. The VSDs in TOF are usually large, resulting in equal systolic pressures in both the right and left ventricles. ⁽¹⁾ The pathophysiology of TOF is dependent on the degree of right ventricular outflow obstruction. Mild obstruction can cause a net left-to-right shunt through the VSD, while severe obstruction leads to a right-to-left shunt, resulting in persistent low systemic arterial saturation (cyanosis) that is not responsive to supplemental oxygen. ⁽⁴⁾

In some cases, individuals with minimal cyanosis can experience dynamic increases in right ventricular outflow tract obstruction, leading to an escalation in right-to-left shunting and the development of cyanosis. In more severe instances, the right ventricular outflow tract can become nearly occluded, resulting in profound cyanosis. These episodes are commonly known as “hypercyanotic spells”. ⁽⁵⁾

A hypercyanotic spell can be triggered “by various events that result in a slight decrease in oxygen saturation (such as crying or defecating) or a sudden decrease in systemic vascular resistance (such as playing or kicking legs upon awakening), as well as sudden onset tachycardia or hypovolemia. The exact mechanism behind hypercyanotic spells remains uncertain, but several factors likely contribute to an increase in right-to-left shunting and a decrease in arterial saturation. These factors may include an escalation in right ventricular outflow tract obstruction, an increase in pulmonary vascular resistance, and/or a decrease in systemic resistance. This creates a vicious circle initiated by the initial decline in arterial oxygen levels, which stimulates the respiratory center, leading to hyperpnea and increased adrenergic tone. The heightened circulating catecholamines then stimulate increased contractility, further exacerbating the outflow tract obstruction. ⁽⁴⁾

Untreated infants with Tetralogy of Fallot (TOF) face a significant natural mortality rate that can be as high as 90%. TOF affects males and females equally. The condition arises when the early embryo fails to undergo normal biological processes, usually occurring within the first eight weeks of development. Timely surgical correction is crucial to improve survival rates and outcomes for infants with TOF.

3. Evaluation

Antenatal diagnosis of Tetralogy of Fallot (TOF) is possible in up to 50% of patients through fetal echocardiography. This early detection allows for the anticipation of postnatal prostaglandin therapy in cases where severe right ventricular outflow obstruction is evident. To aid in the diagnosis and evaluation of TOF, several useful studies can be employed, including chest radiograph, electrocardiogram (ECG), and echocardiogram.

Chest radiographs typically reveal a normal-sized heart silhouette, characterized by an upturned apex and a concave main pulmonary artery segment, often referred to as a “boot-shaped” appearance. On the ECG, common findings include signs of right atrial enlargement and right ventricular hypertrophy, manifested as right axis deviation, prominent R waves anteriorly, S waves posteriorly, an upright T wave in V1, and a qR pattern in the right precordial leads.

Among the various imaging studies, echocardiography is considered the gold standard. It provides comprehensive information regarding the anatomy and severity of right ventricular outflow obstruction, the location and number of ventricular septal defects, and the assessment of any associated anomalies or variations involving the coronary arteries and the aortic arch. ⁽⁶⁾

4. Diagnosis and mRNA profiles of fetal tetralogy of Fallot

Recent studies have revealed the significant association between abnormal expression of long noncoding RNAs (lncRNAs) and the progression and prognosis of Tetralogy of Fallot (TOF). Consequently, there has been growing attention towards exploring the molecular pathology mechanisms of fetal TOF by investigating the role of lncRNAs. lncRNAs are a class of RNA transcripts that are longer than 200 nucleotides and have the ability to regulate gene expression. ⁽²⁾

The correlation analysis between lncRNAs and mRNA profiles has further demonstrated that differentially expressed lncRNAs can be linked to specific mRNAs, suggesting a potential regulatory role of lncRNAs in mRNA expression. ⁽⁷⁾

Researchers have also discovered that differences in lncRNA expression can be detected in the body fluids of TOF patients, providing valuable evidence for further exploring lncRNAs as potential biomarkers for TOF. For instance,

Zhang X identified four hub lncRNAs that regulate mRNA expression through miRNAs in the heart tissues of 22 children with TOF. ⁽⁴⁾ Additionally, Quan Wang et al. reported an increased expression of *lncRNA HA117*, which was associated with adverse short-term outcomes in TOF patients. ⁽⁸⁾ These findings highlight the potential of lncRNAs as important players in the molecular mechanisms and potential biomarkers for TOF.

Abnormal expression of lncRNAs and mRNAs in myocardial tissues of fetal Tetralogy of Fallot (TOF) and control groups plays a crucial role in the development of TOF and may serve as potential candidates for early prevention and intervention strategies. However, the specific roles of these dysregulated lncRNAs in TOF are yet to be reported. Miriam S. Reuter reported that recurrent variants, such as p. (Phe271del) in the *PURA* gene, are associated with structural heart defects, highlighting the importance of genetic variants in TOF. ⁽⁶⁾

EDF1, a transcriptional coactivator of *PPAR γ* , has been found to be necessary for the *VEGF-induced* transcriptional activity of *PPAR γ* in human endothelial cells. *EDF1* initiates the activation of a lipogenic gene program and cooperates with the *lncRNA Blnc1*. These findings suggest that *lncRNA n326323* may negatively regulate the *EDF1* gene, which plays a significant role in heart development. ⁽⁹⁾

Another candidate gene, the prolactin receptor (PRLR), is potentially negatively regulated by the *lncRNA TCONS_00014231-XLOC_006288*. The PRLR gene is known to be important for reproduction and growth. Silencing of the PRLR gene has been associated with the inhibition of hippocampal neuron apoptosis, indicating its role in cell apoptosis. Additionally, increased expression of PRLR protein has been observed in hypertrophic hearts and is involved in the development of cardiac hypertrophy. ⁽²⁾

These findings suggest that the dysregulation of specific lncRNAs and their target mRNAs in fetal myocardial tissues may contribute to the development of TOF and could potentially serve as important targets for early intervention and prevention strategies. Further research is needed to elucidate the precise mechanisms and functional roles of these lncRNAs in TOF pathogenesis.

5. Inheritance

The comprehensive study conducted by Boon et al. (1972) provided valuable insights into the heritability of Tetralogy of Fallot (TOF). Their findings suggested that approximately 54% of the risk for TOF is attributable to genetic factors. Furthermore, in siblings of individuals with TOF, the recurrence risk for Fallot tetralogy was estimated to be around 1%, while the recurrence risk for any cardiac defect was approximately 2%. ⁽¹⁰⁾

In a notable case reported by Der Kaloustian et al. (1985), a unique form of TOF was observed in a family with an autosomal recessive inheritance pattern. Two daughters, who were the offspring of first cousins, were diagnosed with tetralogy and pulmonary valve atresia. Notably, the bronchial circulation and pulmonary valve anatomy were identical in both siblings. The parental consanguinity was of less significance in this case, as the family belonged to the Christian Maronite Lebanese community. Another instance of familial occurrence of TOF was documented by Friedberg (1974) across three generations. However, none of the affected individuals in this particular family had pulmonary valve atresia, distinguishing it from the aforementioned case. ⁽¹¹⁾

These reports highlight the complex genetic aspects of TOF, with varying patterns of inheritance and clinical presentations. Further research is necessary to explore the underlying genetic mechanisms contributing to the development of TOF and its different phenotypic manifestations.

A study conducted by Pankau et al. (1990) presented the occurrence of tetralogy of Fallot in three out of five siblings (two boys and one girl) born to non-consanguineous parents. The observation of tetralogy of Fallot in multiple siblings suggests the involvement of a recessive gene in its inheritance. The variable expression of tetralogy reported by Pankau and Friedberg (1974) does not contradict the possibility of monogenic inheritance. ⁽¹²⁾

In a study by Pacileo et al. (1992), tetralogy of Fallot was observed in three siblings and a cousin, indicating a familial pattern of occurrence. Hirt-Armon et al. (1996) reported a case of a woman with tetralogy of Fallot and absence of the pulmonic valve. She gave birth to a female infant with tetralogy of Fallot, extreme hypoplasia and dysplasia of the pulmonary valve, and type III tracheal agenesis, suggesting a potential distinct syndrome with autosomal dominant inheritance. ⁽¹¹⁾

Empirical risk figures for the recurrence of isolated tetralogy of Fallot were calculated by Digilio et al. (1997) in families after excluding del(22q11) syndrome. The study included relatives of 102 patients. The results showed that the

frequency of congenital heart defects was 3% in siblings, 0.5% in parents, 0.3% in grandparents, 0.2% in uncles or aunts, and 0.6% in first cousins. The recurrence risk rate for siblings was consistent with previous estimates, indicating that genetic counseling for patients with isolated tetralogy of Fallot should not be modified after excluding patients with del(22q11) syndrome. The researchers concluded that genes other than those located on 22q11 must be involved in causing familial aggregation of nonsyndromic tetralogy of Fallot in these cases. ⁽¹³⁾

The prevalence of different phenotypes among patients with congenital heart defects (CHDs) can vary among different ethnic groups, suggesting potential ethnic differences in the underlying genetic factors. Comparative studies between Asians and Europeans have revealed differences in the incidence and characteristics of left- versus right-sided heart phenotypes in patients with complex CHDs. These differences may be influenced by various genetic factors, including variations in genetic predisposition and the variable impact of rare coding variants by common cis-regulatory variants.

In a Chinese cohort, it was observed that the incidence of extracardiac structural malformations was relatively low (2%), which may contribute to some of the observed differences in genetic landscape. This finding suggests that the lack of enrichment of chromatin-modifying genes, which play a role in regulating gene expression and development, could be partially explained by the lower incidence of extracardiac structural malformations in the Chinese population. ⁽¹⁴⁾

6. Cytogenesis

Various studies, ranging from candidate gene investigations to whole-exome sequencing (WES) studies, have revealed the heterogeneous nature of the genetic landscape of Tetralogy of Fallot (TOF). Chromosomal anomalies are commonly associated with TOF, particularly in cases of syndromic TOF. Among these chromosomal anomalies, trisomy 21 (Down syndrome) and 22q11.2 deletion (DiGeorge syndrome) are the most frequently observed, accounting for 7% and 5.2% of TOF cases, respectively. ⁽¹⁵⁾ Additionally, other chromosomal aberrations or submicroscopic copy number changes have been detected in 3% of TOF patients. ⁽¹¹⁾

The presence of microdeletion 22q11 can lead to TOF in the context of DiGeorge and velocardiofacial syndromes. This microdeletion has also been associated with familial conotruncal cardiac defects and congenital heart disease (CHD), with previous reports suggesting a higher recurrence risk for CHD when the affected parent is the mother rather than the father. ⁽¹⁶⁾

7. Molecular genetics

Mutations in various genes have been linked to congenital heart defects (CHDs), including tetralogy of Fallot (TOF), in both humans and animal models. Whole exome sequencing has proven to be a successful method in identifying new candidate genes associated with CHDs. Among the genes known to be associated with conotruncal defects, including TOF, are *GATA4*, *NKX2.5*, *ZFPM2/FOG2*, *GDF1*, and *ISLET1*. ⁽¹⁷⁾

The *NOTCH1* locus is the most frequently identified site of genetic variants that predispose individuals to non-syndromic TOF, followed by *FLT4*. Together, variants in these genes are found in nearly 7% of TOF patients. It is important to note that approximately 80% of TOF cases are non-syndromic, meaning there is typically no identifiable cause. This is largely due to the nonMendelian patterns of inheritance associated with these cases. ⁽¹⁸⁾

7.1. Mutation in the JAG1 Gene

In a significant finding, Eldadah discovered a missense mutation in the *JAG1* gene within a large family affected by autosomal dominant tetralogy of Fallot (TOF) with reduced penetrance. Among the mutation carriers, nine out of eleven individuals exhibited cardiac disease, including classic TOF, ventricular septal defect with aortic dextroposition, and isolated peripheral pulmonic stenosis. The mutation affected a highly conserved glycine residue at position 274, which is present in other EGF-like domains of *JAG1* as well as in other proteins.

The data obtained from this family provided insights into the pathogenesis of TOF associated with *JAG1* mutations. It suggested that the *JAG1* mutation could result in either a relative loss-of-function or a gain-of-function mechanism. This finding is significant as it indicates that *JAG1* mutations may play a significant role in the development of common variants of right heart obstructive disease. These findings contribute to our understanding of the genetic factors underlying TOF and provide potential avenues for further research and therapeutic interventions. ^(11,19)

7.2. Mutation in the *NKX2-5* Gene

The presence of heterozygous *NKX2.5* mutations in individuals with various congenital heart defects indicates the involvement of this transcription factor in multiple pathways during cardiac development. These pathways include the formation of atrial and ventricular septa, conotruncal septation, AV conduction, and AV valve development. It has been observed that individuals carrying *NKX2.5* mutations exhibit cardiovascular abnormalities in 31% of cases.

These abnormalities encompass a range of conditions, such as conoventricular ventricular septal defects (VSD) associated with tetralogy of Fallot, double-outlet right ventricle, and muscular VSDs that spontaneously close during infancy. These findings suggest that, particularly in individuals without a deletion in the 22q11 region, *NKX2.5* mutations may significantly contribute to the occurrence of conotruncal defects. Therefore, *NKX2.5* is considered a strong candidate gene for several forms of cardiovascular disease in young individuals. ⁽²⁰⁾

7.3. Mutation in the *GATA4* Gene

In a study conducted by Zhang et al., the *GATA4* gene was investigated in a cohort of 486 Chinese patients diagnosed with congenital heart defects. Within this group, 12 patients were found to carry heterozygous mutations in the *GATA4* gene, accounting for a prevalence of 2.5% among the study participants. Specifically, among the 64 patients diagnosed with tetralogy of Fallot (TOF), two individuals (3.1%) were identified to have *GATA4* mutations. ^(10, 11)

Similarly, Peng et al. conducted a study involving 135 sporadic pediatric Chinese patients with congenital heart defects. Among the 12 patients diagnosed with tetralogy of Fallot, one individual (8.3%) was found to carry a heterozygous missense mutation in the *GATA4* gene. ⁽²¹⁾

These findings suggest a potential role for *GATA4* gene mutations in the pathogenesis of tetralogy of Fallot in Chinese populations.

7.4. Mutation in the *ZFPM2* Gene

In a study involving 47 patients with sporadic tetralogy of Fallot (TOF), two individuals (4.3%) were found to have heterozygous mutations in the *ZFPM2* gene. Specifically, the identified mutations were ser657 to gly and glu30 to gly. This finding suggests that mutations in the *ZFPM2* gene may play a role in the development of TOF in some sporadic cases. ⁽¹¹⁾

7.5. Mutation in the *GATA6* Gene

Two novel sequence variations in the *GATA6* gene, a cardiac transcription factor, have been identified in studies investigating congenital heart defects (CHD). These mutations are associated with a higher frequency of cardiac septal defects and conotruncal abnormalities. The findings suggest that genetic variations in the *GATA6* gene may contribute to the development of CHD, particularly affecting the cardiac septa and conotruncal region. ^(22, 23)

7.6. Mutation in the *NOTCH1* Gene

The *NOTCH1* gene is responsible for encoding a transmembrane receptor that plays a crucial role in cell-to-cell communication, regulating cell fate decisions during development. It is considered the major susceptibility gene for tetralogy of Fallot (TOF). Studies have shown that approximately 4.5% of TOF patients carry heterozygous variants in the *NOTCH1* gene. This prevalence is significant, as no other single gene locus, except for the 22q11 deletion, has been identified to account for a higher number of TOF cases. ⁽²⁴⁾

Furthermore, *NOTCH1* variants have also been associated with other cardiac malformations, including bicuspid aortic valve, aortic valve stenosis, coarctation of the aorta, hypoplastic left heart syndrome, and TOF. These findings highlight the involvement of *NOTCH1* in the development of various congenital heart defects.

7.7. Mutation in *FLT4* Gene

The *FLT4* gene is responsible for encoding a receptor tyrosine kinase called vascular endothelial growth factor 3 (*VEGFR3*). Variants in the *FLT4* gene have been identified as a significant contributor to the incidence of tetralogy of Fallot (TOF). *VEGFR3* plays a crucial role in angiogenesis, the process of blood vessel formation, which is essential for proper heart development. ⁽¹⁸⁾

The association of *FLT4* variants with TOF suggests the existence of common pathways between heart development and angiogenesis. This finding highlights the importance of further investigating the molecular mechanisms that govern

both cardiac development and blood vessel formation, as they may share overlapping regulatory networks and signaling pathways.

8. Differential Diagnosis

During episodes known as “Tet spells” or in cases of heart failure, patients with tetralogy of Fallot (TOF) may exhibit symptoms of respiratory distress, cyanosis (bluish discoloration of the skin), and failure to thrive. These symptoms can sometimes lead to the consideration of alternative diagnoses, such as bronchiolitis, viral or bacterial pneumonia, pneumothorax, or severe pulmonic or aortic stenosis, as these conditions can present with similar clinical features.

It's important to differentiate TOF from other cardiac malformations that can produce similar symptoms. These include conditions that cause a right-to-left shunt, such as complete (dextro-) transposition of the great arteries with pulmonary stenosis, double outlet right ventricle (including Taussig-Bing anomaly), tricuspid atresia, Ebstein anomaly, and pulmonary atresia with intact ventricular septum. Additionally, there are conditions associated with a bi-directional shunt, including total anomalous pulmonary venous return, hypoplastic left heart syndrome, single ventricle, and truncus arteriosus, which can also present with similar clinical manifestations.

Considering the range of potential underlying cardiac abnormalities, a comprehensive evaluation is necessary to accurately diagnose the specific condition causing the symptoms in order to provide appropriate management and treatment. ⁽¹⁾

9. Treatment

In neonates with severe right ventricle outflow obstruction causing profound hypoxemia and cyanosis, prostaglandin therapy may be necessary to maintain ductal patency and ensure adequate pulmonary blood flow prior to surgical repair. In cases of tet spells, a rapid and aggressive approach is required. This includes positioning the patient to increase systemic vascular resistance, administering oxygen therapy to cause pulmonary vasodilation and systemic vasoconstriction, providing intravenous fluid bolus to improve right ventricle filling and pulmonary flow, administering morphine and intravenous beta-blockers to relax the muscle and improve right ventricle outflow obstruction, and using intravenous phenylephrine to increase systemic afterload.

The first surgical treatment for cyanotic heart conditions, including tetralogy of Fallot, was performed by Dr. Helen Taussig at John Hopkins. She observed that keeping the ductus arteriosus open improved the prognosis for children with these conditions. Currently, most congenital heart surgeons perform a modified Blalock-Taussig (BT) shunt procedure using a posterolateral thoracotomy or median sternotomy approach. This involves creating a prosthetic tube graft made of polytetrafluoroethylene (PTFE) to connect a systemic artery to the pulmonary artery. This modified BT shunt differs from the original direct anastomosis technique described by Taussig, which had a higher risk of thrombosis.

Bonchek and Starr proposed that performing a complete repair earlier in life is beneficial to prevent worse obstruction caused by fibrosis and undergrowth of the right ventricle outflow tract. Currently, large centers report excellent early survival rates (98% to 100%) after a complete primary repair. However, there is still controversy regarding the optimal approach for neonates and infants. Some advocate for primary complete repair, as it promotes normal growth and development, eliminates chronic hypoxemia, improves long-term ventricular function, reduces the incidence of late dysrhythmias, and lowers the risk of tet spells and their complications. Others argue that a two-stage procedure, starting with shunt placement, may be preferable as it allows for growth of the pulmonary valve and branch pulmonary arteries, potentially reducing the need for a transannular patch and the long-term consequences of pulmonary insufficiency. ^(1, 26)

10. Conclusion

Tetralogy of Fallot (TOF) is a complex congenital heart defect that involves abnormalities in the structure of the heart. Extensive research has identified several genes associated with TOF, including *GATA4*, *GATA6*, *NOTCH1*, *FLT4*, and *ZFPM2*. These genes play critical roles in cardiac development and signaling pathways.

Accurate diagnosis of TOF is crucial, considering the overlapping symptoms with other cardiac and respiratory conditions. Neonates with severe TOF may require prostaglandin therapy to maintain pulmonary flow before surgical

repair, while “Tet spells” necessitate a multidimensional approach for symptom relief. Surgical interventions, such as the modified Blalock-Taussig shunt, have revolutionized TOF treatment.

Ongoing research is vital for a deeper understanding of the genetic and developmental mechanisms underlying TOF. Further advancements in genetic sequencing and interdisciplinary collaboration hold promise for identifying new therapeutic targets and improving long-term outcomes. Continued efforts in TOF research are necessary to enhance patient care and provide better quality of life for individuals living with this complex cardiac condition.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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