Introduction

Tetralogy of Fallot (TOF; OMIM# 187500) is the most frequent type of congenital heart disease (CHD). TOF is one of the first CHDs repaired by corrective surgery; females and males are equally involved. TOF is named after French physician Dr. Etienne-Louis Arthur Fallot, who defined a panel of 3 cyanotic patients in 1888 with 4 anatomical characteristics: stenosis of the pulmonary artery, overriding aorta, right ventricular hypertrophy, and ventricular septal defect (VSD). In addition to genetic characteristics, the cause of TOF is not exactly clear due to the interference of other factors, such as environmental factors. Therefore, despite many advances in treatment, some (5%-6%) patients still die. The role of genetics in TOF has been reported in the literature, and the increased risk across generations strongly supports the role of genetics in TOF. The TOF publications focus on two groups of patients, syndromic and non-syndromic. TOF has been observed in the context of several syndromes including Alagille syndrome (AGS), Trisomy 21, CHARGE (coloboma, heart, atresia of the nasal, retarded, genital, ear abnormalities) and DiGeorge syndromes. Nearly 20% of TOF cases are related to genetic syndromes. The presence of mutations in genes Jagged1 (JAG1), NK2 Homeobox 5 (NKX2.5), binding protein GATA (GATA), zinc finger protein, FOG family member 2 (ZFPM2/FOG2), Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 2 (CITED2) and variants of other genes and the interaction between genetic and environmental factors underly the pathogenesis of TOF. Therefore, this review addresses current information regarding the roles of variations in JAG1, NKX2.5, GATA4, ZFPM2/FOG2 and CITED2 genes in the pathogenesis of TOF.
JAG1
The JAG1 gene is an embryological signaling molecule associated with cardiac development.9 JAG1 is a very important ligand that plays a substantial role in the developmental stages of the mammals’ heart. De novo mutations in the JAG1 ligand of transmembrane receptors NOTCH1, NOTCH2 and themselves, are impressive in cardiac development that are involved in isolated TOF (Figure 1).10–12 Jagged1 is a ligand for the NOTCH family receptors that is encoded by JAG1 gene.13 The JAG1 is involved in the decision of the cell’s fate in the early cardiac development through Notch signaling, and mutation in a number of proteins in the pathway is seen in many disorders.14,15 The JAG1 heterozygous mutations are responsible for AGS.16,17 The clinical significance of AGS lies within its cardiac structural defects and hepatic dysfunction.18 Mutations in JAG1 responsible for AGS are basically deletions and truncations resulting in haplo-insufficiency.2 In addition, the mutated JAG1 protein works as NOTCH signaling inhibitor.19 Conversely, population analysis of TOF patients mainly detects missense mutations that produce functional mRNA and a non-functional protein which prevents proper passage in the nuclear membrane due to the effect on post-translational properties. Specifically, TOF patients and carrier parents who had no apparent clinical symptoms showed an increased risk of TOF in next generations.8,20 The JAG1 structural and functional studies have shown that this gene is involved in stem cell maintenance, cell fate, cell growth, several malignancies, tumor angiogenesis, and metastatic behavior in brain, breast, head/neck, ovarian, prostate, kidney, colorectal, gastric, cervical, endometrial, pancreatic, hepatic, adrenocortical and lung cancers.21–24 The tumor invasion is affected by the relative concentration of JAG1 mRNA and is linked to the patient survival.21,22 JAG1 mutations were first identified in TOF and CHD patients with pulmonary stenosis.6 Among the Notch ligands, exon 6 of the JAG1 gene is more conserved than other exons.11,25 Several significant mutations have been identified in exon 6 that are associated with TOF. The missense mutation of G274D was reported in 2001 by Eldadah et al.1 This mutation was also identified in TOF patients in a study in 2009 but was not seen in patients with AGS syndrome.26 In a study by Kola et al, 4 novel variations in exon 6, including 2 missense (E278D and V272F), 1 nonsense (Y255X) and 1 silent variation (N287N) were reported in TOF patients.27 In a recent study in Iran, Safari-Arababadi et al, reported a significant association between synonymous variant Y255Y and TOF in Iranians.28

NKX2.5
NKX2.5 is an essential transcription factor for the development, myogenesis, and function of the heart in the fetus.29 The homologous of NKX2.5 gene in Drosophila is tinman gene. When this gene is knocked out in the Drosophila, the development of the heart is also impaired.30–33 In the development and formation of the heart, the association of NKX2.5 with septal defects is very impressive as a major feature of TOF.34 NKX2.5 mutations lead to various types of cardiac abnormalities and could account for a major proportion of the idiopathic atrioventricular block and TOF.35 In different forms of CHD, including atrial septal defect (ASD), VSD and TOF, over 37 mutations have been reported to be associated with the NKX2.5.35,36 Mutations related to TOF rarely belong to the homeodomain region of the gene, but often result in substitutions of extremely conserved amino acids. While these mutations do not lead to a relative change in the expression of proteins, substitutions in these extremely conserved domains affect the interactions with other molecules, such as DNA, and the downstream function.37 Therefore, the NKX2.5 related mutations that produce truncated and structurally-altered proteins are distinct.38–40 There is a homeodomain region called 60-amino acid in the NKX2.5 protein, which is a helix-turn-helix motif that contains 3 α-helices and interacts with DNA. This is the most important role of NKX2.5 as a transcription factor.41,42 In patients with TOF, there is an association between NKX2.5 methylation and transcriptional regulation, which seems to play a significant role in TOF pathogenesis.43

Figure 1. Gene Mutations Involved in the Tetralogy of Fallot. Gene mutations occur in the genes responsible for the normal morphogenesis of the heart, causing congenital heart disease such as tetralogy of Fallot.

To identify the clinical effects of NKX2.5 mutations associated with TOF, it is necessary to study large populations of patients. However, despite these limitations, significant clinical features related to NKX2.5 mutations have been identified.44 Research has shown that 2 causes of death in TOF patients are arrhythmias and sudden cardiac death, and the only clinical symptom to predict these complications is prolonged QRS complex (QRS duration ≥180 ms).45-47 It is assumed that these clinical symptoms can be related to the NKX2.5 gene, but no study has yet been conducted on the association of NKX2.5 mutations with these symptoms. NKX2.5 plays an outstanding role in the development of normal conduction pathways. Mutations in this gene may disrupt this pathway and cause abnormalities, which can be verified by genetic
research in the future. Therefore, it will be better to study NKK2.5 associated TOF patients with long-term clinical symptoms in the future. In Iran, Kheirrollahiet al studied the association between NKK2.5 gene and TOF, and only one synonymous variant was seen in the patients. Therefore, there was no significant association between this gene and TOF in Iranian patients.

**GATA4**

The complex process of development of the embryonic heart requires the collaboration of processes such as differentiation, cell fate, reproduction, migration and apoptosis. Furthermore, cardiac transcription factors including the GATA family are involved in this process. These transcription factors include a family of DNA binding proteins with zinc finger domains that bind to DNA. In the vertebrate, 6 GATA families have been identified (i.e. GATA1–GATA6), among which GATA4, GATA5 and GATA6 are primarily expressed in the fetal heart. Among these 3 factors, GATA4 plays a vital role in regulating gene expression in heart development and function. In patients with a variety of congenital cardiovascular diseases, including TOF, many GATA4 gene mutations are involved.

In a recent research in China, 3 families with autosomal dominant mutations in the GATA4 gene have been identified. These mutations occurred in 2-3 generations in each family, and the function of the transcription factor and DNA binding capacity also decreased. Furthermore, the mutations disturbed the physical interaction of GATA4 with TBX5, which is an effective signaling molecule in the cardiac development. In addition to GATA4 mutations in familial TOF, these mutations have also been present in sporadic pediatric cases in Chinese CHD patients.

However, further research is required in order to confirm the role of GATA4 mutations in sporadic non-syndromic TOF to determine whether these mutations are linked to TOF in Asian populations as Asia has the highest prevalence of TOF.

**ZFPM2/FOG2**

ZFPM2 gene (formerly known as FOG2) encodes a zinc-finger transcription cofactor called FOG2 (friend of GATA). This protein is a regulator of the paired-like homeodomain transcription factor 2-gamma (PITX2C). ZFPM2 gene plays a significant role in the development of the heart, and mutations in this gene are potential risk factors for CHD including the TOF. The CITED2 is the most important member of the CITED gene family, and the protein sequence of this gene has been coded by the 3 conserved regions (CR1, CR2 and CR3). CITED2 as a cAMP-response element binding protein (CREB) interacts with transcriptional modulator and is suggested as a negative regulator for hypoxia-inducible factor 1α (HIF-1α). HIF-1α is a major transcription factor that is needed for cardiovascular development and CITED2 mutations cause overexpression of HIF-1α. Springer et al in a cohort study in Germany reported for the first time 2 mutations in this gene, namely, c.-91G>A and c.1268A>G, in patients with TOF. CITED2 gene mutations that cause loss of function result in impaired cardiac development by altering the expression of genes such as vascular endothelial growth factor (VEGF) and paired-like homeodomain transcription factor 2-gamma (PITX2C).

In 3 studies in 2010, 2012 and 2014 in China, missense mutation of G184S in the CITED2 was observed in patients with TOF. Liu et al in 2014 observed the P140S mutation of CITED2 gene in Chinese patients with TOF. These mutations affect the expressions of PITX2C and VEGF genes, both of which play an extremely important role in the development of the heart (Figure 2).

**Conclusion**

Many articles and reviews have focused on the role of gene mutations in CHD patients. Further, very few genetic studies have been conducted on TOF patients and the role of gene mutations in the development of TOF in Iranians. The purpose of this review was to provide an overview on highly significant genes that may be involved in the pathogenesis of TOF. The disturbance of balance...
between transcription factors plays a substantial role in the development of the heart in the pathogenesis of non-syndromic TOF. Studies have not yet been conducted to assess the impacts of gene mutations on mortality and morbidity rates in TOF. As most patients with TOF reach reproductive age, genetic studies in TOF patients and genetic counseling can play a very important role in managing TOF.

Ethical Issues
Not applicable.

Conflict of Interests
Authors declare that they have no competing interests.

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References
8. Lu F, Morissette JJ, Spinner NB. Conditional JAG1 mutation shows the developing heart is more sensitive than developing liver to JAG1 dosage. Am J Hum Genet. 2003;72(4):1065-1070. doi:10.1086/374386
22. Dickson BC, Mulligan AM, Zhang H, et al. High-level JAG1 mRNA and protein predict poor outcome in breast...
cancer. Mod Pathol. 2007;20(6):685-693. doi:10.1038/modpathol.3800785


