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GATA.4 and TBX5 gene polymorphism in children with congenital heart disease

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Abstract

Congenital heart defects represent 25% of all congenital malformations and threaten nearly 1% of all newborns and pose a significant threat of infant death. Cardiac development during embryogenesis has been linked to the zinc finger transcription factor GATA4 (found on chromosome 8p23.1) and the T-box transcription factor family member TBX5 (placed on chromosome 12q24.21). Mutations in the GATA4 and TBX5 genes have been implicated in the development of various congenital cardiac disorders. To detect the mutations in GATA4 and TBX5 genes in children with congenital heart diseases by using direct sequencing technique. Methods: Thirty children with congenital heart disorders were studied, and their results were compared to thirty apparently healthy children with matching age and sex and without a family history of cardiac diseases as a control group. All patients underwent clinical examination, echocardiography, and genetic testing for the GATA4 rs55633527, GATA4 rs114868912, and TBX5 rs77357563 polymorphisms using the Sanger sequencing method, as well as other tests. Two variants were detected in GATA4 gene and one variant in TBX5 gene. Conclusions: GATA4 1 rs55633527 CT+TT, GATA4 2 rs114868912 GA+AA and TBX5 rs77357563 GT +TT were significantly associated with higher risk to develop congenital heart disease.

Keywords: Heart, congenital, polymorphism, GATA4, TBX5

Abbreviations

CHD: Congenital Heart Defects; HOS: Holt-Oram Syndrome; CAD: Coronary Artery Disease; ASD: Atrial Septal Defect; VSD: Ventricular Septal Defect; PDA: Patent Ductus Arteriosus; TGA: Transposition of Great Arteries; PS: Pulmonary Stenosis.

Introduction

Congenital heart defects refer to abnormalities in the structure and/ or function of the heart that arise before birth. They make an important contribution to infant mortality and remain a leading cause of death among infants in many countries in the world, where Congenital Heart Defects (CHDs) represent 25% of all congenital malformations and threaten nearly 1% of all newborns and pose a significant threat of infant death. In the United States there are more than 35.000 new cases each year and over 1 million survivors of congenital heart disease in the community. The transcriptional regulatory pathways involved in cardiac morphogenesis are extremely complex, and the effects exerted by the essential genetic transcriptional factors participating in these networks are still being elucidated [1,2]. Genes that encode transcription factors have been previously associated with congenital heart diseases and include TBX5, NKX2.5 and GATA4. There have been many novel and functional GATA4 and TBX5 mutations identified, and these variants have the potential to be exploited as risk predictors in the molecular diagnosis of coronary artery disease in the future.

A number of essential cardiac transcription factors, including Nkx2-5, TBX5, and GATA4, are involved in the process of cardiogenesis. These transcription factors interact with one another and reinforce one another during the process of cardiogenesis. Because it contains two zinc finger domains, GATA4 is involved in DNA binding as well as interaction with other proteins, such as NKX2.5 and TBX5. Due to the presence of an N-terminal domain, GATA4 aids in the stabilization of DNA binding as well as the interaction with a subclass of Zinc Finger Protein Multi-Type (ZFPM) originally known as FOG (friend of GATA) [3,4].

Each of these compartments, the proepicardium, epicardium, and myocardium, expresses the GATA4 gene, and its activity is critical for each of these compartments. Changing the level of GATA4 protein even by a small amount may have a significant impact on heart development and embryonic survival. The TBX5 gene gives instructions for generating a protein known as T-box 5, which is critical in the construction of tissues and organs during embryonic development. The TBX5 gene is found in both humans and mice [5,6]. Other genes' activity is

controlled by this protein, which attaches (binds) to certain sections of DNA and controls the activity of other genes. The Holt-Oram Syndrome (HOS) is caused by mutations in the T-box transcription factor TBX5, which causes heart and limb abnormalities in those who have the disorder HOS. Depending on where they occur within the T-box gene, they are thought to cause either more severe cardiac issues or more severe limb abnormalities. A single amino acid substitution at the N-terminal end of the T-box, which was predicted to disrupt TBX5 binding to the major groove of target DNA, was proven to cause severe heart deformities in mice but only minor limb abnormalities in the same animals [7,8]. The laboratory mouse was also observed to have mild cardiac abnormalities but severe limb deformities when amino acid modifications were made to the C-terminal end of the T-box, which was predicted to enable TBX5 to get dislodged from the minor groove of the target DNA. Following the publication of these findings, it was revealed that TBX5 genotypes may be predictive of Holt-Oram syndrome expressivity, which was previously unknown. The identification of novel and functional TBX5 mutations has the potential to be useful in the molecular diagnosis of coronary artery disease, particularly in the context of risk prediction. The aim of this study was to assess the role of GATA4 rs55633527, GATA4 rs 114868912 and TBX5 rs77357563 polymorphism in children with congenital heart diseases [9,10].

Materials and Methods

This study was a case control study including pediatric patients who were diagnosed with congenital heart diseases in Pediatric Cardiology Clinic of Benha and Menoufia University Hospitals (case-control study design). Laboratory investigations were done in Clinical and Chemical Pathology department, Benha University Hospital and Clinical and Chemical department, Cairo University Hospital. The case group consisted of 16 females and 14 males with mean age 7.4 months. The normal control group consisted of thirty children who seemed to be in good condition, were the same age and gender, and had no family history of congenital heart diseases. Parental permission was gained in writing via an official process [11,12]. The ethics committees gave its approval for this research. All subjects in our study were subjected to full history taking, review of medical records, physical examination, 2-dimentional trans-thoracic echocardiography with color flow Doppler and laboratory investigations. In order to extract DNA, two milliliters of venous blood were taken in an EDTA tube and stored frozen at-40°C until the analysis was completed. Analyses of the GATA4 rs55633527, GATA4 rs114868912, and TBX5 rs77357563 polymorphisms were performed using PCR and the sanger sequencing method, respectively [13,14].

The following procedures were followed to genotype all participants for GATA4 and TBX5 gene mutations at five exons using the Sanger sequencing technique: By using the QIA amp DNA Mini Kit, we were able to extract genomic DNA from EDTA blood leucocytes (Qiagen, Germany, cat. no. 51304). The extracted DNA was amplified by Polymerase Chain Reaction (PCR) using a specified set of primers.

Polymerase chain reactions were used to amplify the coding portions of the GATA4 and TBX5 genes from genomic DNA, including exon/intron boundaries. It was possible to amplify 5 exons situated on the GATA4 gene and 5 exons located on the TBX5 gene using the PCR method, which was previously reported [15,16]. PCR was used to accomplish enzymatic amplification of the isolated DNA. The PCR Master Mix (2X) (Metabion, Germany) (Cat#7022S) was used for this purpose, Primers for PCR (Metabion, Germany) Two sets of PCR primers for each of the five exons were used. For 100 pmol/µl, lyophilized primers were dissolved in the proper amount of nuclease free water and PCR thermal cycler (Biometral thermal cycler). Trans-illumination of Ultraviolet (UV) light was used to view the amplified products after they were separated by gel electrophoresis on a 2 percent agarose gel containing ethidium bromide. Using the MEGA quick-spin purification kit, the separated products were processed to enable selective adsorption of DNA molecules within certain size ranges, allowing for the detection of small DNA molecules (iNtRON-Korea, cat.no. 17286). The purified products were sequenced using Sanger sequencing technique. PCR products were sequenced through Sanger sequencing method. Fragments were sequenced using the BigDye sequencing kit (Applied Biosystems-USA, cat. no. 4337454) which contains specific terminating nucleotides then, the reaction products were separated on the automated sequencer, the reaction products were separated on ABI 310 PRISM automated sequencer. The sequenced products were analyzed by in silico analysis procedure for analysis of data and prediction of mutations functionality through certain software algorithms and through minor allele frequency [17,18].

Results

The study included 30 cases with congenital heart disease (14 males and 16 females) with mean age (7.4 ± 1.6) months and 30 healthy subjects (age and sex matched) as a control group, including 11 males and 19 females, their mean age was (8.2 ± 2.1) months. Regarding age, gender, positive family history, and consanguinity, there was no statistically significant difference between the cases and the controls [19,20]. However, as compared to the control group, there was a statistically significant decline in the percentiles of weight (p0.001) and height (p0.013) in the CHD patients. We discovered three single nucleotide polymorphisms (SNPs) that resulted in missense mutations in the GATA4 gene: GATA4 rs55633527 and GATA4 rs11468912, and one TBX5 SNP: TBX5 rs77357563. The GATA4 gene contains two SNPs GATA4 rs55633527 and GATA4 rs11468912, and one according to the results of this study, when it comes to GATA4-rs55633527, there is a statistically significant decrease in the frequency of genotype CC in cases when compared to the control group. A further finding is that, when the patients are compared to the control group, there is a statistically significant rise in the frequency of genotype CT and TT (P=0.005; Tables 1-4).

Table 1. *The Sequence of PCR primers of TBX5 9.*

Primers		Sequence(5'-----3')	Size (bps)
Exon9p1	F	TTGGCCAAATAACTGTCTC	465
	R	GCTGGAACTTCCCTCTCC	

Table 2. *The Sequence of PCR primers of GATA4 9.*

Primers		Sequence(5'-----3')	Size(bps)
Exon 2p1	F	GTGGGTTCTGAAAGCTCTGG	
	R	CCTCGGTGTCCTCTCTCTCC	497
Exon 2p2	F	CACGCATATTATCGTTGTTGC	267
	R	GCCCTGGAGGTAGGACAGG	

Table 3. *Demographics of the studied groups.*

	Control N=30		Cases N=30	P	
	Mean	± SD	mean		± SD
Age (months)	8.2	2.1	7.4	1.6	0.675
	N	%	N	%	
Males	11	36.7	14	46.7	0.432
Female	19	63.3	16	53.3	
Weight (percentile)	52.1	15.6	18.6	5.3	<0.001
Height (percentile)	48.3	13	36.8	8.7	0.013
Positive family history	No	%	No	%	
	0	0	2	6.7	0.472
Positive consanguinity	3	10	6	20	0.469

Table 4. *Echocardiographic findings of the studied cases.*

	Cases N=30	
	N	%
Cardiac enlargement	16	53.3
VSD	16	53.3
PDA	10	33.3
ASD	9	30
PS	2	6.7
TGA	1	3.3
Aortic coarctation	1	3.3
Epstein anomaly	1	3.3
Fallot tetralogy	1	3.3

A statistically significant increase in allele T frequency is seen when patients are compared to the control group, while a statistically significant decrease in allele C frequency is observed when patients are compared to the control group Table 5.

Table 5. *Genotype and allele frequency in the studied groups.*

		Control N=30	Cases N=30	P	OR	95% CI			
		N	%				N	%	
GATA4-rs55633527	CC	21	70	10	33.3		R		
	CT	8	26.7	14	46.7	0.024	2.246	1.11	4.546
	TT	1	3.3	6	20	0.015	4.609	1.338	15.88

	CT+TT	9	30	20	66.7	0.005	2.6	1.341	5.038
	C	50	83.3	34	56.7	0.001	3.824	1.635	8.942
	T	10	16.7	26	43.3				
GATA4-rs114868912	GG	24	80	12	40		R		
	GA	5	16.7	14	46.7	0.005	2.899	1.383	6.075
	AA	1	3.3	4	13.3	0.059	3.569	0.952	13.388
	GA+AA	6	20	18	60	0.002	3.02	1.514	6.023
	G	53	88.3	38	63.3	0.001	4.383	1.7	11.301
	A	7	11.7	22	36.7				
TBX5-rs77357563	GG	22	73.3	13	43.3		R		
	GT	7	23.3	11	36.7	0.099	1.841	0.892	3.798
	TT	1	3.3	6	20	0.025	4.038	1.187	13.736
	GT+TT	8	26.7	17	56.7	0.019	2.216	1.141	4.305
	G	51	85	37	61.7	0.004	3.523	1.462	8.486
	T	9	15	23	38.3				

R, reference; OR, odds ratio; CI, confidence interval.

Logistic regression test was used when the GATA4-rs114868912 variant was examined in this research, it was shown that there was a statistically significant drop in the frequency of genotype GG in cases when compared to the control group (P 0.001). And when comparing the patients to the control group, there is a statistically significant rise in the frequency of genotype GA and AA (P=0.002). In addition, when comparing the patients to the control group, there is a statistically significant drop in the frequency of allele G, and a statistically significant rise in the frequency of allele T in the cases (both P=0.001)(Table 5) [21,22]. In this research, when TBX5-rs77357563 is compared to the control group, there is a statistically significant drop in the frequency of genotype GG in the patients (P 0.001) compared to the control group. In addition, when comparing the patients to the control group, there is a statistically significant increase in the frequency of genotype GT and TT (P=0.019). Additionally, when comparing patients to the control group, there is a statistically significant drop in the frequency of allele G, and a statistically significant rise in the frequency of allele T (P=0.004) in cases. GATA4 rs55633527 CT+TT, GATA4 rs114868912 GA+AA, TBX5 rs77357563 GT+TT were significantly associated with higher risk to develop CHD, in univariable and multivariable analyses (Table 6).

Table 6. *Regression analysis for prediction of CHD within control subjects.*

	Univariable				**Multivariable**			
	p	OR	95% CI		p	OR	95% CI	
Age	0.67	0.985	0.921	1.054				
Gender	0.433	0.662	0.236	1.858				
Family history	1	1	0.131	7.605				
Consanguinity	0.286	2.25	0.507	9.993				
GATA4 1 rs55633527 CT +TT	0.001	3.824	1.635	8.942	0.001	5.568	2.018	15.362
GATA4 2 rs114868912 GA +AA	0.001	4.383	1.7	11.301	0.002	3.766	1.614	8.791
TBX5 rs77357563 GT +TT	0.004	3.523	1.462	8.486	0.002	5.123	1.809	14.505

Discussion

Among all birth abnormalities, congenital heart disease is the most frequent form, accounting for roughly 30 percent of all significant congenital anomalies that cause death in the first year of life. There are about 150 million live births every year around the globe, with 1.35 million of them being impacted with CHDs. As previously stated, the etiology of CHDs comprises both genetic and environmental components that are currently being investigated. This is due to the fact that mutations in linked genes can only explain a tiny proportion of CHD occurrences. The pathophysiology of sporadic occurrences of Coronary Artery Disease (CAD), the most frequent kind of CHD, is still poorly understood. There are many genes that have been identified as being critical for heart development. In the case of GATA4, NKX2-5, and TBX5, for example, it is possible that they work in concert to control a

subset of genes essential for heart development. As a result, it is probable that certain changes in each gene have an effect on how they interact. Discovered that a mutation in the GATA4 gene had an influence on transcription factor GATA4 protein trafficking by impairing normal nuclear localization and causing partial protein distribution in the cytoplasm alongside the nucleus, as previously reported [23,24]. Approximately 85 percent of TBX5 gene mutations are novel either missense or nonsense variants, with the majority of them occurring in the T-box domain. Because of these mutations, either a lack of protein or reduced DNA binding results in loss of function, or a gain of function results in enhanced DNA binding, resulting in a gain of function. A substantial number of non-coding variants of TBX5 located in the intronic region, promoter region, and enhancer area have been discovered to be associated with coronary heart disease. These variations are found in the intronic region, promoter region, and enhancer region (CHD).

In the present study, we detected two GATA4 SNPs: GATA4 rs55633527, GATA4 rs11468912. According to our study, there was no significant difference in age and sex among the studied groups as shown in table. The results reported in this study are in agreement with who reported that there were no statistically significant difference in age, sex among the study groups which included 100 CHD patients (ASD, VSD, TOF and AVSD) and 200 controls in their study. In this study, we reported three Single Nucleotide Polymorphisms (SNPs) which caused missense mutation which include two GATA4 SNPs: GATA4 rs55633527, GATA4 rs11468912 and one TBX5 SNP TBX5 rs77357563. The GATA4-rs55633527 variant was shown to be associated with a statistically significant reduction in the frequency of genotype CC in cases when compared to the control group (P=0.001) in this investigation. In addition, when comparing the patients to the control group, there was a statistically significant increase in the frequency of genotype CT and TT (P=0.005).GATA4 rs55633527 TT genotype in our study showed significantly highest consanguinity proportion, with significant differences between CC, CT, and TT genotypes. There was a statistically significant drop in the frequency of genotype GG in patients when compared to the control group for GATA4-rs114868912 in this research (P 0.001) when comparing cases and controls. And when comparing the patients to the control group, there was a statistically significant increase in the frequency of genotype GA and AA (P=0.002).. GATA4 rs114868912 GG genotype in our study was significantly associated with consanguinity when compared to GA+AA genotypes. reported several GATA4 mutations in 3'UTR, 5'UTR and GATA4 exons after isolation of the genomic DNA then amplification reactions were done and the purified products were directly used as templates for sequencing using dideoxy chain terminator sequencing technique (Sanger sequencing method) and the sequenced products were analyzed using PolyPhen and SIFT in silico analysis algorithms. On the contrary, did not report any role of GATA4 Single Nucleotide Polymorphisms (SNPs) in non-syndromic CHDs. This might be owing to the fact that they evaluated the GATA4 gene using Multiplex Ligation-Dependent Probe Amplification (MLPA) and subsequently studied the MLPA products using an ABI PRISM 3130 automated sequencer, while they analysed the GATA4 gene using a conventional sequencing. The results of the MLPA study indicated that the normalised MLPA signals for all exons in all patients were all determined to be within the normal range values und that no significant correlation between the type of defect and the mutation frequency, because several reported GATA4 mutations overlapped in producing various types of septal defects. TBX5-rs77357563 is associated with a statistically significant decrease in the frequency of genotype GG among patients (P=0.001) when TBX5-rs77357563 is evaluated in comparison to the control group in this study. Furthermore, when comparing the patients to the control group, there is a statistically significant increase in the frequency of genotypes GT and TT (P=0.019), which is consistent with previous findings as seen in figure. According to, a previously published research clarified the function played by the mutation spectrum of GATA4 and TBX5 in the development of VSD. The findings of this study vary from ours in that they describe another heterozygous GATA4 missense mutation. In a recent study, found that the link between the TBX5 genotype and the severity of the clinical aspects of HOS has remained a controversial matter of contention. The great majority of TBX5 mutations are associated with severe cardiac and skeletal problems, according to the genetic testing company. Due to the fact that missense mutations may arise anywhere within the DNA-binding region of the gene, they can generate an extremely broad variety of phenotypes in the heart and limbs of the person who has the mutation. Others feel that missense mutations at the amino terminus of the DNA-binding domain cause severe cardiac difficulties, while others believe that they induce skeletal abnormalities of lower severity. Genetic mutations in the TBX5 gene that affect the C-terminus, on the other hand, are associated with less severe cardiac problems but severe skeletal malformations. Despite this, when a greater number of mutations and persons were studied, this relationship could not be established. As a result, it is still necessary to identify the criteria and variables that influence the genotype-phenotype link in HOS. Observe that the position of the mutation in the TBX5 gene is related to the phenotype expression of HOS as their study confirms that mutations in an elongated TBX5 protein underlie HOS. Their results support the idea that any TBX5 mutation not prominently in the T-box region has the potential to affect the development of the heart and limbs in HOS. The severity of impairments seems however to depend on the precise location of the mutation. Basson and colleagues discovered that some of the mutations are predicted to function as null alleles, while others are missense mutations that seem to encode an active mutant protein, according to the results of their study. In this study, they demonstrate that there is a link between the locations of missense mutations and the clinical problems that arise as a consequence of them. A single amino acid alteration towards the amino-terminal end of the T-box results in very significant cardiac defects. This substitution should have an impact on interactions with the major groove of the target DNA sequence, yet it does not. While mutations in the carboxyl terminus of the T-box should have an influence on the contacts between TBX5 and the minor groove of a DNA target sequence, mutations in the amino acid sequence of the T-

box produce severe limb abnormalities in mutant mice. It is hypothesized that structurally variable TBX5-target DNA interactions have distinct functional implications in the developmental pathways of limb and heart shape, as well as in other developmental pathways. This hypothesis is supported by the findings of this study. Similarly, Al-Qattan and Abou Al-Shaar agreed with Basson and noted that extended protein variations are more likely to cause severe bilateral skeletal deformities and more severe cardiac problems than shorter protein variants, which is in line with previous research. on the other hand, discovered that neither the kind of mutation nor the site of a mutation is a significant influence in the development of CHD symptoms, contrary to what has previously been reported hypothesised that a gain of function variation would have negative consequences on heart development but would not have such negative effects on upper limb development, and their findings were confirmed. TBX5 genotypes are not predicted to be associated with the expressivity of HOS, according to the results of this is due to the fact that all prior investigations that revealed a genotype-phenotype association relied on a limited number of independent instances. In order to further study the association between TBX5 genotypes and HOS manifestations, they sequenced the coding and non-coding areas of TBX5 in 55 probands with HOS to see whether there was a causal relationship. Their findings included the discovery of 14 unique variations in 17 different kindreds, as well as six missense mutations in affected people with symptoms ranging throughout the spectrum of HOS presentations. A total of 17 distinct kindreds were tested, and they discovered the mutation. According to the findings, neither the type of mutation in TBX5 nor the location of the mutation in TBX5 was connected with the degree of limb or cardiac abnormalities in a patient suffering from haemophilia A. According to the results of Al-Qattan and Abou Al-Shaar, there is controversy in the literature about the genotype-phenotype relationship in the case of TBX5 missense mutations. Researchers discovered that the same missense mutation can cause either a severe or mild radial-ray deficiency in different family members, although certain mutations such as (Asp61Tyr, Gly80Arg, Trp121Gly), (Gly169Arg, and His170Leu) tend to result in a mild degree of radial-ray deficiency in different family members, according to the researchers. It was discovered that three genetic variants in the gene GATA4 (rs55633527 CT+TT, gta4 rs114868912 GA+AA, and TBX5 rs77357563 GT+TT) were significantly associated with a higher risk of coronary heart disease in both univariable and multivariable analyses, according to this study.

Conclusion

In conclusion, the current study proved a link between GATA4 rs55633527, GATA4 rs11468912 and TBX5 rs77357563 mutations and congenital heart defects. The information gathered was tabulated and evaluated with the help of the SPSS version 16 program (SPSS Inc, Chicago, ILL Company, USA). Numerical and percentage representations of categorical data were used. When the p value was less than 0.05, it was regarded statistically significant, and when it was less than 0.01 it was declared highly statistically significant. We used the Chi square test (X2), Odds Ratios (OR), and the related 95 percent Confidence Interval (CI) to determine whether or not there was a significant difference.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper

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