ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

NKX2-5 Gene Variants Associated with Congenital Heart Defects in Turkish Population

Türk Popülasyonunda Konjenital Kalp Hastalıkları ile İlişkili NKX2-5 Gen Varyantları

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Abstract

Introduction: Congenital heart defects (CHDs) are the most common congenital anomaly of the newborn with high mortality and morbidity rates. Genetic and environmental risk factors have affect on cardiogenesis. NKX2-5 (NK2 homeobox 5) is a homeobox containing gene which is essential for cardiac differentiation. In this study, our aim was to detect NKX2-5 gene variants associated with CHDs in Turkish population and to better understand genotype- phenotype correlations.
Materials and Methods: In this study, we designed primers specific for NKX2-5 gene and sequenced the gene in 80 isolated CHD and 50 control group patients. Patients with chromosomal anomalies, DiGeorge syndrome and multiple congenital anomalies were not included.
Results: Most common CHDs seen in the patients were ventricular septal defects (VSD) and atrial septal defects (ASD) (20%), atrioventricular septal defects (AVSD) and tetralogy of Fallot (TOF) (8.75%). We have detected NKX2-5 gene variants in 3.75% of the patients. We found A119S, R161P and C270Y changes in TOF; PFO (patent foramen ovale) with transient supraventricular, ventricular arrhythmia; and ASD patient, respectively.
Conclusion: This study is designed to contribute to the genetic variations associated with CHD in Turkish population. NKX2-5 gene R161P variant which is on homeobox domain, was previously reported as pathogenic in an individual with thyroid ectopy and PFO. Further studies are needed to evaluate a possible role of these changes. Genetic testing is important in the follow-up and treatment of patients.

Keywords
Congenital heart defects, NKX2-5 gene, tetralogy of fallot, patent foramen ovale, atrial septal defect

Öz

Giriş: Konjenital kalp hastalıkları (KKH), yenidoğan döneminde yüksek mortalite ve morbidite oranları ile en sık görülen konjenital anomalidir. Kardiyogenezde genetik ve çevresel faktörlerin etkisi vardır. Homeobox içeren NKX2-5 (NK2 homeobox 5) geninin kardiyak farklılaşmada önemli rolü vardır. Bu çalışmamızda amaç, Türk polpulasyonunda KKH ile ilişkili NKX2-5 gen varyantlarının saptanması ve genotip-fenotip korelasyonlarına katkı sağlanmasıdır.
Gereç ve Yöntem: Bu çalışmamızda 80 izole KKH hastasında ve 50 kontrol grup hastasında NKX2-5 genine özgü primerler design edilerek gen sekanslanmıştır. Kromozomal anomalisi, DiGeorge sendromu ve multipl konjenital anomalileri olan hastalar çalışmaya alınmamıştır.

Anahtar kelimeler
Konjenital kalp hastalıkları, NKX2-5 geni, fallot tetralojisi, patent foramen ovale, atrial septal defekt

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Bulgular: Çalışmamızda hastalarda en sık ventriküler septal defekt (VSD) ve atrial septal defekt (ASD) (%20) ile atrioventriküler septal defektler (AVSD) ve Fallot tetralojisi (TOF) (8.75%) saptanmıştır. Hastalarda NKX2-5 geni varyantları %3.75 oranında görülmüştür. A119S, R161P and C270Y değişimleri sırasıyla TOF; geçici supraventriküler, ventriküler aritminin eşlik ettiği PFO (patent foramen ovale); ve ASD hastasında mevcuttu.
Sonuç: Bu çalışma, Türk populasyonunda KKH ile ilişkili varyantların saptanmasına katkı sağlanması amacıyla sunulmuştur. Tiroid ektopisi olan PFO hastasında NKX2-5 geni homeobox domainde bulunan R161P varyantı daha önceden patojenik olarak tanımlanmıştır. Varyantların olası etkilerinin değerlendirilebilmesi için daha fazla çalışmaların yapılması gerekmektedir. Genetik testlerin yapılması hastaların takibi ve tedavisi için önemlidir.

Introduction

Congenital heart defects (CHDs) are the most common congenital anomaly of the newborn with high mortality and morbidity rates even with advances in surgery (1). Genetic mechanisms involved are complex with genetic and environmental risk factors affecting cardiogenesis. A wide range of CHD spectrum includes septal defects, valve defects and lesions affecting the outflow tract (2). *NKX2-5* (NK2 homeobox 5) is a homeobox containing gene which is essential for cardiac differentiation (3). *NKX2-5* gene mutations have been found in patients with atrial septal defect (ASD) 7, with or without atrioventricular (AV) conduction defects (OMIM#108900), conotruncal heart malformations, variable (OMIM#217095), hypoplastic left heart syndrome 2 (OMIM#614435), Tetralogy of Fallot (TOF) (OMIM#187500) and ventricular septal defect (VSD) 3 (OMIM#614432).

In this study, we sequenced the *NKX2-5* gene in 80 isolated CHD patients and 50 control patients. Our aim was to evaluate the variants of the *NKX2-5* gene related to isolated CHD in the Turkish population and to better understand genotype- phenotype correlations.

Materials and Methods

In our study, 80 CHD patients and 50 control group healthy participants were included. Patients with chromosomal abnormalities, DiGeorge syndrome and multipl congenital anomalies were excluded from the study. The approval for this study was obtained from the Marmara University Faculty of Medicine Clinical Research Ethics Committee (date: 08.01.2016, approval number: E-70737436-050.06.04). Informed consents were obtained from all the study participants.

All the participants were evaluated by a pediatric cardiologist at the Marmara University School of Medicine.This included a clinical history, physical examination, electrocardiogram, echocardiography, and catheterization. Participants were also evaluated by medical geneticist for pedigrees, family history and physical examination.

Participants genomic DNA was isolated from peripheral blood leucocytes using RINA™ M14 nucleic acid extraction kit (IVD biotechnolojy, Istanbul, Turkey) according to the manufacturers' protocols. Samples DNA quantification and qualification measurements were done by NanoDrop™ 2000/2000c Spectrophotometer (Thermo Scientific, Inc., Waltham, MA, USA). For qPCR, 3 forward (F) and 3 reverse (R) primers were designed according to referential genomic DNA sequence of NKX2-5 in GenBank database (accession no. NT_023133) (Table 1). qPCR was performed with Premix Ex Taq DNA polymerase (Cat no.# RR039W) (Takara Bio Inc., Shiga, Japan) on a Biorad CFX96 Touch thermal cycler (Biorad; Berkeley, CA, USA). The PCR cycling parameters were as follows: Pre-denaturation of template and activation of the DNA polymerase at 95˚C for 10 min, followed by 45 cycles of denaturation at 95˚C for 20 sec, annealing at temperatures specific for the primers for 20 sec and extension at 72˚C for 25 sec. Amplified products were visualised on 1% agarose gels.

PCR products were sequenced with the BigDye® Terminator v1.1 Cycle Sequencing kit (Life Technologies, Carlsbad, CA, USA) with an ABI PRISM 3130XL DNA Analyzer (Applied Biosystems, Waltham, MA, USA). In order to evaluate the pathogenicity of the novel variants, we used in silico prediction tools, mutation databases (Human Gene Mutation Database and Clinvar), allele frequency in population studies (1000 Genome, Genome Aggregation Database) and the American College of Medical Genetics and Genomics (ACMG) genetic variant classification criteria (4).

Table 1. Primer pairs designed for NKX2-5				
Primer	**Forward (5'-3')**	**Reverse (5'-3')**	**Amplicon size (bp)**	**Annealing temperature (^{0}C)**
Exon 1	caaaaggagacccttccaaa	cgacaacaccaggcatcttac	844	59
Exon2a	caagcgtctctctgcctctc	gggtcccttccctaccag	685	60
Exo2b	gccgccaacaacaacttc	ggttccagcaagggttaggt	724	60

Statistical Analysis

Were done by chi-square test.

Results

Patients included in the study were aged ranging between 7 days to 17 years (median age, 13 months). Patients were 36 females (45%) and 44 males (55%). Consanguinıty of parents was seen in 12 (15%) and family history of CHD was in 10 (12.5%) patients. 16 (20%) patients had VSD and ASD, respectively. AVSD and TOF were each seen in 7 patients (8.75%) (Table 2). Healthy control group partiicipants included 30 females (60%) and 20 males (40%) aged ranging between 2 years to 45 years (median age, 24 years).

Of the 3 patients *NKX2-5* (NM_ 004387) gene variants were found in Exon 2. In a TOF female patient heterozygous c.355G>T (p.A119S) variant was found (Figure 1). Segregation analysis for this patient couldn' t have been done. In the second patient with PFO and transient supraventricular and ventricular arrhythmia in the newborn period c.482G>C (p.R161P) variant was found (Figure 2). His healthy father had this variant in a homozygous state. In an ASD male patient heterozygous c.809G>A (p.C270Y) variant was found (Figure 3). His father was found to be a heterozygous carrier (Table 3). These variants were not detected in the control group.

Discussion

NKX2-5 (NK2 homeobox5) gene which is on 5q35.1, has 2 exons and encodes a 324 aa protein. Functional domains of the NKX2-5 protein and our patients aminoasit changes are shown in Figure 4.

NKX2-5 gene variants have been found in about 3% of CHD patients, similar to our study (3.75%) (5). Previously, Akçaboy et al. (6) reported 72 conotruncal anomalies in Turkish patients and the p.R25C variant was found where together with previous studies its pathogenicity is not concluded.

Our TOF patient had A119S change that has been previously reported in patients each with adult onset cardiomyopathy and hypoplasic left heart syndrome where in functional studies a significant reduction in transcriptional activities was determined (7,8).

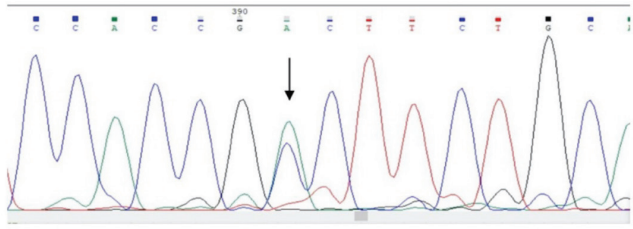

Figure 1. NKX2-5 (NM_004387) gene heterozygous c.355G>T (p.A119S) variat in TOF patient (reverse strand)

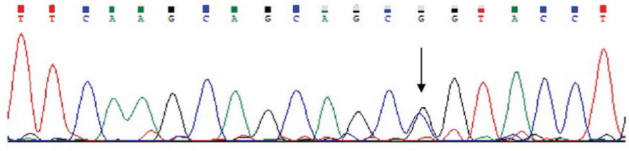

Figure 2. NKX2-5 (NM_004387) gene heterozygous c.482G> (p.R161P) variant in PFO patient

PFO: Patent foramen ovale, TOF: Tetralogy of fallot

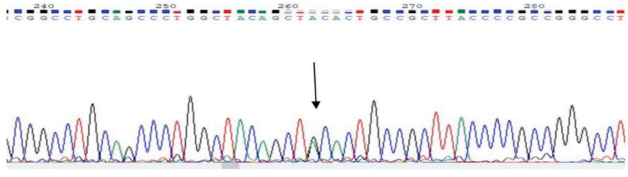

Figure 3. NKX2-5 (NM_004387) heterozygous c.809G>A (p.C207Y) variant in ASD patient

ASD: Atrial septal defect

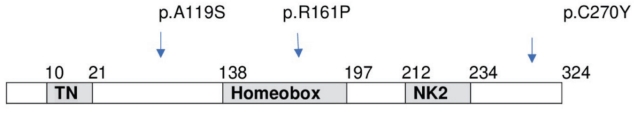

Figure 4. Functional domains of the NKX2-5 protein showing the TN, Homeobox and NK2 domains with domains with the corresponding amino acid numbers. Our three patients amino said changes are given with arrows

TN: Tinman

Table 2. Clinical characteristics of CHD patients	
Age (years)	Avr.: 3 years 9 months
Age at diagnosis (months)	Avr.: 12 months
	Number (%)
Female	36 (45%)
Consanguinity	12 (15%)
Family history	10 (12.5%)
VSD	16 (20%)
ASD	16 (20%)
AVSD	7 (8.75%)
TOF	7 (8.75%)
AQ	4 (5%)
AQ+VSD	2 (2.5%)
PDA	2 (2.5%)
PS+VSD	2 (2.5%)
VSD+PFO	2 (2.5%)
PA+VSD	2 (2.5%)
ASD+PDA	1 (1.25%)
PS	1 (1.25%)
VSD+PDA+PFO	1 (1.25%)
VSD+TA	1 (1.25%)
PS+PDA	1 (1.25%)
PA+ASD+VSD	1 (1.25%)
AQ+ASD+VSD	1 (1.25%)
TOF+ASD	1 (1.25%)
TOF+PDA+PFO	1 (1.25%)
TOF+RAA	1 (1.25%)
TGA+AVSD	1 (1.25%)
LVH+TGA	1(1.25%)
TGA+DOLV+VSD+ PS	1 (1.25%)
SV	1 (1.25%)
LVH+DORV+PA+RAA	1(1.25%)
PA, VSD, TGA, RAA	1 (1.25%)
PFO	1 (1.25%)
TrA	1 (1.25%)
Other	2 (2.5%)

Avr.: Avarage, ASD: Atrial septal defect, AQ: Aort quarctation, AVSD: Atrioventricular septal defect, DOLV: Double outlet left ventricule, DORV: Double outlet right ventricule, LVH: Left ventricular hypoplasia, PA: Pulmonary atresia, PDA: Patent ductus arteriosus, PFO: Patent foramen ovale, PS: Pulmonary stenosis, RAA: Right aortic arch, SV: Single ventricule, TA: Tricuspid atresia, TGA: Transposition of great arteries, TOF: Tetralogy of fallot, TrA: Truncus arteriosus, VSD: Ventricular septal defect

This variant has also been reported previously in an individual with thyroid ectopy, but also in relatives with normal thyroid function, which showed reduced DNA binding affinity (9). This variant is likely to be a rare, disease-modifying polymorphism (7,10). In a family with left ventricular noncompaction *MYH7, MLK2* gene variants were found and *NKX2-5* A119S change was suggested to be a modifier (11).

R161P variant found in our patient with PFO was only previously reported in an individual with thyroid ectopy. This reported patient also had PFO at birth that resolved spontaneously and had minor mitral valve insufficiency. There was minor mitral valve insufficiency in the father transmitting the R161P. This variant showed reduced DNA binding affinity (9). This variant is located on the homeobox domain (HD), and it was shown that truncation or missense mutations in the HD had severely reduced DNA binding activity and little or no transcriptional activation function (12). These variants in HD also lead to secundum ASD with AV block with a prevalence of 97.2%. Our patient also had transient supraventricular and ventricular arrhythmia in the newborn period. This variant was transmitted from a healthy father, which maybe attributed to incomplete penetrance, but we don't know if the father had PFO as a child (13).

Our patient with ASD had the C270Y variant which is on tyrosine -rich domain. This variant was previously reported as VUS (variant of unknown significance) in a patient with dilated cardiomyopathy (14). A previously reported ASD patients family members segregation analysis suggested that this variation was not correlated with CHD (13). In a RAA (right aortic arch) patient with this variant, no functional impairment in transcriptional assay was shown, still it may have caused alterations not detected by their testing system (15). In-silico analyses to examine the effects on the secondary structures of proteins showed that there was no apparent distinction between the NKX2–5 mutant protein and the wild-type protein (16). This variant is not sporodic in our patients family.

Table 3. Variants in *NKX2-5* gene and associated CHD

Patient	Exon	Nucleotid change	Amino-acid change	Domain	Familial/sporadic	CHD
1	2	c.355G>T	p.A119S	-	NA	TOF
2	2	c.482G>C	p.R161P	Homeobox	Familial	PFO
3	2	c.809G>A	p.C270Y	-	Familial	ASD

ASD: Atrial septal defect, CHD: Congenital heart defect, NA: Not available, PFO: Patent foramen ovale, TOF: Tetralogy of fallot

Conclusion

As there are few studies related with the *NKX2-5* gene in the Turkish population, we have reported our three *NKX2-5* gene variants to contribute to the genetic variations associated with CHD. We recommend genetic testing as defining a variant is important in the follow-up and treatment of patients. Further studies are needed to evaluate a possible role of these changes and to determine the genetic variations associated with CHD.

Ethics

Ethics Committee Approval: The approval for this study was obtained from the Marmara University Faculty of Medicine Clinical Research Ethics Committee (date: 08.01.2016, approval number: E-70737436-050.06.04).

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Footnotes

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, et al. Heart disease and stroke statistics-2018 update: a report from the American Heart Association. Circulation. 2018;137:67-492.
2. Andersen TA, Troelsen Kde L, Larsen LA. Of mice and men: molecular genetics of congenital heart disease. Cell Mol Life Sci. 2014;71:1327-52.
3. Shiojima I, Komuro I, Inazawa J et al. Assignment of cardiac homeobox gene CSX to human chromosome 5q34. Genomics. 1995;27:204-6.
4. Richards S, Aziz N, Bale S, Bick D, Das S, Foster JG, et al. ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-24.
5. McElhinney DB, Geiger E, Blinder J, Benson DW, Goldmuntz E. NKX2.5 mutations in patients with congenital heart disease. J Am Coll Cardiol. 2003;42:1650-5.
6. Akçaboy MI, Cengiz FB, Inceoğlu B, Uçar T, Atalay S, Tutar E, et al. The effect of p.Arg25Cys alteration in NKX2-5 on conotruncal heart anomalies: mutation or polymorphism? Pediatr Cardiol. 2008;29:126-9.
7. Costa MW, Guo G, Wolstein O, Vale M, Castro ML, Wang L, et al. Functional characterization of a novel mutation in NKX2-5 associated with congenital heart disease and adult-onset cardiomyopathy. Circ Cardiovasc Genet. 2013;6:238-47.
8. Reamon-Buettner SM, Sattlegger E, Ciribilli Y, Inga A, Wessel A, Borlak J. Transcriptional defect of an inherited NKX2-5 haplotype comprising a SNP, a nonsynonymous and a synonymous mutation, associated with human congenital heart disease. PLoS One. 2013;8:83295.
9. Dentice M, Cordeddu V, Rosica A, Ferrara AM, Santarpia L, Salvatore D, et al. Missense mutation in the transcription factor NKX2-5: a novel molecular event in the pathogenesis of thyroid dysgenesis. J Clin Endocrinol Metab. 2006;91:1428–33.
10. Engelen KV, Mommersteeg MTM, Baars MJH, Lam J, Ilgun A, Paul AS, et al. The ambiguous role of NKX2-5 mutations in thyroid dysgenesis. PLoS One. 2012;7:52685.
11. Gifford CA, Ranade SS, Samarakoon R, Salunga HT, de Soysa TY, Huang Y, et al. Oligogenic inheritance of a human heart disease involving a genetic modifier. Science. 2019;364:865-70.
12. Kasahara H, Lee B, Schott JJ, Benson DW, Seidman JG, Seidman CE, et al. Loss of function and inhibitory effects of human CSX/NKX2.5 homeoprotein mutations associated with congenital heart disease. J Clin Invest. 2000;106:299-308.
13. Hassan OKA, Fahed AC, Batrawi M, Arabi M, Refaat MM, Depalma SR, et al. NKX2-5 mutations in an inbred consanguineous population: genetic and phenotypic diversity. Sci Rep. 2015;5:8848.
14. Monaco I, Santacroce R, Casavecchia G, Correale M, Bottigliero D, Cordisco G, et al. Double de novo mutations in dilated cardiomyopathy with cardiac arrest. J Electrocardiol. 2019;53:40-3.
15. Rauch R, Hofbeck M, Zweier C, Koch A, Zink S, Trautmann U, et al. Comprehensive genotype-phenotype analysis in 230 patients with tetralogy of Fallot. J Med Genet. 2010;47:321-31.
16. Uysal F, Turkgenc B, Toksoy G, Boston OM, Evke E, Uyguner O, et al. Homozygous, and compound heterozygous mutation in 3 Turkish family with Jervell and Lange-Nielsen syndrome: case reports. BMC Med Genet. 2017;18:114.