TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2017) 47: 217-221
© TÜBİTAK
doi:10.3906/sag-1605-166

A novel mutation in exon 1 of *GATA4* in Egyptian patients with congenital heart disease

Olfat SHAKER[1], Salwa OMRAN[2], Eman SHARAF[2], Gehan A. HEGAZY[3,4], Mohamed MASHALY[2], Nagwa E.A. GABOON[5,6,*]

[1]Departments of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt
[2]Department of Pediatrics, Faculty of Medicine, Cairo University, Cairo, Egypt
[3]Department of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia
[4]Department of Medical Biochemistry, National Research Center, Cairo, Egypt
[5]Department of Genetic Medicine, Faculty of Medicine, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia
[6]Medical Genetic Center, Faculty of Medicine, AinShams University, Cairo, Egypt

Received: 29.05.2016 • **Accepted/Published Online:** 05.06.2016 • **Final Version:** 27.02.2017

Background/aim: Congenital heart disease (CHD) is a common birth defect. Many studies have reported *GATA4* mutations in patients with CHD, mainly septal defects. This study aimed to investigate the *GATA4* exon 1 mutation in Egyptian patients with isolated congenital heart defects as a possible causative mutation.

Materials and methods: Screening for mutations or any sequence variations in exon 1 of the *GATA4* gene was carried out by PCR amplification followed by direct sequencings in 165 Egyptian patients with different nonsyndromic congenital heart diseases and 93 controls who were matched in terms of age and sex. Thorough clinical assessments were done for all subjects, along with X-ray, 2D echocardiography, and Doppler examinations.

Results: The most common CHD among our cases was isolated ventricular septal defect (VSD) in 47.3% (78/165), followed by isolated atrial septal defect. A novel nonsynonymous sequence variation in fragment 2 (P193H) of exon 1 of *GATA4* was detected in 15 (9.1%) of the subjects with septal defects. This mutation was not seen in any of the control group subjects.

Conclusion: There is a high prevalence of exon 1 *GATA4* mutation (9.1%) in our study compared to other studies in different populations, which may correlate with different ethnic populations.

Key words: Congenital heart disease, cardiac septal defects, *GATA4* mutation

1. Introduction

Congenital heart diseases (CHDs) are the most widely recognized noninfectious cause of mortality in the neonatal period. The incidence of CHD ranges from 19 to 75/1000 (1–3). However, almost 15% of patients with CHD have been distinguished as having single gene or chromosomal defects, which are considered as a part of syndromes or environmental factors, while the rest of the patients (85%) show multifactorial inheritance (4).

GATA4, which maps to chromosome 8p23, is a main transcription factor that regulates different physiological processes and has an important role in heart development (5,6). Numerous heterozygous mutations have been distinguished in previous mutation screenings of *GATA4* (MIM: 600576) with sporadic or familial CHD (2,5,7). Not much is known about the common mutations in patients with CHD in Egypt. In the present study we investigated the possible causative mutation in Egyptian patients with CHDs.

2. Subjects and methods

2.1. Subjects

This case control study enrolled 165 patients (81 males and 84 females) and 93 controls (51 males and 42 females). They were all Egyptian and recruited between May 2013 and October 2014 from the outpatient cardiac clinic or the Pediatric Cardiology Department of Abu-Elreish Hospital with echocardiographic evidence of CHD. Patients with any dysmorphic features, 22q deletion, Down syndrome, or any other numerical or structure chromosomal abnormality were excluded. The controls were matched to the patients in terms of age and sex. The cases were classified according to the type of CHD into isolated (VSD, ASD, PDA, PS, TOF, AP window) or combined (VSD with ASD/ASD with PDA).

The research protocol was approved by ethical committee of Kasr al Ainy hospital. Written informed consent was obtained from the parents of each patient

* Correspondence: nogaota5000@yahoo.com

prior to enrollment in the study. Following enrollment, a detailed medical history was obtained, and a clinical assessment and physical examination for malformation and dysmorphology were done with exclusion of any affected or suspected syndromes. A family pedigree was constructed for each subject, and a high resolution chromosomal study showed that all subjects had a normal karyotype. FISH microdeletion studies for our suspected cases of DiGeorge spectrum were done with exclusion of positive cases. All our patients and controls had chest X-ray study, 2D echocardiography was used to detect cardiac septal defects, and the direction of the shunt color flow mapping was obtained. Doppler examinations were done to assess the pulmonary blood flow. The images were obtained according to the usual standardization (8). Some patients underwent cardiac catheterization. A pediatric cardiologist diagnosed and classified CHD.

2.2. Methods

2.2.1. Genetic testing for *GATA4* sequence variations

DNA was extracted from peripheral blood leukocyte samples using the QIAamp DNA minikit (Qiagen, USA) following the manufacturer's instructions. DNA samples were subjected to DNA quantitation and purity assessment using the NanoDrop (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, DE, USA). The amplified PCR products of fragment 1 were shown at 422 bp (Figure 1). All DNA samples were screened for mutations or any sequence variations in exon 1 of the *GATA4* gene. Screening was carried out by PCR amplification of exon 1 using two overlapping primers followed by direct sequencings (9). Cycle sequencing PCR was carried out on a PerkinElmer thermal cycler (Applied Biosystem 2720, Singapore) (Table 1).

2.2.2. PCR amplification of exon 1

The reaction mixtures were made in a total volume of 50 µL containing 0.5 µg of genomic DNA, 10X buffer, 0.25 mM dNTPs, 2.5 pmol of each primer (MWG-Biotech, Germany), and 2 units of Taq polymerase. PCR was carried out using the following conditions: denaturation at 94 °C for 10 min, followed by 30 cycles of denaturation at 93 °C for 50 s, annealing at 60 °C for 50 s, and elongation at 72 °C for 50 s, followed by a final extension at 72 °C for 5 min. The PCR products were analyzed for successful amplification on 2% agarose gel electrophoresis stained with ethidium bromide.

Purification of the PCR products was performed using the QIAquick PCR purification kit (Qiagen, Germany). According to this kit protocol 5 volumes of Buffer PB were added to 1 volume of the PCR product and mixed well. The samples were then applied to the QIAquick column and centrifuged for 1 min at 10,000 rpm. The washing step was carried out using 0.75 mL of Buffer PE followed by centrifugation at 10,000 rpm for 1 min. PCR products were then eluted in 30 µL of sterile H_2O followed by spinning the column at 10,000 rpm for 1 min. Cycle sequence PCR was carried out using a BigDye Terminator kit (Applied Biosystems, Foster City, CA, USA). To prepare the reaction mixtures, the following components were mixed: 8.0 µL of the terminator-ready reaction mix, 10–30 ng of PCR products, and 3.2 pmol of primer (forward or reverse primer), and then sterile H_2O was added to reach a 20 µL final reaction volume. Cycle-sequencing PCR was carried out with a PerkinElmer thermal cycler (Applied Biosystems 2720, Singapore) using the following conditions: denaturation at 96 °C for 1 min, followed by 25 cycles of denaturation at 96 °C for 10 s, annealing at 58 °C for 5 s, and elongation at 60 °C for 4 min. CENTRI-SEP purification spin columns were used to

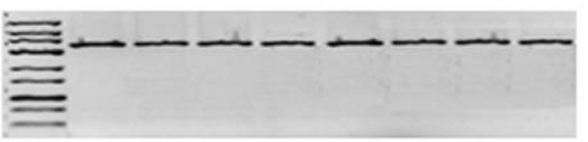


Figure 1. The amplified PCR products of fragment 1 were shown at 422 bp.

Table 1. PCR primers for exon 1 *GATA4*.

Primer	Sequence
Fragment 1	F:5'- TGTTGCCGTCGTTTTCTCTC -3' R:5'- GTCCCCGGGAAGGAGAAG-3'
Fragment 2	F:5'- CGACGGAGCCGCTTACAC -3' R:5'- CGACGGAGCCGCTTACAC -3'

remove dye terminators before applying the sample to the sequencer (Applied Biosystems, Foster City, CA, USA). This purification provides excellent recovery of DNA fragments and removes >98% of salts, dNTPs, and other low-molecular-weight compounds. The samples were injected into an automatic sequencer and the data were analyzed using the ABI Prism DNA sequencing analysis software.

3. Results

3.1. Patients

The 165 CHD patients (81 males and 84 females) were children (age range 1 month–16 years). The controls were healthy children matched to the patients in terms of age and sex with no family history of CHD.

The most common CHD among our cases was isolated ventricular septal defect (VSD) (47.3%, 78/165), followed by isolated atrial septal defect (ASD) (16.4%, 27/165), combined VSD and ASD (14.6%, 24/165), tetralogy of Fallot (TOF) (7.3%, 12/165), and 24 (15.4%) other abnormalities (Table 2).

3.2. Consanguinity

Positive consanguinity has been elicited in 48/165 cases (29%)**.** Five of the patients with sequence variation showed positive consanguinity (4/15, 26.6%).

3.3. *GATA4* nonsynonymous sequence variation

No sequence variations were identified in fragment 1 PCR amplification of exon 1 of the *GATA4* gene. While a nonsynonymous sequence variation in fragment 2 was detected in 15/165 (9.1), all of them had cardiac septal defect 15/129 (11.6%) including 9 of 78 with isolated VSD (11.5%) and 6 of 24 with combined VSD and ASD (25%). This variant conferring a base substitution (A) instead of (C) (578C > A) resulting in amino acid change from proline (CCC) to histidine (CAC) at codon 193 (P193H) (Figure 2). This mutation was not found in the control group.

4. Discussion

The most common nonsyndromic CHDs are ASD, VSD, and TOF, which are reported in about 60% of all CHDs (4). The *GATA4* gene is a zinc finger protein belonging to an evolutionarily conserved *GATA* family that consists of six members.

It is located on chromosome 8p23, and is one of the key transcription factors that is involved at all stages in the regulatory pathways of heart development (4,5,9–11).

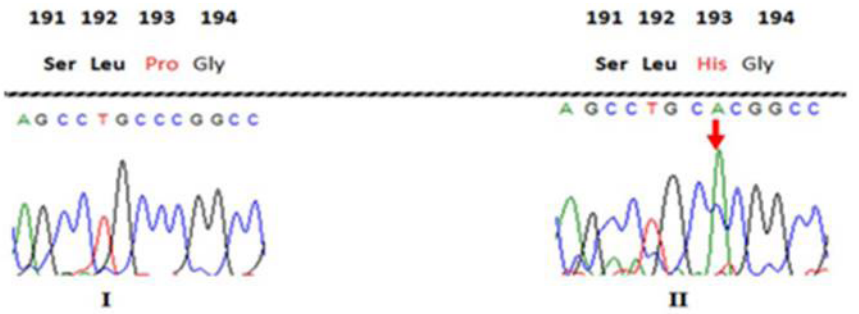
Over 20 heterozygous mutations in the *GATA4* gene have been described in previous publications mainly in cases of ASD, VSD, atrio-ventricular septal defects, pulmonary stenosis (PS), and TOF (2,5,12,13).

In this study, we detected a novel single-base nucleotide substitution (578C > A) that altered a single amino acid (P193H) in the *GATA4* gene. This heterozygous missense mutation was detected in 15 of 165 (9.1%) of our patients. The high frequency of our results is consistent with the 10% *GATA4* mutation found in a previous study done by Chen et al. (14). However, this high prevalence is different from the 2.1% (8/384) and 2.5% (12/486) in 2 studies done by Zhang et al. and Wang et al. in sporadic Chinese CHD patients (5,7). Our prevalence is also higher when compared to the 0.8% (5/628) *GATA4* mutation detected in American patients in a study done by Tomita-Mitchell et al. (9). All the previous mentioned studies examined larger panels of cases compared to ours.

Positive consanguinity was determined in 48/165 cases (29%). Four of the patients with sequence variation showed positive consanguinity (4/15, 26.6%). However, consanguinity has no role in this situation, as all detected

Table 2. Phenotypes of the patients with congenital heart disease.

Cardiac defect	Patients (165)	%
VSD	78	47.3
ASD	27	16.4
VSD, ASD	24	14.6
TOF	12	7.3
PARTIAL AV CANAL	6	3.6
COMPLETE AV CANAL	6	3.6
PS	6	3.6
ASD, PDA	3	1.8
AP WINDOW	3	1.8



Sequencing analysis of fragment 2 in **a patient with P193H mutation(II)**in comparison with a **control case (I)**.

The figure shows **portion of the sequencing** electrophoregram **of fragment 2**. The second base of codon 193 was substituted (A) instead of (C) leading to its change from proline (CCC) to histidine (CAC).

Figure 2. Sequencing analysis of fragment 2 in a patient with P193H mutation (II) in comparison with a control case (I). The figure shows a portion of the sequencing electropherogram of fragment 2. The second base of codon 193 was substituted (A) instead of (C) leading to its change from proline (CCC) to histidine (CAC).

mutation showed heterozygous defect (autosomal dominant mode) (MIM: 600576) (4,5,7,12,15).

Our study population was composed of small numbers for each type of cardiac malformation. However, this cannot rule out the likelihood that mutations in *GATA4* have a higher frequency in certain sporadic cases of CHD such as ASD, VSD, TOF, or pulmonary stenosis.

In this study, we had a diversity of lesions including 78 (47.3%) cases of VSD, 27 (16.4%) ASD, 24 (14.6%) combined VSD and ASD, 12 TOF (7.3%), and 24 (15.4%) other abnormalities. Similar diversity of lesions was found in the study by Zhang et al. (10) that included 24 cases of VSD (39%), 14 cases of ASD (23%), 7 cases of TOF (11%), 3 cases of PS (5%), and 2 cases of AV canal defect (3%) and other lesions. Similar diversity of lesions was found in other studies done by Butler et al. and Tomita-Mitchell et al. (9,12). The study was limited to VSD cases only in the study by Wu et al. (16) and septal defects as in the study done by Chen et al. (14).

The nonsynonymous sequence variation in exon 1 of *GATA4* (P193H) was detected in cases of septal defects: isolated VSD and combined VSD and ASD. Another study revealed 4 missense mutations in 4 out of 300 nonsyndromic patients (1.3%); all of them had VSD with other minor anomalies, two mutations in exon 1 of *GATA4* (G69D and P163R) in addition to 2 mutations in 3 cases in exon 6 (A411V and D425N) (13), while in the study done by Tomita Mitchell et al. four mutations were identified in 5 patients, 4 of them with septal defect and one with TOF (G93A, Q316E, A411V, D425N); they were distributed in exon 1 and exon 4 and 2 mutations in 2 cases in exon 6 (9). Chen et al. did not detect any mutation in exon 1 but there was mutation in 5 cases in exon 2 and 7. Moreover, the study done by Hussein et al. (17) showed no mutation in exon 1 but it showed 1 mutation in 2 cases in exon 6.

One of the most important outcomes in our study was the presence of a high frequency of mutations in exon 1 of *GATA4* (9.1%) compared with other studies in different populations. However, most of them shared the fact that they mainly had cardiac septal defects. The different CHD cases in different ethnic groups, however, may be the result of several factors, such as epigenetic regulation, genetic background (different races), modifier genes and environmental factors, and even the personalized genetic signature (4,5,15,16). This finding highlights the importance of studying *GATA4* sequence variation on a large scale in Egyptian children with CHD.

References

1. Richards AA, Garg V. Genetics of congenital heart disease. Curr Cardiol Rev 2010; 6: 91.
2. Xiang R, Fan LL, Huang H, Cao BB, Li XP, Peng DQ, Xia K. A novel mutation of GATA4 (K319E) is responsible for familial atrial septal defect and pulmonary valve stenosis. Gene 2014; 534: 320-323.
3. Hoffman JI, Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol 2002; 39: 1890-1900.
4. Matsuoka R. GATA4 mutation and congenital cardiovascular diseases: importance of phenotype and genetic background clarification. J Mol Cell Cardiol 2007; 43: 667-669.
5. Wang E, Sun S, Qiao B, Duan W, Huang G, An Y, Xu S, Zheng Y, Su Z, Gu X et al. Identification of functional mutations in GATA4 in patients with congenital heart disease. PloS One 2013; 8: e62138.
6. Giglio S, Graw SL, Gimelli G, Pirola B, Varone P, Voullaire L, Lerzo F, Rossi E, Dellavecchia C, Bonaglia MC et al. Deletion of a 5-cM region at chromosome 8p23 is associated with a spectrum of congenital heart defects. Circulation 2000; 102: 432-437.
7. Zhang W, Li X, Shen A, Jiao W, Guan X, Li Z. GATA4 mutations in 486 Chinese patients with congenital heart disease. Eur J Med Genet 2008; 51: 527-535.
8. Schiller NB. Two-dimensional echocardiographic determination of left ventricular volume, systolic function, and mass. Summary and discussion of the 1989 recommendations of the American Society of Echocardiography. Circulation 1991; 84: I280-287.
9. Tomita-Mitchell A, Maslen C, Morris C, Garg V, Goldmuntz E. GATA4 sequence variants in patients with congenital heart disease. J Med Genet 2007; 44: 779-783.
10. Nemer G, Nemer M. Transcriptional activation of BMP-4 and regulation of mammalian organogenesis by GATA-4 and -6. Dev Biol 2003; 254: 131-148.
11. Huang WY, Cukerman E, Liew CC. Identification of a GATA motif in the cardiac alpha-myosin heavy-chain-encoding gene and isolation of a human GATA-4 cDNA. Gene 1995; 155: 219-223.
12. Butler TL, Esposito G, Blue GM, Cole AD, Costa MW, Waddell LB, Walizada G, Sholler GF, Kirk EP, Feneley M et al. GATA4 mutations in 357 unrelated patients with congenital heart malformation. Genet Test Mol Bioma 2010; 14: 797-802.
13. Wang J, Fang M, Liu XY, Feng XY, Min LZ, Chen X, Wang X, Fang W, Liu X et al. A novel GATA4 mutation responsible for congenital ventricular septal defects. Int J Mol Med 2011; 28: 557-564.
14. Chen MW, Pang YS, Guo Y, Pan JH, Liu BL, Shen J, Liu T. GATA4 mutations in Chinese patients with congenital cardiac septal defects. Pediatr Cardiol 2010; 31: 85-89.
15. Nemer M. Genetic insights into normal and abnormal heart development. Cardiovasc Pathol 2008; 17: 48-54.
16. Wu G, Shan J, Pang S, Wei X, Zhang H, Yan B. Genetic analysis of the promoter region of the GATA4 gene in patients with ventricular septal defects. Transl Res 2012; 159: 376-382.
17. Hussein I, El-Rubi M, Helmy N, Hussein H, El-Gerzawy A, Bassyouni R, Ahmed A, Fayez A, Shehata G, Abdel-Rahman N. Genetic studies of congenital heart defects in Egyptian patients. Res J Med Med Sci 2009; 4: 55-66.