Mutat Res Fund Mol Mech Mutagen 803–805 (2017) 26–34

Contents lists available at ScienceDirect

Mutat Res Fund Mol Mech Mutagen

journal homepage: www.elsevier.com/locate/mut

Identification of intronic-splice site mutations in *GATA4* gene in Indian patients with congenital heart disease

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ARTICLE INFO

Keywords:
Intronic variants
Branch point mutation
Congenital heart disease
Enhancer motif
Silencer motif

ABSTRACT

Congenital Heart Disease (CHD) is the most common birth defect among congenital anomalies that arise before birth. *GATA4* transcription factor plays an important role in foetal heart development. Mutational analysis of *GATA4* gene in CHD patients revealed five known heterozygous mutations (p.T355S, p.S377G, p.V380M, p.P394T and p.D425N) identified in exons 5 and 6 regions and fifteen intronic variants in the non-coding regions (g.76885T > C/Y, g.76937G > S, g.78343G > R, g.83073T > Y, g.83271C > A/M, g.83318G > K, g.83415G > R, g.83502A > C/M, g.84991G > R, g.85294C > Y, g.85342C > T/Y, g.86268A > R, g.87409G > A/R, g.87725T > Y, g.87813A > T/W). *In silico* analysis of these intronic variants identified two potential branch point mutations (g.83271C > A/M, g.86268A > R) and predicted effects of these on intronic splice sites as enhancer and silencer motifs. This study attempts to correlate the pattern of intronic variants of *GATA4* gene which might provide new insights to unravel the possible molecular etiology of CHD.

1. Introduction

Congenital Heart Disease (CHD) is the most common birth defect in humans affecting 1% of all live births in the first year of life and it is one of the major causes of morbidity and mortality in infants [1]. In India, nearly 1,80,000 children are born with CHD with the prevalence ranged from 8 to 10 of every 1000 live births [2]. CHD is a complex multifactorial disorder with genetic and environmental factors playing an important role in disease development [3]. It is categorized mainly into four groups namely septal defects, cyanotic heart disease, obstruction defects and hypoplasia, where genetic factors are implied in the etiology [4]. A group of highly conserved transcription factors such as *GATA4, NKX2-5, MYH6, NOTCH, TBX5, TBX20, ZIC3, TFAP2B* and others are involved in foetal heart development and regulation [5–7]. Among these *GATA4* is well studied and established gene known to cause sporadic and familial non-syndromic CHDs which include atrial septal defect (ASD), ventricular septal defect (VSD), patent ductus arteriosus (PDA), atrioventricular septal defect (AVSD), tetralogy of fallot (TOF) and pulmonary value stenosis (PS) [5–8].

GATA proteins (DNA binding proteins) are involved in cell differentiation, survival and proliferation of tissues that regulate gene expression. They recognize and bind to "*GATA*" consensus sequence of target genes [9]. Six members have been indentified in vertebrates *GATA* family. *GATA1, GATA2,* and *GATA3* are mainly involved in hematopoietic cell expression, whereas *GATA4, GATA5* and *GATA6* are expressed in heart, liver and gonadal tissues [10]. *GATA4* (Gene Id: 2626, OMIM: 600576) is a critical transcription factor expressed in embryo and adult cardiomyocytes and it promotes cardiac morphogenesis, survival and function of the heart [11]. Human *GATA4* gene maps to chromosome 8p23.1- p22 region, a hypermutable protein coding gene encodes 442 amino acids with two transcriptional activation domains (TAD1, 1–74 amino acids; TAD2, 130–177 amino acids), two zinc finger domains (ZF1, 215–240 amino acids; ZF2, 270–294 amino acids) and one nuclear localization signal (NLS, 254–324 amino acids) [12–14]. Mutations associated in *GATA4* gene had been reported in several cardiac diseases such as congenital heart disease, abnormal ventral folding and hypoplasia of ventricular myocardium [15,16]. Till date, more than 120 mutations have been reported in the *GATA4* gene. These mutations in *GATA4* are well-

Abbreviations: CHD, congenital heart disease; ASD, atrial septal defect; VSD, ventricular septal defect; PDA, patent ductus arteriosus; AVSD, atrioventricular septal defect; TOF, tetralogy of fallot; PS, pulmonary value stenosis; DORV, double outlet right ventricle; TAD, transcriptional activation domain; ZF, zinc finger domain; NLS, nuclear localization signal; EDTA, ethylenediaminetetraacetic acid; PolyPhen-2, polymorphism phenotyping version 2; SIFT, sorting intolerant from tolerant; ESE, exonic splicing enhancer; ESS, exonic splicing silencer; ISE, intronic splicing enhancer; ISS, intronic splicing silencer

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http://dx.doi.org/10.1016/j.mrfmmm.2017.08.001
Received 7 April 2017; Received in revised form 1 July 2017; Accepted 8 August 2017
Available online 18 August 2017
0027-5107/

Table 1
List of *GATA4* primers.

Exon	Forward primer (5′–3′)	Reverse Primer (5′–3′)	Annealing temperatue
Exon 2	GAACTCTCAGTGTCTGGGATTAG	GTGGCTCCAGCTAACTCTAAA	61 °C
Exon 3	TGACGGTGAATGATGGTTAGG	GGCCAGCAAAGTAGTTGAAAG	61 °C
Exon 4	CATCACACAGGTGCTCGATAAG	CCAAAGATGAAAGGACCGAGTA	61 °C
Exon 5	TGTAGCCCTCCGCAGATAA	GTCATGTCCACATCACCATCT	61 °C
Exon 6	TTCTGGGCAACCACAGTATC	AGTCCCATCAGCGTGTAAAG	61 °C
Exon 7	GCTCCTTCACTTCCAACATCTC	ACCCTCTCCCAGGAAATTAAAG	61 °C

Table 2
Cardiac septal defects of the subjects.

CHD condition	Number of subjects
ASD	22
VSD	12
PDA	3
ASD, VSD	4
ASD, PDA	9
ASD, VSD, PDA	4
VSD, DORV	1
ASD, PFO	1
VSD, TOF, PS	1
VSD, TOF	1
PS	1
PFO	1
Total Number of Patients	60

studied and reported from different countries in CHD patients and are implied as reasons for CHD in humans [17].

There are only few studies conducted in Indian population on CHD to identify the causes of this disease condition [18–20]. The present study was designed to look for mutations in the exonic and intronic regions of *GATA4* gene of patients with CHD.

2. Materials and methods

2.1. Subjects

Sixty patients diagnosed with isolated CHDs at R.L. Jalappa Hospital and Narayana Hrudayalaya Heart Centre from Kolar were recruited in this study. Pediatric cardiologists confirmed the CHD by two-dimensional echocardiography with color flow doppler. After getting the Institutional Ethics committee approval and written informed consent from the patients, whole peripheral blood samples were collected for further genetic study.

2.2. DNA extraction

Genomic DNA from all patients was isolated from blood lymphocytes collected in EDTA vacutainers using standard salting out method. Three ml of blood was mixed thoroughly and incubated for erythrocyte lysis. After erythrocyte lysis, the packed white blood cells pellet was kept for Proteinase-k digestion overnight in the presence of 20% SDS. The genomic DNA was precipitated by the addition of 5 M NaCl and the DNA was spun down and washed with alcohol to remove salts and eluted with Tris-EDTA buffer [21]. DNA concentration and purity was assessed by optical density ratios (260/280 nm) using UV–vis Spectrophotometer (Lambda 35, Perkin Elmer, Waltham, Massachusetts, USA) and stored at −20 °C until use.

2.3. Sequence analysis

The referential genomic DNA sequence of *GATA4* was retrieved from Genbank (Accession No. NC_000008) and sequence specific primer pairs were designed to amplify the coding exons and exon-intron boundary regions of *GATA4* gene with the help of Primer Quest tool, IDT DNA software (https://www.idtdna.com/) and listed in Table 1. Polymerase chain reaction (PCR) was carried out with these specific primers using C1000 Touch Thermal cycler (Bio-Rad Laboratories, Hercules, California, USA). Reactions contained 100 ng of genomic DNA, 10× PCR buffer, 10 mM dNTPs, 10 picomole of each primer, 1.5 mM $MgCl_2$, and 1 unit *Taq* DNA polymerase (Bangalore Genei, India) and the conditions followed with an initial denaturation at 95 °C for 5 min followed by 33 cycles of denaturation at 95 °C for 30 s, annealing at 61 °C for 30 s, 72 °C for 30 s and final extension at 72 °C for 10 min. The PCR products were analyzed on 1% agarose gel and purified with GeneJET PCR Purification kit (Thermo Fisher Scientific). Sequence analysis was performed for all the six exons with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) under ABI-3500 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). DNA Sequences were

Table 3
Exonic variants in *GATA4* coding region.

Exon	Nucleotide change	Aminoacid change	dbSNP id	Mutation type	Mutation Prediction		
					Polyphen-2	SIFT	Mutation Taster
5	c.1064C > G	T355S	rs200167770	Missense	Benign	Tolerated	Polymorphism
5	c.1129A > G	S377G	rs3729856	Missense	Benign	Tolerated	Polymorphism
5	c.1138G > A	V380M	rs114868912	Missense	Benign	Tolerated	Polymorphism
6	c.1180C > A	P394T	rs200319078	Missense	Benign	Tolerated	Polymorphism
6	c.1273G > A	D425N	rs56208331	Missense	Probably damaging	Tolerated	Disease causing