Prevalence and spectrum of GATA5 mutations associated with congenital heart disease

Jin-Qi Jiang [a], Ruo-Gu Li [b], Juan Wang [c], Xing-Yuan Liu [d], Ying-Jia Xu [b], Wei-Yi Fang [b], Xiao-Zhong Chen [e], Wei Zhang [e], Xiao-Zhou Wang [e], Yi-Qing Yang [f,*]

[a] Department of Emergency, Shanghai Chest Hospital, Medical College of Shanghai Jiaotong University, Shanghai 200030, China
[b] Department of Cardiology, Shanghai Chest Hospital, Medical College of Shanghai Jiaotong University, Shanghai 200030, China
[c] Department of Cardiology, East Hospital, Tongji University School of Medicine, Shanghai 200120, China
[d] Department of Pediatrics, Tongji Hospital, Tongji University School of Medicine, Shanghai 200065, China
[e] Department of Cardiac surgery, Shanghai Chest Hospital, Medical College of Shanghai Jiaotong University, Shanghai 200030, China
[f] Department of Cardiovascular Research, Shanghai Chest Hospital, Medical College of Shanghai Jiaotong University, Shanghai 200030, China

ARTICLE INFO

Article history:
Received 12 June 2012
Accepted 15 September 2012
Available online 30 September 2012

Keywords:
Congenital heart disease
Transcription factor
Genetics

Congenital heart disease (CHD) is the most prevalent form of developmental abnormality with an incidence of approximately 1% in live newborns, and is the leading non-infectious cause of infant mortality with more than 29% of neonates who die of birth defects having cardiac anomalies [1]. Despite its striking prevalence and important clinical significance, the etiology responsible for CHD remains largely unknown. Now it is generally understood that abnormal cardiovascular development during embryogenesis may be attributed to an aberrant biological process that is heterogeneous and complex, with both environmental and genetic risk factors involved [2]. Great advance in developmental biology has contributed to the discovery of numerous transcriptional regulators, signaling molecules and structural proteins that are crucial for normal cardiogenesis. This mechanistic understanding of cardiac morphogenesis results in the identification of multiple CHD related genes by using positional cloning or direct candidate gene strategies [3]. Aggregating evidence demonstrates that cardiac transcription factor genes *GATA4* and *NKX2-5* are most commonly causally implicated in the pathogenesis of CHD [4]. Nevertheless, CHD is genetically heterogeneous and the genetic determinants underlying CHD in a vast majority of patients are still to be identified.

GATA transcription factors are a group of DNA binding proteins characterized by preferential binding to the consensus DNA sequence GATA of target gene promoters. The GATA family comprises six members (GATA1 to GATA6), of which GATA4, GATA5 and GATA6 are broadly expressed in various mesoderm and endodermally derived tissues, especially in embryonic and adult heart [5]. Of these three GATA transcription factors, GATA4 has been most extensively explored, and a long list of GATA4 mutations have been identified in patients with a wide variety of CHD, including ventricular septal defect, atrial septal defect, atrioventricular septal defect, tetralogy of Fallot, pulmonary stenosis, and patent ductus arteriosus [4]. Recently, mutations in GATA6 have also been associated with various kinds of CHD [6,7]. GATA5 is another member of the GATA family, and its expression and function overlap partially with those of GATA4 and GATA6 during cardiovascular development [5], which makes GATA5 a logical candidate as the genetic cause of variable CHD.

To determine the prevalence and spectrum of *GATA5* mutations in patients with CHD, a cohort of 320 unrelated CHD patients identified among the Chinese Han population was included in this study. All subjects were evaluated by medical history, physical examination, electrocardiography, and echocardiography. The cardiac catheterization and operative reports were also reviewed when available. The patients with known chromosomal abnormalities or syndromic CHD were excluded from the study. The baseline demographic and clinical characteristics of the study population are summarized in Table 1. The control population is composed of 200 unrelated ethnically matched healthy individuals. The control individuals did not have known congenital heart deformities but subclinical cardiovascular malformations such as bicuspid aortic valve were not excluded. The study protocol was reviewed and approved by the local institutional ethics committee and written informed consent was obtained from the participants or their guardians prior to study.

Peripheral venous blood specimens were prepared from all subjects and genomic DNA was extracted as described previously [8]. According to the genomic DNA sequence of *GATA5* (GenBank accession no. NT_011362), the primer sequences were designed as shown elsewhere [8]. The coding exons (exons 2–7) and their flanking splice junction sites of *GATA5* were screened for genetic variations by means of polymerase chain reaction, followed by DNA sequencing with Big Dye chemistry under an ABI 3130 XL DNA Analyzer.

As a result, four novel heterozygous GATA5 mutations, c.394C>G equivalent to p.R132G, c.569T>C equal to p.V190A, c.667A>C corresponding to p.A266P, and c.821A>G the same as p.H274R, were identified in 4 of 320 unrelated patients with CHD, respectively (Fig. 1), with a mutation prevalence of 1.25%. Genetic scan of the four mutation carriers' families showed that in each family the mutation was present in all affected living family members, but absent in unaffected family members examined except for member I-1 from family 3, which may be ascribed to delayed echocardiographic measurement for some minor defects that are likely to close spontaneously during the first few years after birth, different genetic background, incomplete penetrance, allele-specific expression, epigenetic modifiers, or environmental factors. Analysis of the pedigrees showed that CHD was transmitted in an autosomal dominant pattern in each family (Fig. 2). The phenotypic characteristics and results for genetic screening of the affected family members are presented in Table 2. The identified sequence variations, that altered the amino acids highly conserved evolutionarily across species (Fig. 3) and were absent in 400 normal chromosomes, were all automatically predicted to be disease-causing mutations by MutationTaster, with p values of 0.730109 for c.394C>G, 0.999774 for c.569T>C, 0.998256 for c.667A>C, and 0.998995 for c.821A>G, indicating that mutated GATA5 leads to or confers susceptibility to CHD in these families. No other single nucleotide polymorphisms in the altered regions were found in the MutationTaster database (http://www.mutationtaster.org).

Interestingly, atrial fibrillation was documented in 2 CHD patients (I-1 and II-3 from family 2), consistent with the previous report on the association of GATA5 with familial atrial fibrillation [8]. Similarly, mutations in other cardiac transcriptional factor genes, such as *GATA6*,

* Corresponding author at: 241 West Huaihai Road, Shanghai 200030, China. Tel.: +86 21 62821990; fax: +86 21 62821105.
E-mail address: yang99yang66@hotmail.com (Y.-Q. Yang).

Table 1
Clinical characteristics of the study population with CHD.

Parameter	Number or mean value	Percentage or range
Male	153	48
Age at the present study (year)	6.85	0–32
Age at initial diagnosis of CHD (year)	3.62	0–12
Positive family history	143	45
Distribution of different types of CHD		
Isolated CHD	198	62
ASD	71	22
VSD	84	26
PDA	13	4
DORV	8	3
PS	6	2
TAPVC	4	1
COA	4	1
TGA	3	1
CAVC	3	1
Cor triatriatum	2	1
Complex CHD	122	38
TOF	35	11
ASD + VSD	20	6
VSD + PDA	16	5
VSD + PFO	12	4
VSD + DORV	10	3
VSD + TGA	7	2
VSD + PFO + PDA	6	2
ASD + PDA	4	1
ASD + TGA	4	1
ASD + VSD + DORV	3	1
ASD + VSD + PDA	3	1
COA + PDA	2	1
Incidence of arrhythmias	18	6
Atrioventricular block	13	4
Atrial fibrillation	5	2
Treatment	296	93
Surgical repair	176	55
Transcatheter closure	120	38

CHD = congenital heart disease; ASD = atrial septal defect; VSD = ventricular septal defect; TOF = tetralogy of Fallot; PDA = patent ductus arteriosus; DORV = double outlet right ventricle; PS = pulmonary stenosis; TAPVC = total abnormal pulmonary venous connection; COA = coarctation of the aorta; TGA = transposition of great arteries; CAVC = common arteriovenous canal; and PFO = patent foramen ovale.

GATA4, and *NKX2-5*, were also implicated in atrial fibrillation [9–12]. These observations imply that atrial fibrillation may share a common genetic origin with CHD.

The findings that mutated GATA5 predisposes to cardiac deformations have been substantiated in animals. In zebrafish, targeted disruption of the *GATA5* gene resulted in embryonic lethality due to defects in endocardial and myocardial differentiation migration, a similar phenotype to that (cardia bifida) of *GATA4*-null zebrafish [13]. Although mice null for GATA5 were viable and without obvious cardiac aberrations, mice that were compound heterozygous for both *GATA5* and *GATA4* or for both *GATA5* and *GATA6* died embryonically or perinatally due in large part to severe defects of the outflow tract development including double outlet right ventricle and ventricular septal defect [14]. These results demonstrate an exquisite sensitivity of the developing cardiovascular system to the levels of GATA4, GATA5 and GATA6 and suggest that these GATA factors act cooperatively to regulate downstream target genes.

The identification of *GATA5* mutations in patients with CHD is of great clinical significance, because emerging evidence shows that the patients affected with genetic CHD have significantly increased late morbidity and mortality, even though they have undergone surgical repair or catheter-based treatment of CHD. This information will be very useful for genetic counseling for these families, given that genetic testing for these mutations in new family members can be followed by careful medical examination and prophylactic intervention if CHD is observed.

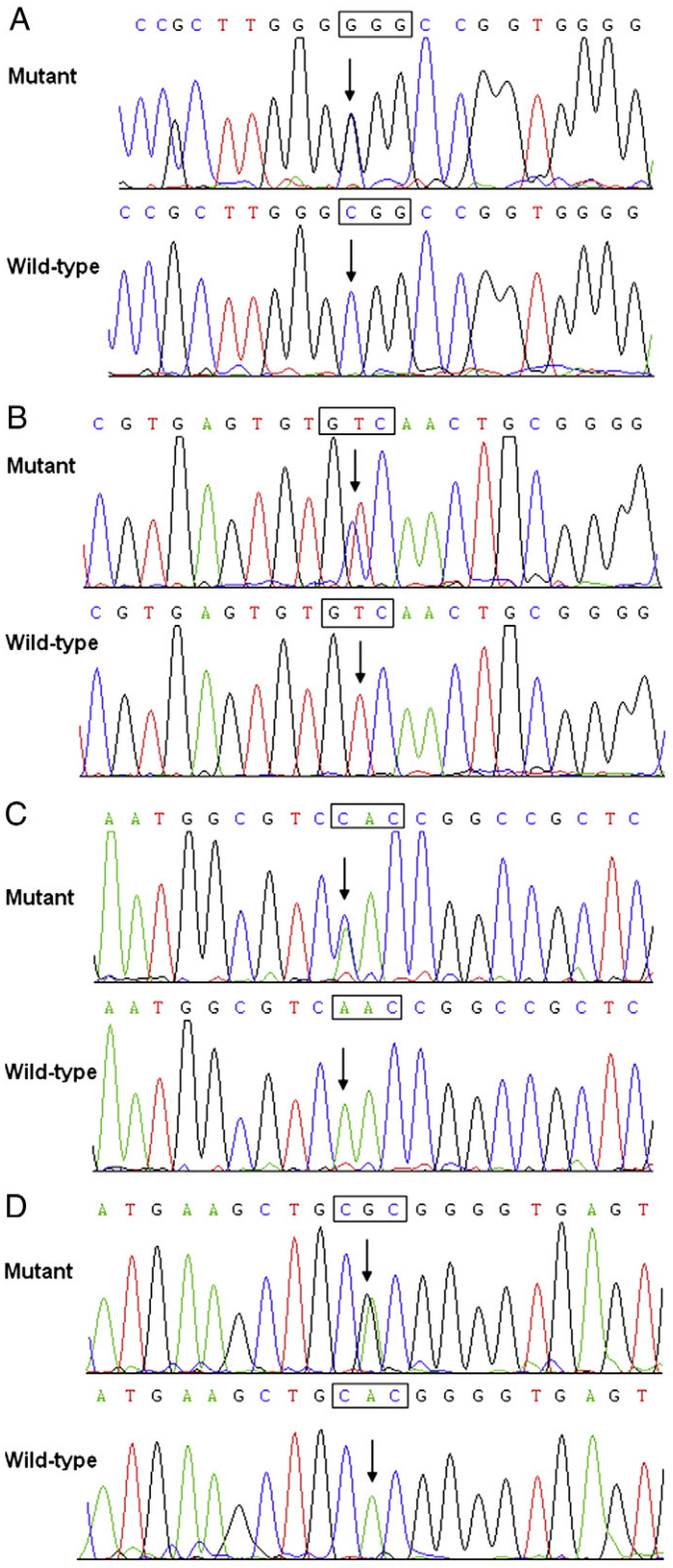

Fig. 1. Sequence electropherograms of *GATA5* in patients and controls. The arrow indicates the heterozygous nucleotides of C/G (A), T/C (B), A/C (C) or A/G (D) in the patient (mutant) or the homozygous nucleotides of C/C (A), T/T (B), A/A (C) or A/A (D) in the corresponding control individual (wild-type). The square denotes the nucleotides comprising a codon of GATA5.

In conclusion, this work firstly provides the genetic evidence of GATA5 linked to human CHD, suggesting the potential implications for the early prophylaxis and gene-specific therapy of CHD.