Associations of GATA4 genetic mutations with the risk of congenital heart disease
A meta-analysis

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Abstract
Background: GATA4 gene is a cardiac transcriptional factor playing important role in cardiac formation and development. Three GATA4 gene mutations, 99 G>T, 487 C>T, and 354 A>C, have been reported in congenital heart disease (CHD). Therefore, a meta-analysis was performed to explore the associations between 99 G>T, 487 C>T, or 354 A>C mutations and the risk of CHD.

Methods: We searched the relevant studies in electronic databases, including ISI Science Citation Index, Embase, PubMed, CNKI, and Wanfang, from January 2006 to March 2016. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to estimate the associations between 99 G>T, 487 C>T, or 354 A>C mutations and the risk of CHD.

Results: A total of 11 studies including 2878 CHD cases and 3339 controls were evaluated. There was no significant association between GATA4 99 G>T (OR = 1.22, 95% CI = 0.74–2.01, P = 0.43) or 487 C>T (OR = 1.16, 95% CI = 0.48–2.78, P = 0.74) mutations and the risk of CHD, whereas GATA4 354 A>C (OR = 1.49, 95% CI = 1.15–1.93, P = 0.003) mutation was significantly associated with CHD risk. Subgroup analysis was further performed for GATA4 99 G>T or 487 C>T mutations and the risk of CHD was found in all subgroups, whereas GATA4 354 A>C mutation was significantly associated with CHD risk in large-sample-size and Asian subgroups. However, subgroup analysis by types of CHD indicated that there was no significant association between GATA4 354 A>C mutation and the risk of ventricular septal defects.

Conclusions: Our findings suggested that GATA4 99 G>T and 487 C>T mutations may not be related to the incidence of CHD. However, GATA4 354 A>C mutation was significantly associated with CHD risk.

Abbreviations: ASD = atrial septal defect, CHD = congenital heart disease, CI = confidence interval, ORs = Odds ratios, VSD = ventricular septal defects.

Keywords: congenital heart disease, gata4, meta-analysis, mutations

1. Introduction
Congenital heart disease (CHD) is the most common congenital malformation, caused by abnormal development of great vessels and the fetal heart. CHD is the most common birth defects in human disease and is the main cause of death in infant’s noninfectious diseases. It affects nearly 8 per 1000 live births in America, whereas the incidence increased sharply from 1.2 to 5.4 per 1000 live births between 2000 and 2011 in China, making it the number one killer among all birth defects. Although rapid advance has been made in drug therapy and surgical treatment during the several decades, the mortality of patients with CHD remained significantly increasing, and its related complications such as sudden cardiac death, arrhythmia, or heart failure may occur even after effective treatment.

To date, more and more genetic studies showed that CHD had significant genetic basis. Pathogenic gene mutations, microRNA lesion, and chromosomal aberrations could all lead to CHD. Therefore, it is important to identify the effect of genetic defects on CHD formations. Moreover, many genes related to CHD have been identified in genetics research, and transcription factors coded by most of these genes, such as GATA4, NKX2-5, TBX5, and TBX20, could regulate heart development. Recently, an increasing number of mutations in these genes have been identified in CHD patients, suggesting their potential roles in CHD development.

GATA4 belongs to the GATA family of zinc finger transcription factors, which consists of 6 members, GATA1 to GATA6. Members of this family could identify the GATA motif presenting in the promoters of many genes. Therefore, these proteins could regulate genes involved in embryogenesis and in myocardial differentiation. GATA1, GATA2, and GATA3 are mainly expressed in the hematopoietic cells, whereas GATA4, GATA5,
and GATA6 are predominantly expressed in tissues such as the heart, gonads, and liver.\textsuperscript{[15]} GATA4 expression during cardiac development is essential for proper cardiovascular formation and function. GATA4 inactivation, with GATA4-null mice, results in the formation of congenital cardiac defects including myocardial hypoplasia, double outlets of the right ventricle, and common atrioventricular canal.\textsuperscript{[16]} Moreover, GATA4 mutations can lead to many kinds of human CHDs.\textsuperscript{[17]} So far, >90 mutations of GATA4 gene have been reported in CHD patients,\textsuperscript{[18]} and abnormal expression levels of GATA4 were also found to be associated with multiple cardiac defects.\textsuperscript{[19]}

A growing number of studies have been done to identify the GATA4 mutations in CHD; however, no meta-analysis is found to report the association between GATA4 mutations and CHD. Several studies reported 3 mutations of GATA4 in CHD, 99 G>T, 487 C>T, and 354 A>C.\textsuperscript{[20–30]} Therefore, we performed a meta-analysis to assess the associations between GATA4 99 G>T, 487 C>T, or 354 A>C mutations and the risk of CHD.

2. Materials and methods

This meta-analysis was approved by the institutional review board of Henan Provincial People’s Hospital. A systematic search of the electronic databases including PubMed, Embase, ISI, CNKI, and Wang fang was performed to screen eligible articles. The key words used to retrieve related literatures were as follows: “GATA4,” “congenital heart defect,” “congenital heart disease,” “congenital cardiovascular malformation,” “mutations,” and “variants.”

2.1. Inclusion criteria

The inclusion standards are as follows: studies that have been published as a full test; case-control studies containing CHD patients and healthy controls; studies that investigate the relationship of GATA4 mutations and CHD; studies that evaluate the GATA4 99 G>T, 487 C>T, or 354 A>C variants and CHD risk. The excluded standards are as follows: duplicate publications; studies without available data; reviews, letters, case reports, and expert opinions.

2.2. Data extraction

The extracted data included the first author, publication year, country of origin, ethnicity, sex, number of patient cases and controls, types of mutations, and types of CHD. Two investigators performed the data extraction independently. Any discrepancies between 2 investigators were resolved by discussion until reaching a consensus.

2.3. Statistical methods

We used a random- or fixed-effects model to estimate the odds ratios (ORs) with 95% confidence intervals (95% CIs) by RevMan (version 5).\textsuperscript{[31]} The heterogeneity of the studies was evaluated by the $\chi^2$ value and the $I^2$ value. Significant heterogeneity was defined as a $\chi^2$ test $P<.10$ or as an $I^2 >50\%$.\textsuperscript{[32]} If $I^2 \leq 50\%$, a fixed-effects model was used for analysis. If not ($I^2 >50\%$), a random-effects model was used. Subgroup analysis was performed based on sample size, ethnicity, and types of CHD. The types of CHD included ventricular septal defects (VSD), atrial septal defect, and others. The study was regarded as large-sample-size if the number of case is $>150$; otherwise, the study was defined as small-sample-size according to previous studies\textsuperscript{[33,34]}. To determine whether the results could be driven by one specific study, we conducted the sensitivity analysis by removing one study each time. Visual assessment of a funnel plot was used to estimate possible publication bias.\textsuperscript{[35]}

3. Results

3.1. Study selection

A total of 72 records were obtained for this meta-analysis through electronic searches. The process of literature retrieval is presented in Figure 1. Fifty-two records were excluded because of reviews, duplicates, letters, case reports, and expert opinions. The remaining 20 full-text articles were examined in detail. Nine of these full-text articles were excluded. Finally, 11 studies were included in the meta-analysis. The characteristics of 11 eligible studies with 6217 participants were shown in Table 1. Of the 11 studies,\textsuperscript{[20–30]} 7 were from Asian populations\textsuperscript{[21,22,25–27,29,30]} and 4 studies were from whites.\textsuperscript{[20,23,24,28]} The earliest study was in June 2007,\textsuperscript{[23]} whereas the latest study was in May 2015.\textsuperscript{[29]} The number of patient cases ranged from 12 to 628, and the number of control cases was from 100 to 957.

3.2. GATA4 99 G>T mutation and CHD risk

The associations of GATA4 99 G>T mutation with CHD risk were shown in Figure 2. Six studies, including 1863 patients and 1073 controls, were analyzed for associations between the 99 G>T mutations of GATA4 and CHD. As there was no significant heterogeneity ($I^2$ for the heterogeneity $= 0.90$, $I^2 = 0\%$), a fixed-effects model was applied. No significant association of GATA4 99 G>T mutation with the risk of CHD was found (OR $= 1.22$, 95% CI $= 0.74–2.01$, $P = .43$) (Fig. 2).

3.3. GATA4 487 C>T mutation and CHD risk

The associations of GATA4 487 C>T mutation with CHD risk were reported in Figure 3. Five studies including 1249 patients and 2271 controls were analyzed. A fixed-effects model was used.
because of no significant heterogeneity ($P$ for the heterogeneity $= .19$, $I^2 = 35\%$). There was no significant association between GATA4 487 C>T mutation and CHD risk (OR = 1.16, 95% CI = 0.48–2.78, $P = .74$) (Fig. 3).

### 3.5. Subgroup analysis

For GATA4 99 G>T mutation and CHD risk, subgroup analysis was performed based on sample size and ethnicity. In the large-sample-size and small-sample-size subgroups, no significant

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Table 1

| First author | Year | Country | Ethnicity | Sex (M/F) | Case/Control | Mutations | Types of CHD |
|--------------|------|---------|-----------|-----------|-------------|-----------|--------------|
| Zhang et al[27] | 2008 | China | Asian | 295/191 | 290/196 | 99 G>T | VSD/ASD/others (319/37/130) |
| Peng et al[22] | 2010 | China | Asian | NA | NA | 487 C>T | VSD/ASD/others (82/19/34) |
| Wang et al[23] | 2010 | China | Asian | NA | NA | 135/114 | VSD/ASD/others (82/19/34) |
| Ting et al[11] | 2012 | China | Asian | NA | NA | 120/110 | NA |
| Wang et al[24] | 2013 | China | Asian | 185/199 | NA | 384/957 | VSD/ASD/others (162/48/34) |
| Butler et al[20] | 2010 | Australia | White | NA | NA | 357/100 | VSD/ASD/others (63/157/137) |
| Rajagopal et al[23] | 2007 | USA | White | NA | NA | 107/600 | VSD/ASD/others (NA/NA/NA) |
| Tomita-Mitchell et al[24] | 2007 | American | White | NA | NA | 628/159 | VSD/ASD/others (137/122/369) |
| Reamon-Buettner et al[28] | 2007 | Germany | White | NA | NA | 12/100 | VSD/ASD/others (91/71/147) |
| Li[29] | 2015 | China | Asian | 105/98 | 98/103 | 203/201 | VSD/ASD/others (NA/NA/NA) |
| Li[30] | 2010 | China | Asian | 170/139 | 252/156 | 300/408 | VSD/ASD/others (NA/NA/NA) |

ASD = atrial septal defect, CHD = congenital heart disease, F = female, M = male, NA = not available, VSD = ventricular septal defect.
association for GATA4 99 G>T mutation was found (large-sample-size, OR=1.08, 95% CI=0.42–2.80, P=.87; small-sample-size, OR=1.28, 95% CI=0.71–2.30, P=.41) (Table 2). In addition, there was no significant association between GATA4 99 G>T mutation and the risk of CHD in the Asian and white subgroups (Asian, OR=1.29, 95% CI=0.77–2.16, P=.33; white, OR=0.44, 95% CI=0.06–3.38, P=.43) (Table 2).

For GATA4 487 C>T mutation and CHD risk, subgroup analysis was carried out by stratifying available data based on sample size and ethnicity. In the large-sample-size and small-sample-size subgroups, we failed to detect any significant association for GATA4 487 C>T mutation (large-sample-size, OR=0.67, 95% CI=0.22–2.08, P=.49; small-sample-size, OR=4.30, 95% CI=0.66–27.82, P=.13) (Table 2). In addition, no significant association was found between GATA4 487 C>T mutation and CHD risk in the Asian subgroup (OR=0.90, 95% CI=0.34–2.33, P=.82) (Table 2).

For GATA4 354 A>C mutation and CHD risk, subgroup analysis was also carried out by stratifying available data based on sample size, ethnicity, and types of CHD. In the large-sample-size subgroups, a significant association between GATA4 354 C>T mutation and CHD risk was found (large-sample-size, OR=1.41, 95% CI=1.10–1.87, P=.007) (Table 2). In addition, significant association was found between GATA4 354 C>T mutation and CHD risk in the Asian subgroup (OR=1.41, 95% CI=1.10–1.87, P=.007) (Table 2). However, no statistical evidence for the association between GATA4 354 C>T mutation and VSD was detected (OR=1.15, 95% CI=0.82–1.62, P=.42) (Table 2).

### 3.6. Sensitivity analysis

To determine whether the results could be driven by one specific study, we conducted the sensitivity analysis by removing one study each time. For GATA4 99 G>T, 487 C>T, and 354 A>C mutations, the sensitivity analysis indicated that none of the studies significantly affected the results.

### 3.7. Publication bias

Visual inspection of funnel plots shown in Figures 5–7 revealed a slight asymmetry for GATA4 99 G>T, 487 C>T, and 354 A>C mutations. These results suggested that there was no obvious publication bias among these studies.

### 4. Discussion

The present meta-analysis suggested that GATA4 99 G>T and 487 C>T mutations may not be associated with CHD risk, whereas 354 A>C mutations of GATA4 gene was significantly associated with CHD risk. Moreover, no significant association of GATA4 99 G>T and 487 C>T mutations with the risk of CHD was detected.
CHD were detected by different sample size and ethnic groups. There was also no significant association between GATA4 354 A>C mutations and VSD. However, GATA4 354 A>C mutations were significantly associated with CHD risk in large sample size and Asian subgroups. Furthermore, the stability of our study was well supported by the sensitivity analysis and publication bias. In addition, no obvious heterogeneity was observed in the overall analysis and the subgroup analysis.

GATA4, a cardiac transcription factor, is well acknowledged as a critical regulator of gene expression and cellular activity in heart development. The coding region of GATA4 includes 2 transactivation domains, a nuclear localization signal, and 2 forms of type IV zinc-finger motif, and the C-terminal zinc finger motif of GATA4 could interact with transcription factors to regulate the expression of cardiac genes. GATA4 gene deletions, as well as gene duplications, have been reported to be related to CHD. Zhang et al. reported a novel mutation, c.C931T (p.R311W), decreased the ability of GATA4 to activate its downstream target gene. A previous study reported another GATA4 mutation, p.R43W, and the mutant protein resulted in a significant suppression in transcriptional activity. In a recent study, common mutations in a specific region of GATA4 3' UTR may lead to CHD susceptibility, likely by changing the miRNA posttranscriptional gene regulation. These studies indicated that GATA4 gene mutations could contribute to the susceptibility of CHD.

Mounting studies have found more and more GATA4 mutations in CHD, including GATA4 99 G>T, 487 C>T, and 354 A>C mutations. Although these mutations have been identified, their association with CHD risk remains undefined. Moreover, there is no meta-analysis to assess the association between GATA4 mutations and CHD risk. Considering that GATA4 99 G>T, 487 C>T, and 354 A>C mutations were reported in several studies, we performed this meta-analysis to explore the association between these 3 mutations and CHD. For the 99 G>T mutation, our overall results indicated that this mutation may not be associated with CHD risk, which was consistent with several previous studies. For instance, Wang et al. indicated no significant association between this mutation and CHD risk. In addition, no document reported the role of this mutation in CHD yet, which may because of the fact that 99 G>T mutation has no effect on the amino acid sequence. For another mutation 487 C>T, no association of this variant with CHD was found, which suggested that 487 C>T mutation may not lead to the susceptibility of CHD. Moreover, 354 A>C mutation was found to be closely related to the incidence of CHD, consistent with previous studies. However, the result should be treated with caution because of lack of sufficient studies.

Subgroup analyses were performed based on sample size and ethnicity of study population. Furthermore, the sensitivity analysis implied that no single study significantly affected the results. In addition, no obvious publication bias was observed among these studies. All these analyses suggested our results were robust.

Several limitations in our study should be taken into consideration when construing the findings. First, the number of involved studies on GATA4 99 G>T, 487 C>T, and 354 A>C mutations was limited. Second, we did not well describe the association of 99 G>T, 487 C>T, and 354 A>C mutations with clinical stage of CHD and time to developing CHD owing to lack of data. Third, gene-environmental and gene-gene interactions could influence the associations of certain GATA4 mutation with CHD. Certain site mutation may increase the CHD susceptibility, but interactions with multiple genes and environmental factors may lead to the absence of the association. Therefore, these factors should be taken into account to draw a more accurate conclusion. However, the information of other genes and environment factors including age, smoking, and alcohol are
lack of enough data to analyze. Owing to these limitations, the results presented by the present study should be interpreted with caution.

In summary, the current meta-analysis first explored the association between GATA4 mutations and CHD risk. The results suggested that GATA4 99 G>T and 487 C>T mutations may not contribute to the pathogenesis of CHD, whereas 354 A>C mutation was significantly associated with the risk of CHD. However, considering that the present results are based on limited number of studies, further researches with more published studies are needed to confirm our results. In addition, given that GATA4 plays a crucial role in heart development, further researches are needed to explore the potential role of other mutations of GATA4 in the development of CHD.

References

[1] Sorrell VL, Panczyk E, Alpert JS. A new disease: bicuspid aortic valve aortopathy syndrome. Am J Med 2012;125:322–3.
[2] Sadowski SL. Congenital cardiac disease in the newborn infant: past, present, and future. Crit Care Nurs Clin North Am 2009;21:37–48.
[3] Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics—2012 update a report from the American heart association. Circulation 2012;125:e2–20.
[4] Cai B, Zhang T, Zhong R, et al. Variant in MTRR, but not MTR, is associated with risk of congenital heart disease: an integrated meta-analysis. PloS One 2014;9:e89609.
[5] Van Der Bom T, Zomer AC, Zwinderman AH, et al. The changing epidemiology of congenital heart disease. Nat Rev Cardiol 2011;8:50–60.
[6] Verheugt CL, Uiterwaal CS, van der Velde ET, et al. Mortality in adulthood of congenital heart disease. Eur Heart J 2010;31:1220–9.
[7] Cecchetto A, Rampazzo A, Angelini A, et al. From molecular mechanisms of cardiac development to genetic substrate of congenital heart diseases. Future Cardiol 2010;6:373–93.
[8] Bruneau BG. The developmental genetics of congenital heart disease. Nature 2008;451:943–8.
[9] He A, Gu F, Hu Y, et al. Dynamic GATA4 enhancers shape the chromatin landscape central to heart development and disease. Nat Commun 2014;5:4907.
[10] Zhang L, Nomura-Kitabayashi A, Sultana N, et al. Mesodermal Nkx2.3 is necessary and sufficient for early second heart field development. Dev Biol 2014;390:68–79.
[11] Stennard FA, Costa MW, Lai D, et al. Murine T-box transcription factor Tbx20 acts as a repressor during heart development, and is essential for adult heart integrity, function and adaptation. Development 2005;132:2451–62.
[12] Tong YF. Mutations of NKK2.5 and GATA4 genes in the development of congenital heart disease. Gene 2016;588:86–94.
[13] Granados-Riveron JT, Pope M, Bu’Lock FA, et al. Combined mutation screening of NKK2.5, GATA4, and TBX5 in congenital heart disease: multiple heterozygosity and novel mutations. Congenit Heart Dis 2012;7:151–9.
[14] Ferreira R, Ohnedal K, Yamamoto M, et al. GATA1 function, a paradigm for transcription factors in hematopoiesis. Mol Cell Biol 2005;25:1215–27.
[15] Pikkarainen S, Tokola H, Kerkeila R, et al. GATA transcription factors in the developing and adult heart. Cardiovasc Res 2004;63:196–207.
[16] Pu WT, Ishiwata T, Jurasek AL, et al. GATA4 is a dosage-sensitive regulator of cardiac morphogenesis. Dev Biol 2004;275:235–44.
[17] Yang YQ, Li L, Wang J, et al. A novel GATA4 loss-of-function mutation associated with congenital ventricular septal defect. Pediatr Cardiol 2012;33:539–46.
[18] Zhang X, Wang J, Wang B, et al. A novel missense mutation of GATA4 in a Chinese family with congenital heart disease. PloS One 2016;11:e0158904.
[19] Mazarrer-Naeini M, Sabbagh SK, Shahrarai M, et al. Expression analysis of GATA4, Tbx5 and Nkx2.5 genes involved in congenital heart disease. Zahedan J Res Med Sci 2016;18:e6448.
[20] Butler TL, Esposito G, Blue GM, et al. GATA4 mutations in 357 unrelated patients with congenital heart malformation. Genet Test Mol Biomarkers 2010;14:797–802.
[21] Ting P, Li W, Cheng Y, et al. Effects of parental generation GATA4 allelic variants on bilateral generation congenital heart defects. Chinese Journal of Healthy Birth & Child Care 2012;18:117–21.
[22] Peng T, Wang L, Zhou SF, et al. Mutations of the GATA4 and NKX2.5 genes in Chinese pediatric patients with non-familial congenital heart disease. Genetica 2010;138:1231–40.
[23] Rajagopalan SK, Ma Q, Obler D, et al. Spectrum of heart disease associated with murine and human GATA4 mutation. J Mol Cell Cardiol 2007;43:677–85.
[24] Tomita-Mitchell A, Maslen C, Morris C, et al. GATA4 sequence variants in patients with congenital heart disease. J Med Genet 2007;44:779–83.
[25] Wang J, Hu D, Li X, et al. [Novel GATA4 mutations identified in patients with congenital heart disease]. Zhong Hua Yi Xue Za Zhi 2010;90:667–71.
[26] Wang E, Sun S, Qiao B, et al. Identification of functional mutations in GATA4 in patients with congenital heart disease. PloS One 2013;8:e62118.
[27] Zhang W, Li X, Shen A, et al. GATA4 mutations in 486 Chinese patients with congenital heart disease. Eur J Med Genet 2008;51:527–35.
[28] Reamon-Buettner SM, Cho SH, Borlik J. Mutations in the 3’ untranslated region of GATA4 as molecular hotspots for congenital heart disease (CHD). BMC Med Genet 2007;8:38.
[29] Li D. Study on differently expressed plasma microRNA of congenital heart disease and its association with GATA4 gene target sequence polymorphism. 2015;WANFANG DATA, Shandong University.
[30] Li J. Studies on association of GATA4 gene polymorphism with ventricular septal defect in northwest Chinese population. 2010; China National Knowledge Infrastructure, Lanzhou University.
[31] The Cochrane Collaboration, Review Manager (RevMan) [computer program]. Version 5.0. The Nordic Cochrane Centre, Copenhagen:2008.
[32] Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. Bmj 2003;327:557.
[33] Zou L, Chen W, Shao SS, et al. Genetic variant in KIAA0319, but not in DYX1C1, is associated with risk of dyslexia: an integrated meta-analysis. Bmj 2003;327:557–60.
[34] Zou L, Chen W, Shao SS, et al. Genetic variant in KIAA0319, but not in DYX1C1, is associated with risk of dyslexia: an integrated meta-analysis. Am J Med Genet B Neuropsychiatr Genet 2012;159:970–6.
[35] Song RR, Zou L, Zhong R, et al. An integrated meta-analysis of two variants in HOXA1/HOXB1 and their effect on the risk of autism spectrum disorders. PloS One 2011;6:e25603.
[36] Egger M, Smith GD, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629–34.
[37] Fahed AC, Gelb BD, Seidman J, et al. Genetics of congenital heart disease. Future Cardiol 2010;6:373–82.
[38] Yu S, Zhou XG, Fiedler SD, et al. Cardiac defects are infrequent in the glass half empty. Cir Res 2013;112:707–20.
[39] Lourenço D, Brauner R, Rybczynska M, et al. Loss-of-function mutation in GATA4 causes anomalies of human testicular development. Proc Natl Acad Sci U S A 2011;108:1597–602.
[40] Yu S, Zhou XG, Fiedler SD, et al. Cardiac defects are infrequent findings in individuals with 8p23.1 genomic duplications containing GATA4. Circ Cardiovasc Genet 2011;4:620–5.
[41] Pulignani S, Vecoli C, Sabina S, et al. 3’UTR SNPs and haplotypes in the GATA4 gene contribute to the genetic risk of congenital heart disease. Rev Esp Cardiol (Engl Ed) 2016;69:760–5.