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# Association between TGFBR2 gene polymorphisms and congenital heart defects in Han Chinese population

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**Abstract**

***Background*: Transforming growth factor-β receptor II (TGFBR2) is a key component of TGF-β signaling pathway. TGFBR2 can be detected in the generation of heart. The mouse embryos of TGFBR2 gene knockout exhibited congenital heart defects.**

***Methods*: We conducted a case-control study to investigate the association between TGFBR2 gene polymorphisms and congenital heart defects in Han Chinese population. 125 patients with congenital heart defects and 615 unrelated controls were recruited. Two tagging single nucleotide polymorphisms (tagSNPs) in 5' upstream of TGFBR2 gene (rs6785358, -3779A/G; rs764522, -1444C/G) were selected and genotyped by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assay.**

***Results*: A significant difference was seen in the distribution of genotypes between patients with congenital heart defects and controls for SNP rs6785358 (P=0.043). For SNP rs6785358 the carrier of the G allele (AG/GG genotype) showed a significantly higher risk of congenital heart defects compared with AA homozygotes (OR=1.545, 95% CI: 1.013–2.356). Further analysis by sex stratification indicated that individuals carrying G allele (AG/GG genotype) for SNP rs6785358 have a higher susceptibility to congenital heart defects (OR=2.088, 95%CI: 1.123-3.883, P=0.019) in males, but not females (OR=1.195, 95%CI: 0.666-2.146, P=0.55). No statistical significance was detected in the distribution of genotypes and allele frequencies for SNP rs764522 between patients and controls.**

***Conclusion*: Our result suggested that SNP rs6785358 of TGFBR2 gene was associated with increased risk of congenital heart defects in Han Chinese men and further research would be warranted.**

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Key words: *Congenital heart defects. TGFBR2. Gene polymorphisms. Case-control study.*

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## ASOCIACIÓN ENTRE POLIMORFISMOS DEL GEN TGFBR2 Y DEFECTOS CONGÉNITOS DEL CORAZÓN EN LA POBLACIÓN CHINA HAN

**Resumen**

***Antecedentes:* Factor de crecimiento transformante β receptor II (TGFBR2) es un componente clave de la via de señalización de TGF - β.TGFBR2 puede ser detectado en la generación de corazón. Los embriones de ratón de TGFBR2 gene knockout mostraron defectos congénitos del corazon.**

***Métodos:* Hemos realizado un estudio de casos y controles para investigar la asociación entre polimorfismos del gen TGFBR2 y defectos congénitos del corazón en la población china han. 125 pacientes con defectos congénitos del corazón y 615 unrelated controles fueron reclutados. Marcado de dos polimorfismos de nucleótido único (tagsnps) en 5 'aguas arriba del gen TGFBR2 (rs6785358, - 3779a / g; rs764522, - 1444c / g) fueron seleccionados y genotipados por reacción en cadena de la polimerasa (PCR) - polimorfismos de longitud de fragmentos de restricción (RFLP) de ensayo.**

***Resultados:* Se observó una diferencia significativa en la distribución de genotipos entre pacientes con defectos congénitos del corazón y controles para SNP rs6785358 (P = 0043). La SNP rs6785358 el porteador del alelo G (AG / GG genotipo) mostraron un importante crecimiento y mayor riesgo de defectos congénitos del corazón en comparación con AA homocigotos (OR = 1.545, IC del 95%: 1.013–2.356). Más análisis por sexo estratificación indicó que los individuos con alelo G (AG / GG genotipo) para SNP rs6785358 tienen una mayor susceptibilidad a defectos congénitos del corazón (OR = 2.088, IC del 95%: 1.123-3.883, p = 0.019) en machos, pero no en las mujeres (OR = 1.195, IC del 95%: 0.666-2.146, p = 0.55). No hay significación estadística fue detectado en la distribución de los genotipos y frecuencias de alelos de SNP rs764522 entre pacientes y controles.**

***Conclusión:* Nuestros resultados sugieren que el SNP rs6785358 de gen TGFBR2 se asoció con un mayor riesgo de defectos congénitos del corazón en los chinos han hombres y más investigación estaría justificada.**

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Palabras clave: *Los defectos congenitos del corazon, TGFBR2, Gene polymorphisms, estudio de casos y controles.*

## Background

Congenital heart defects (CHD) usually refer to abnormalities in the heart's structure or function that arise before birth. The incidence of congenital heart defects ranges from 19 to 75 per one thousand live births, depending on the types of defect that are included [1]. It is reported that genetic predisposition of the individual interacts with the environment to cause the congenital heart diseases[2]. Series of studies reported that the transforming growth factor-β (TGF-β signaling pathway played a crucial role in cardiac development[3-6].

TGF-β signaling pathway regulates a wide range of biological functions, including cell growth, differentiation, matrix production and apoptosis across a large variety of cell types [7-8]. To initiate TGF-β signaling pathway, TGF-β ligands must first bind to the TGF-β type II receptor (TGFBR2) on the cell surface, this binding leads to activation of the TGF-β type I receptor (TGFBR1), which then phosphorylates SMAD2 and SMAD3 proteins to allow association with SMAD4 and translocation to the nucleus, in the nucleus SMAD proteins interact with other transcription factors to regulate transcription of target genes to control the cell response[7, 8]. In the development of heart, TGF-β signaling pathway promotes epithelial-to-mesenchymal (EMT) transformation, which is important during the formation of endocardial cushions (EC)[9]. Remarkably, EC are the primordial of the valves and septa of the adult heart and disruptions in the signal transformation might result in valvuloseptal heart defect[10].

The expression level of TGFBR2 can influence the pathway activation status of TGF-β signaling pathway and the specific response of cells to TGF-β[11]. It has been proved that during the developing mouse heart, TGFBR2 is expressed in the walls of the primitive cardiac tube at embryonic day (E) 8, in the myocytes of the atrium and ventricle at E10, and in the endothelial cells and endocardial cushion at E10.5[12, 13]. Endothelial cells-specific TGFBR2 knockout mice embryos exhibited deficient ventricular septation[6]. And the deletion of TGFBR2 gene in expressing the smooth muscle cell-specific protein SM22-α of mice embryos had heart defects, including ventricular myocardium hypoplasia and septal defect[5].

As those intensive researches above have been shown the crucial role of TGFBR2 in the development of the heart, here we conducted a case-control study to investigate the association between TGFBR2 gene polymorphisms and congenital heart defects in Han Chinese population.

## Materials and Methods

### *Subjects*

From October 2008 to December 2010, a total of 115 unrelated Han patients (55 males and 60 females; mean age 32.7±16.8 years, age range 2-69 years) with congenital heart defect were consecutively recruited in the department of Thoracic and Cardiovascular Surgery, Nanjing First Hospital Affiliated to Nanjing Medical University, Nanjing, China. Of the 115 patients with congenital heart defect, 53 had atrial septal defect, 40 had ventricular septal defect, 2 had atrial septal defect and ventricular septal defect, and 20 had tetralogy of Fallot. All patients had been diagnosed by echocardiography, with the defect confirmed surgically. The study enrolled 615 healthy controls (207 males and 408 females; mean age 60.2±9.1 years, aged 41-85 years) who had no historical or clinical signs of congenital heart defects. We excluded individuals who had structural malformations involving another organ system or known chromosomal abnormalities. The study was approved by the institutional review board of Nanjing Medical University, and informed consent was obtained from each subject or their relatives.

### *Single nucleotide polymorphisms (SNP) selecting and genotyping*

The SNPs were searched using the database of CHB (Han Chinese in Beijing, China) population of the International HapMAP Project (HapMap Data Rel 24/phase II Nov08, on NCBI B36 assembly, dbSNP b126;http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap24 B36/). 109 SNPs with minor allele frequency greater than 5% were obtained from 5 kb upstream of TGFBR2 gene to 2kb downstream. Tagging SNPs (tagSNPs) were selected using Haploview version 4.2 and the threshold of pairwise linkage disequilibrium (LD) was set as $r^2 = 0.80$. 49 tagSNPs were obtained capturing 109 subject's genotyped alleles. Out of the candidate tagSNPs cover TGFBR2 gene, we only selected two tagSNPs (rs6785358, -3779A/G; rs764522, -1444C/G) located in 5 kb upstream of TGFBR2 gene to investigate whether promoter region harbored any genetic variants susceptible to heart septal defect in the present study.

An approximately 5ml venous blood sample with EDTA-containing receptacle was collected. Genomic DNA from blood specimens was isolated using proteinase K digestion and phenol-chloroform extraction. Polymerase chain reaction (PCR)-Restriction fragment length polymorphism (RFLP) assay was used for genotyping and the primers of the rs6785358 and rs764522 were 5'-GAACTGCAAACAAGAGAATGGAT-3' (forward) and 5'-TTAGAATTCT- ACCCTAATGATTGTAAGG-3' (reverse), and 5'-GAGTGAAAGAGCCCAGAACG-3' (forward) and 5'-GGGCTAGGCATCTTCTTTCC-3' (reverse) respectively. PCR was performed in a total volume of 10$\mu$L containing 10 ng of genomic DNA, 0.5 pmol of each primer, 1×PCR buffer, 2.5 mM MgCl, 0.2mM dNTPs, and 0.5 U of Taq DNA polymerase. The PCR amplifications were performed in the ABI PRISM 9700 thermal cycler. The

PCR program was set as: 1 cycle at 95°C for 5 minutes, 30 cycles at 95°C for 30 seconds, 61°C (rs6785358) /63°C (rs764522) for 30 seconds, 72°C for 30 seconds and a final cycle of extension at 72°C for 8 minutes. The PCR products were respectively digested with restriction enzymes BsuRI and MvaI for rs6785358 and rs764522. The digested PCR fragments were separated by a new high throughout electrophoresis method with 96-sample agrose gel block. For rs6785358 (-3779A/G), the G allele was cut into 147 bp and 29 bp fragments, and the A allele remained intact as a single 176bp band. For rs764522 (-1444C/G), the G allele was cut into 41bp and 151bp fragments, the C allele was not digested and was a single 192bp band.

*Statistical Analysis*

Hardy–Weinberg equilibrium was tested by Fisher's exact $\chi^2$ test using the program HWE14. Statistical analysis was performed using SPSS for Windows version 13.0 (SPSS Inc, Chicago, USA). The allele frequencies and genotype distributions between cases and controls were compared by the Chi-square ($\chi^2$) test. Association was expressed as odds ratios (OR) as risk estimates with 95% confidence intervals (95% *CI*). Binary logistic regression (Enter method) was applied to adjust for sex. Statistical significance was set at $P < 0.05$ (two tails).

## Results

The observed genotype distributions in the controls and cases did not deviate significantly from Hardy-Weinberg equilibrium for rs6785358 (*P*=0.305 and *P*=0.727, respectively) or rs764522 (*P*=0.332 and *P*=0.137, respectively). We evaluated the association between genotypes and congenital heart defects with a dominant model. Distributions of genotypes and allele frequencies for the two SNPs tested were showed in table I. We observed significant difference in the distribution of genotypes of rs6785358 between patients and controls (*P*=0.043). G allele carriers (AG/GG genotype) of rs6785358 had a 1.545-fold increased risk (95% *CI*: 1.013-2.356) of congenital heart defects. There was no significant difference in the distribution of allele frequencies of rs6785358 between patients and controls with a boundary *P* value (0.054). Distribution of genotypes and allele frequencies of rs764522 was comparable between cases and controls (*P*=0.750 and *P*=0.938, respectively).We also evaluate the effects of the TGFBR2 gene rs6785358 and rs764522 polymorphisms on certain types of congenital heart defects, but we observed no significant difference in the distribution of genotypes and allele frequencies for the two SNPs between patients with certain types of congenital heart defects and controls. The results were showed in table II.

Furthermore, we compared the distribution of genotypes and allele frequencies of rs6785358 and rs764522 after stratification by sex and results were listed in table III. The carrier of the AG/GG genotype of rs6785358 showed a significantly higher risk of congenital heart defects compared with the AA genotype in male subjects and *OR* (95%*CI*) was 2.088 (1.123-3.883), *P* value was 0.019, whereas no significant association presented in female subjects, and *OR* (95%*CI*) was 1.195 (0.666-2.146), *P* value was 0.550. Additionally, the allele frequencies for rs6785358 were also significantly different between cases and controls in male subjects (*P*=0.022), and the allele G had a 1.842-fold increased risk (95%*CI*: 1.088-3.118) of congenital heart defects. However no statistical significance was detected for females (*P*=0.711). For rs764522, there was still no statistical difference in the distribution of genotypes and allele frequencies between cases and controls in female subjects (*P*=0.878 and *P*=0.629, respectively) or male subjects (*P*=0.529 and *P*=0.711, respectively).

## Discussion

TGF-βsignaling pathway is essential for normal heart development in several different cell types con-

**Table I**
*Distribution of genotypes and allele frequencies for rs6785358 and rs764522 in cases and controls*

| *SNP* | *Group* | *Genotype* | | | | *Allele* | | |
|---|---|---|---|---|---|---|---|---|
| | | *WT* | *Ht+MT* | *P* | *OR(95%CI)* | *Major/Minor* | *P* | *OR(95%CI)* |
| rs6785358 | | AA | AG+GG | | | A/G | | |
| | Case | 73 | 38+4 | | | 184/46 | | |
| | Control | 448 | 150+17 | 0.043 | 1.545(1.013-2.356) | 1046/184 | 0.054 | 1.421(0.992-2.035) |
| rs764522 | | CC | CG+GG | | | C/G | | |
| | Case | 87 | 28+0 | | | 202/28 | | |
| | Control | 475 | 128+12 | 0.750 | 1.079(0.675-1.724) | 1078/152 | 0.938 | 0.983(0.639-1.511) |

**Table II**
*Association between TGFBR2 gene rs6785358 and rs764522 polymorphisms and three types of congenital heart defects*

| SNP | Genotype/allele | Control(N) | Atrial septal defect | | | Ventricular septal defect | | | Tetralogy of Fallot | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | N | P | OR(95%CI) | N | P | OR(95%CI) | N | P | OR(95%CI) |
| rs6785358 | AA/AG+GG | 448/150+17 | 35/15+3 | 0.283 | 1.386(0.764-2.516) | 24/16+0 | 0.076 | 1.824(0.940-3.540) | 12/7+1 | 0.211 | 1.822(0.712-4.666) |
| | A/G | 1046/184 | 85/21 | 0.184 | 1.404(0.850-2.322) | 64/16 | 0.225 | 1.421(0.804-2.513) | 31/9 | 0.191 | 1.650(0.773-3.524) |
| rs764522 | CC/CG+GG | 475/128+12 | 43/10+0 | 0.503 | 0.784(0.384-1.600) | 29/11+0 | 0.504 | 1.280(0.620-2.644) | 13/7+0 | 0.203 | 1.812(0.726-4.521) |
| | C/G | 1078/152 | 96/10 | 0.376 | 0.739(0.377-1.448) | 69/11 | 0.715 | 1.131(0.585-2.184) | 33/7 | 0.333 | 1.504(0.654-3.461) |

taining the cardiac neural cells, the cushion mesenchymal cells, the epicardial cells and vascular smooth muscle cells[7]. TGFBR2 is a transmembrane protein that includes an extracellular domain, a single hydrophobic transmembrane domain, and a cytoplasmic serine/threonine kinase domain[15]. It is a key component of TGF-βsignaling pathway and controls TGF-βsignaling pathway activation[11]. During mouse embryonic heart development, the TGFBR2 expression can be detected[12, 13].

In recent years, several studies propped up to determine the role of TGFBR2 in heart development based on mouse models of depleting TGFBR2 in special-cells. Kai et al. reported that endocardial depletion of TGFBR2 resulted in double-inlet left ventricle (DILV) defect and a ventricular septal defect (VSD)[4]. The mice embryos with conditional deletion of TGFBR2 gene in cells expressing the smooth muscle cell-specific protein SM22α, all died during the last third of gestation, about half of them exhibited heart defects including hypoplasia of the compact zone of the myocardium, ventricular and atrial defects[5]. And Andrew et al. proved that TGFBR2 played a critical role in the endothelial cells during heart development and inactivation of TGFBR2 in endothelial cells resulted in deficient ventricular septation[6].

TGFBR2 gene was mapped on human chromosome 3p22[16]. Many human congenital diseases are associated with genetic variation of TGFBR2 gene, containing Marfan syndrome, Loeys-Dietz syndrome, neoplasm, aortic aneurysms and dissections, nonsegmental vitiligo, intracerebral hemorrhage, and sudden cardiac arrest in patients with coronary artery disease[17-25]. However, whether genetic variation of TGFBR2 gene is associated with congenital heart defects has not yet been demonstrated in human.

We designed the present case-control study to investigate the association between TGFBR2 gene promoter region polymorphisms and congenital heart defects in Han Chinese population. The mutation in the TGFBR2 gene promoter results in significantly decreased transcriptional activity and loss of gene expression [1]. So we mainly focus on the variants on the promoter region of TGFBR2 gene in this study. The results revealed that SNP rs6785358 had significant association with congenital heart defects and the carrier of the AG/GG genotype were associated with a significantly increased risk of congenital heart defects compared with AA genotype. Because of our small sample size, our results showed that TGFBR2 gene rs6785358 and rs764522 polymorphisms had no association with certain types of congenital heart defects. Further stratification analysis by sex indicated that SNP rs6785358 was significantly associated with congenital heart defects and the individuals carrying G allele (AG/GG genotype) had an increased risk of congenital heart defects in males, but not females. There was no association between SNP rs764522 and congenital heart defects either in males or females. As

**Table III**
*Analysis of sex stratified association of rs785358 and rs764522 polymorphisms with congenital heart defects*

| SNP | Sex | Group | Genotype | | | | Allele | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | WT | Ht+MT | P | OR(95%CI) | Major/Minor | P | OR(95%CI) |
| rs6785358 | | | AA | AG+GG | | | A/G | | |
| | Male | Case | 32 | 21+2 | | | 85/25 | | |
| | | Control | 154 | 49+4 | 0.019 | 2.088(1.123-3.883) | 357/57 | 0.022 | 1.842(1.088-3.118) |
| | Female | Case | 41 | 17+2 | | | 99/21 | | |
| | | Control | 294 | 101+13 | 0.550 | 1.195(0.666-2.146) | 689/127 | 0.587 | 1.151(0.693-1.912) |
| rs764522 | | | CC | CG+GG | | | C/G | | |
| | Male | Case | 40 | 15+0 | | | 95/15 | | |
| | | Control | 159 | 45+3 | 0.529 | 1.242(0.632-2.441) | 363/51 | 0.711 | 1.124(0.606-2.086) |
| | Female | Case | 47 | 13+0 | | | 107/13 | | |
| | | Control | 316 | 83+9 | 0.878 | 0.950(0.493-1.832) | 715/101 | 0.629 | 0.860(0.466-1.586) |

reported that activation of the TGF-β signal pathway is subject to hormone regulation[26], and this might explain why the association between TGFBR2 gene and congenital heart defects risk depend on sex. The exact biological effect of SNP rs6785358 is unknown at the present and the association evidence arise in this study would warrant further investigation on potential function for the certain mechanism.

There are some limitations in our study. First, our cases were relatively small for genetic epidemiology studies, and larger sample study is warranted to replicate the association between TGFBR2 gene polymorphism and congenital heart defects. Second, our population exclusively consisted of Han Chinese subjects, and so, future studies in other ethnics are needed to confirm and expand our findings. Moreover, we just proved the rs6785358 polymorphism on promoter region of TGFBR2 gene was susceptible marker for congenital heart defects in males based two tagSNPs association analysis. Therefore, we hope this report will stimulate studies to investigate whether tagSNPs or functional SNPs coved TGFBR2 gene harbor any susceptible variants for congenital heart defects.

## Conclusion

This study showed that the SNP rs6785358 on 5' upstream promoter region of TGFBR2 gene was associated with congenital heart defects for the Han Chinese male population. However, the SNP rs764522 had no significant association with congenital heart defects in Han Chinese population. Further applicable evaluation of clinical diagnosis and prognosis prediction and functional research would be warranted.


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## Author Disclosure Statement

The authors have no conflicts of interest.

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