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DGCR8 expression is altered in children with congenital heart defects

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ABSTRACT

Aim: To explore the correlation of DGCR8 expression in children with congenital heart defects (CHD) and its clinical significance.

Methods: Full blood samples were collected from children with congenital heart disease(n = 40) and healthy children(n = 40), respectively.Real-time PCR was used to detect the expression of DGCR8 in the blood of healthy children and CHD. Myocardial tissues were collected from children with ventricular septal defect (VSD)(n = 25), and tetralogy of Fallot (TOF)(n = 16),. Real-time PCR and Western blotting were used to detect the expression of DGCR8 in myocardial tissues. Analyze the correlation between DGCR8 expression and congenital heart disease.

Results: The expression levels of DGCR8 was significantly lower in CHD than healthy children (P = 0.037), and lower in TOF tissues compared with VSD tissues (P = 0.046). There was no significant correlation between the expression of DGCR8 and the size of VSD(r = −0.022, P = 0.917).

Conclusions: The low expression of DGCR8 was significantly correlated with the occurrence of CHD, which may affect the development of heart and the formation of blood vessels. The lower expression of DGCR8 was correlated with severe CHD. However, DGCR8 expression did not associate with the size of VSD.

1. Introduction

Congenital heart diseases (CHD) refers to abnormal changes in morphology and structure of heart and/or large vessels caused by various developmental defects or partial developmental retardation in fetal heart and large vessels during embryonic development stage. CHD is the major reason for disability and neonatal or infant death. Currently, the overall incidence of CHD is approximately 10‰-12‰ in China [1]. The prevalence of birth defects has been increasing annually in China, which is the largest developing country, and this has resulted in substantial economic burdens for both the family of the person with birth defects and society [2]. For the children with CHD, approximately 30% of the fetuses died during gestation, and 40%–60% died during neonatal stage. CHD became a major public health problems that affect physical and mental health of children as well as the quality of population.

Recent studies have demonstrated that microRNAs (miRNAs) as a biomarker for the diagnosis and prognosis of CHD, is closely related to cardiovascular diseases. miRNAs play an important role in cardiac morphogenesis, cardiomyocyte growth and differentiation [3–6]. DiGeorge syndrome critical region 8 (DGCR8) is an RNA-binding protein that interacts with the RNase III enzyme Drosha to process miRNAs. DGCR8 converts long primary miRNAs (pri-miRNAs) into precursor miRNAs (pre-miRNAs). Thus, DGCR8 facilitates miRNA maturation, and miRNAs regulates their target gene expression [7].

It is well recognized that genetic factors including chromosomal abnormality and gene mutation, and environmental factors together lead to CHD. Transcriptional factors including GATA4、TBX5、EZC3、NKX2.5 were found to be associate with the occurrence of CHD. Datas have shown that the function of DGCR8 is closely related to the occurrence and development of many human diseases. Some studies suggested that DGCR8 gene participate in vascular development by

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regulating VSMC proliferation, apoptosis, and differentiation [8]. However, the relationship between DGCR8 and CHD remains to be defined. This study described the correlation between the occurrence and development of CHD and DGCR8 expression. This study may help us to identify potential biomarker for prenatal diagnosis of CHD fetus and to develop new interference strategies for CHD.

2. Materials and methods

Human studies including cardiac tissues and blood collection have been approved by the institutional ethical committee.All patients were provided by the Center of Henan Provincial People's Hospital and the Third Affiliated Hospital of Zhengzhou University.

2.1. Blood

2 ml of preoperative blood was collected from the CHD children (n = 40,mean age10.3 ± 11.1 months, 18 boys and22 girls)and healthy children (n = 40,mean age12.5 ± 13.3 months, 20 boys and 20 girls), respectively. 6 ml of cell lysis buffer was added to blood and the mixture was placed on ice for 15 min. After gentle mixing, the mixture was centrifuged with 450 × g for 10 min at 4 °C. The supernatant was discarded and 4 ml of red blood cell lysate to the Leukocyte sediments. After 450 × g centrifugal for 10 min at 4 °C, the supernatant was removed and 1 ml Trizol reagent was added to white blood cells and the sample was stored in −80 °C freezer.

2.2. Myocardial tissues

Myocardial tissues were collected from children with ventricular septal defect (VSD) (n = 25) and Tetralogy of Fallot (TOF) (n = 16). All children with CHD were diagnosed by color Doppler echocardiography, electrocardiogram and confirmed by surgery.

2.3. Detect mRNA expression by real-time fluorescence quantitative PCR

Total RNA was extracted from veinal blood (children with CHD and health children)and myocardial tissues (children with CHD) using Trizol reagent. Primers for individual miRNAs were listed in Table 1. The SYBR Green-based real-time PCR was performed using the Quant-Script RT Kit (TIANGEN Biotech, Beijing co). Melting curve analysis was performed to examine the specificity of PCR product.The relative levels of DGCR8 mRNA was normalized by those of GAPDH mRNA with the $2^{-\Delta\Delta Ct}$ method and were expressed as mean ± standard deviation (SD).

2.4. Western blotting

Myocardial tissues from children with CHD were collected in RIPA buffer (Thermo Scientific; Rockford, IL) containing 1% oroteinase inhibitor cocktail (Thermo Scientific; Rockford, IL). An equal amount of protein (50 g/lane) was loaded on 10% SDS-PAGE and transferred onto nitrocellulose membranes.The membrane was blocked with 5% non-fat milk for 1 h and incubated with primary antibodies (1:1000 dilution) against DGCR8 (Abcam, Shanghai) and glyceraldehyde 3-phosphate dehydrogenase (Abcam, Shanghai).

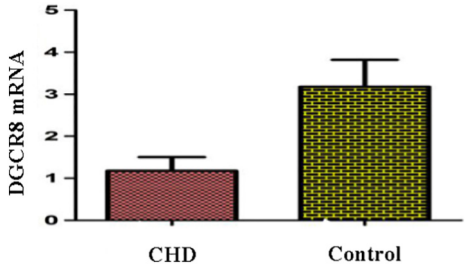

Fig. 1. DGCR8 expression was significantly low in blood of CHD compared with control(health children)(P = 0.037).

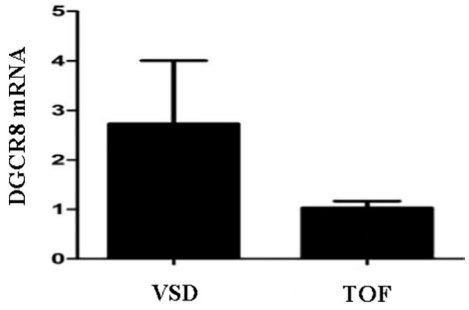

Fig. 2. In myocardial tissues, DRCG8 mRNA is significantly lower in TOF than VSD(p = 0.046).

2.5. Statistical analysis

Data shown represent the mean ± standard deviation (SD) from at least three different experiments. The differences were analyzed using Wilcoxon rank sum test of two independent samples. The correlation between the expression of DGCR8 and the size of VSD was analyzed by Spearman rank correlation analysis. P values < 0.05 was considered significant.

3. Results

3.1. DGCR8 expression was downregulated in the blood of CHD

Real-time PCR was performed to detect DGCR8 mRNA levels in 40 patients with CHD and 40 normal controls.The results showed that the expression of DGCR8 was lower in children with CHD compared with healthy children (Fig. 1), indicating that DGCR8 expression was downregulated in CHD at the transcriptional level.

3.2. The expression of DGCR8 in heart tissues was lower in TOF compared with VSD

As shown (Fig. 2), the real-time results demonstrated that the expression of DGCR8 was lower in TOF heart tissues compared with VSD heart tissues. The DGCR8 protein decreased in TOF group compared with VSD group was confirmed by Western blotting (Fig. 3).

Table 1
qRT-PCR primer sequences and amplified fragment length.

Primers	Primer pairs	Prime length(bp)	Products length(bp)
DGCR8	Forward:5′-AACGGGAAATCCGAGGTCTG-3′	20	101
	Reverse:5′-GGCTCACTTGGGTTCTCACA-3′	20	
GAPDH	Forward:5′-TCGTGGAAGGACTCATGACC-3′	20	116
	Reverse:5′-AGGGATGATGTTCTGGAGAG-3′	20	

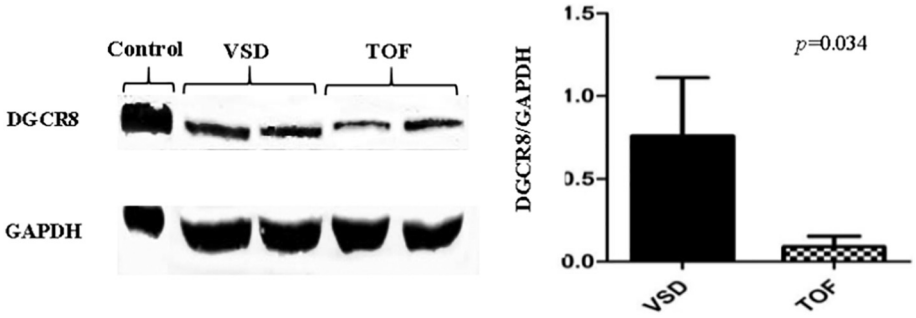

Fig. 3. The expression of DGCR8 in heart tissues was lower in TOF compared with VSD.

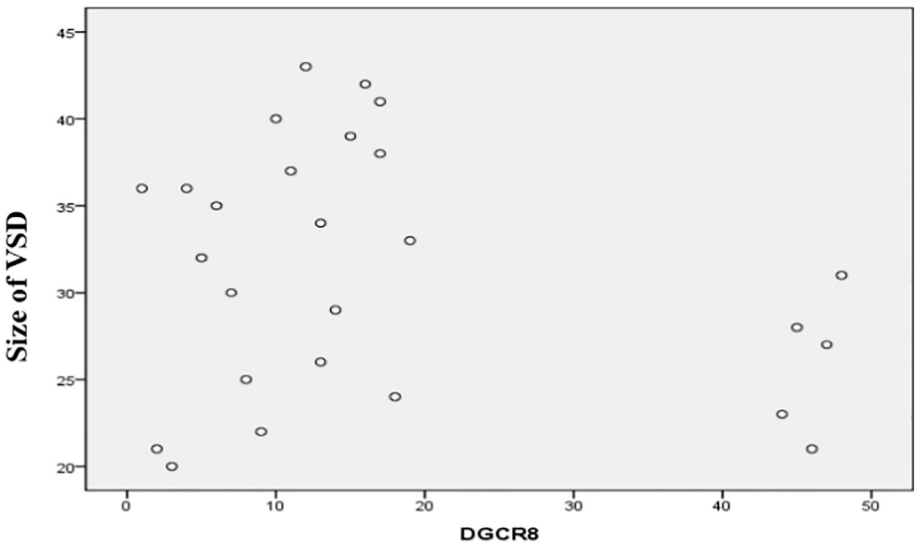

Fig. 4. DGCR8 expression in children with CHD was not significantly correlated with the size of VSD analyzed by spearman rank correlation analysis ($r = -0.22$, $P = 0.917$).

3.3. DGCR8 expression was not significantly associate with the size of VSD

To examine the correlation between DGCR8 blood level and the size of VSD, we performed real-time PCR. The results showed that DGCR8 expression in children with CHD was not significantly correlated with the size of VSD (Fig. 4).

4. Discussion

The DGCR8 gene encodes a double-stranded RNA binding protein and one of genes monoallele deleted in chromosome 22 related to DiGeorge syndrome(DGS) is a key component of miRNA biogenesis pathway and facilitates miRNA maturation by interacting with RNAase III enzyme Drosha [9].Studies have shown that miRNAs regulate the expression of key proteins in the process of cardiac formation [6]. miRNA may promote the process of cardiac remodeling [10,11], and their variations may lead to the pathogenesis of CHD and nerve-craniofacial-heart defects [12]. miRNAs were used as markers for the diagnosis of CHD and pulmonary hypertension (PH) [13]. Landthaler et al. [14] found that knockdown RNAi in fly and DGCR8 resulted in the accumulation of pri-miRNAs and the reduction of pre-miRNAs and maturation of miRNAs, and the increase of target mRNA levels.

Sellier.et a l [15] studied Digeorge syndrome (chromosome 22q11.2 deletion syndrome, 22q11DS) and found that the expression of DGCR8 in the blood of healthy people was low. Low expression of DGCR8 prevents the miRNA formation in neural crest cells, leading to craniofacial malformations and ventricular septal defect, double outlet ventricle and aortic arch defects. Chapnik et al. [16] investigated the potential contribution of DGCR8 insufficiency to cardiovascular development, and found that DGCR8 mutants displayed a wide spectrum of malformations, including persistent truncus arteriosus (PTA) and ventricular septal defect (VSD).This study revealed that the expression level of DGCR8 mRNA in the blood of CHD was statistically significant different from that in healthy children,suggesting that DGCR8 is closely related to the occurrence and development of blood vessels, heart block in CHD.

Chen,et al. [8] found that deletion of DGCR8 in VSMCs resulted in the larger heart and liver while smaller lung and stomach. Besides, the left and right ventricles of the heart were enlarged, and the ventricular wall was thinner, which are similar to clinical symptoms of advanced heart failure. Rao et al. [17] observed that knockout of DGCR8 gene in heart striated muscle induced left ventricular remodeling and promoted the development of heart failure. Our studies have shown that the relative expression level of DGCR8 in myocardial tissue of children with TOF was lower than that in VSD , suggesting that DGCR8 may contribute to CHD, and reduced DGCR8 expression may prevent the formation of the normal structure of fetal heart. The size and location of VSD and pulmonary arterial hypertension are important indexes to evaluate the operation of CHD, which is directly related to the postoperative recovery and the final outcome. This study analyzed the relationship between DGCR8 expression level and septal defect size, but found no significant correlation between DGCR8 and the size of VSD. Although DGCR8 is involved in the formation of CHD, but it may not directly affect the size of VSD.

Our study showed that reduced DGCR8 expression is significantly correlated with CHD. Further study of the potential molecular

mechanism of DGCR8 in regulating miRNA and participating in CHD, it can provide a basis for clinical detection and diagnosis of CHD children, which will help us to reduce the birth of children with disabilities.

Conflicts of interest

The authors declare no competing financial interests.

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Collection of blood samples and myocardial tissues from CHD and control were approved by institutional ethics committee.

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