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Associations between IL-6 Variations and Congenital Heart Disease Incidence among Chinese Han People

Authors' Contribution:

Study Design A

Data Collection B

Statistical Analysis C

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Background: Our research explored if Interleukin-6 (IL-6) variants held substantial connection to congenital heart disease (CHD) susceptibility among Chinese Han children.

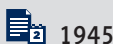
Material/Methods: A total of 102 CHD children were recruited as the case group while 98 healthy persons were recruited as the control group. We used polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) completed genotyping for IL-6 variants rs1800795 and rs1800796. The Hardy-Weinberg equilibrium (HWE) among controls was tested using χ^2 analysis. Genotype and allele frequencies for variants were compared between groups. Odds ratio (OR) accompanied by 95% confidence interval (CI) reflected the potential link of IL-6 variants to CHD occurrence.

Results: A remarkable increased trend of rs1800795 CC genotype and C allele was detected in the CHD patient group ($P < 0.05$). Individuals carrying rs1800795 CC genotype showed higher risk for CHD (OR=3.763, 95% CI=1.162-12.190). In addition, rs1800795 C allele could increase CHD incidence (OR=1.766, 95% CI=1.101-2.832). No significant differences were detected in IL-6 gene rs1800796 polymorphism in both genotype and allele distributions between the case group and the control group ($P > 0.05$). These associations had no significant alteration after the adjustment of age, gender, maternal smoking history, and maternal history of diabetes.

Conclusions: IL-6 variant rs1800795 exhibited a close relation to CHD susceptibility among Chinese Han people while the mutant C allele promoted CHD incidence. But rs1800796 variant showed no such influence.

MeSH Keywords: **CCAAT-Enhancer-Binding Protein-beta • DNA Copy Number Variations • Heart Defects, Congenital**

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Background

Congenital heart disease (CHD) refers to a defect in the structure or function of the heart existing at birth [1]. CHD is the most widespread type of congenital deformity and a predominant reason for birth defect-related deaths [2]. There are many types of CHD, including ASD (atrial septal defect), VSD (ventricular septal defect) and PDA (patent ductus arteriosus), as well as PAPVR (partial anomalous pulmonary venous return) and PDAC (pulmonary hypoplasia/agenesis, diaphragmatic hernia/eventration, anophthalmia/microphthalmia, and cardiac defect) syndrome which are rare types of CHD [3,4]. CHD can be influenced by environmental factors or genetic factors, or a combination of factors [5,6]. Various environmental factors have been identified which may increase CHD risk, such as excessive alcohol consumption during pregnancy, taking certain medications, and maternal viral infection [7]. But research has shown that not all pregnant women will bear CHD infants who are exposed to risk factors, suggesting individual differences in CHD susceptibility that may vary with hereditary factors. Genetic changes have been confirmed to play a role in CHD onset, and an accumulating number of candidate genes have been identified in recent years [8,9]. Identifying more candidate genes responsible for CHD will bring new insights to the diagnose and treatment of CHD.

Interleukin-6 (IL-6) represents a pleotropic inflammatory cytokine that can be generated by activated T cells, B cells, monocytes, and malignant cells. IL-6 exerts significant influence on the homeostasis of the immune and neuroendocrine systems, as well as influence on the balance of proinflammatory/anti-inflammatory pathways [10,11]. In humans, the IL-6 gene is located at chromosome 7q21–24, consisting of 4 introns and 5 exons [12]. Numerous single genetic variations have been found in the IL-6 gene [13]. IL-6 degree and secretion could be affected by IL-6 variants, thus further causing relevant biological responses. A number of human diseases are associated with IL-6 variants, like systemic lupus erythematosus, gastric cancer, and degenerative lumbar scoliosis [14–16].

Growing evidence suggests that the level of anti-inflammatory cytokines in the blood shows a remarkably positive association with CHD events [17]. Furthermore, Wang et al. detected elevated IL-6 degree among CHD children, suggesting vital impacts of IL-6 on CHD initiation and advancement [18].

Two common and widely studied SNPs (single nucleotide polymorphisms) are rs1800795 (–174G/C) and rs1800796 (–572C/G), which have been shown to be related to cardiovascular diseases. Therefore, we selected these 2 SNPs to explore their association with CHD risk. We checked genotype frequencies for the IL-6 variants rs1800795 (–174G/C) and rs1800796 (–572C/G) in CHD patients and healthy persons. And potential

link of IL-6 variant to CHD susceptibility was assessed among Chinese Han people.

Material and Methods

Study participants

For our research, the case group comprised 102 children (43 males and 59 females) with CHD seen at Emergency General Hospital, Beijing. All cases fulfilled the criteria of CHD. For the study control group, we recruited another 98 healthy children who had a physical examination at The People's Hospital of Rongchang District, during the same time period. All healthy controls had no CHD or family history of CHD, and had no other heart disease. All cases and controls were aged 0–11 years old and were age and gender matched with each other. All participants involved in this study were from the Chinese Han population and had no migration history or history of marriage with other nationalities within 3 generations.

This case-control study was conducted with prior approval and review of the Ethics Committee of Emergency General Hospital, Beijing. The sample extraction followed the ethnics criteria for national human genome research. Written informed consents were obtained.

DNA extraction

For DNA extraction, 5 mL fasting venous blood samples was collected from each participant into the anticoagulative tube with EDTA-disodium salt. The genomic DNA was extracted by TaKaRa Genome DNA Extraction Kit (Dalian Biological Engineering Co., Ltd., China), and then stored at –20°C for future application.

Genotyping

Genotype frequencies for IL-6 variants were determined using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). The primer sequences for IL-6 rs1800795 (–174G/C) and rs1800796 variants were designed by Primer Premier 5.0 software, and generated in Sangon Biotech (Shanghai, China) (Table 1). The PCR program started with an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 51°C for 60 seconds, extension at 72°C for 60 seconds, and an ultimate extension at 72°C for 10 minutes.

Subsequently, the PCR products were digested by different restriction enzyme: *CviAII* for rs1800795 and *Bsp6I* for rs1800796. Then the digested fragment of IL-6 gene rs1800795 and rs1800796 polymorphisms were examined employing 2% agarose gel electrophoresis and visualized by ultraviolet (UV) light.

Table 1. Primer sequences of IL-6 gene two polymorphisms rs1800795 and rs1800796.

SNP		Primer sequences	Annealing temperature (°C)
rs1800795	Sense	5'-TGCAAGACATGCCAAAG-3'	50.2
	Reverse	5'-CTTTGTTGGAGGGTGAGG-3'	
rs1800796	Sense	5'-TGAAGCAGGTGAAGAAAGT-3'	51.3
	Reverse	5'-CTGGGATTATGAAGAAGGTA-3'	

Table 2. The basic characteristics of subjects.

Characteristic		Case, n=102	Control, n=98	P
Age	The range	0–11	0–11	0.476
	Mean age	4.21±2.91	4.5±2.91	
Gender (%)	Male	43 (42.16)	40 (40.82)	0.559
	Female	59 (57.84)	58 (59.18)	
Maternal smoking history (%)	Yes	12 (11.76)	7 (7.14)	0.265
	No	90 (88.24)	91 (92.86)	
Maternal history of diabetes (%)	Yes	14 (13.73)	9 (9.18)	0.314
	No	88 (86.27)	89 (90.81)	

Statistical analysis

The data analysis was conducted using PASW Statistics 18.0 statistical software. The genotype and allele distribution for IL-6 variants rs1800795 and rs1800796 polymorphisms was determined through direct counting. Hardy-Weinberg equilibrium (HWE) test was used to assess the representativeness of the study population. Genotype and allele frequencies for IL-6 gene 2 polymorphisms were examined between the 2 groups employing χ^2 analysis. The link of IL-6 variants to CHD susceptibility was calculated by odds ratio (OR) and 95% confidence interval (CI). The results were adjusted by basic characteristics of participants through logistic regression analysis. The *P*-value less than 0.05 was considered statistically significant.

Results

Demographic characteristics of participants

The 102 CHD patients consisted of 43 males and 59 females; the age range was 0–11 years old (average age was 4.21±2.91 years). Healthy controls included 40 males and 58 females, aged 0–11 years (average age was 4.50±2.91 years). There was no significant difference in age, gender, maternal history

of smoking, or maternal history of diabetes between the CHD group and the control group (*P*>0.05 for all, Table 2).

HWE test

Table 3 presented genotype and allele frequencies for IL-6 variants rs1800795 and rs1800796 in cases and controls. Using the chi-square test, we found that the genotype distributions of each SNP did not deviate from HWE in control group (*P*>0.05), indicating our study sample was from the same Mendelian population and had a good representativeness.

Distributions of IL-6 gene polymorphisms between groups

Data comparison demonstrated significant differences for IL-6 gene rs1800795 polymorphism in both genotype and allele distributions between groups. A remarkable increase trend of rs1800795 CC genotype frequency was detected in the CHD patients group compared with the healthy control group (12.75% versus 4.08%), while the GG genotype frequency decreased obviously in the case group (55.88% versus 67.35%), and the difference reached significance level (*P*<0.05). But the heterozygous GC genotype distribution showed no significant differences between groups (31.37% versus 28.57%, *P*>0.05). Besides, the G and C allele frequencies also showed significant differences between groups (71.57% versus 81.63%, 28.43%

Table 3. Genotype and allele distributions of IL-6 gene rs1800795 and rs1800796 polymorphisms in case and control groups.

Genotype/allele	Case n=102(%)	Control n=98(%)	χ^2	P	OR (95% CI)	P^a	OR ^a (95%CI)
rs1800795							
GG	57 (55.88)	66 (67.35)	–	–	1	–	1
GC	32 (31.37)	28 (28.57)	0.789	0.374	1.323 (0.713–2.457)	0.334	1.361 (0.728–2.542)
CC	13 (12.75)	4 (4.08)	5.423	0.020	3.763 (1.162–12.190)	0.029	3.762 (1.114–12.374)
G	146 (71.57)	160 (81.63)	–	–	1	–	1
C	58 (28.43)	36 (18.37)	5.632	0.018	1.766 (1.101–2.832)	0.018	1.781 (1.106–2.868)
rs1800796							
CC	36 (35.29)	45 (45.92)	–	–	1	–	1
CG	47 (46.08)	42 (42.86)	1.187	0.276	1.399 (0.764–2.560)	0.259	1.420 (0.772–2.612)
GG	19 (18.63)	11 (11.22)	3.125	0.077	2.159 (0.912–5.114)	0.094	2.115 (0.879–5.088)
C	119 (58.33)	132 (67.35)	–	–	1	–	1
G	85 (41.67)	64 (32.65)	3.474	0.062	1.473 (0.979–2.216)	0.072	1.461 (0.967–2.207)

versus 18.37%, $P<0.05$). Mutant C allele of rs1800795 variant elevated CHD onset, and the CC genotype exerted a similar effect on CHD (OR=3.763, 95% CI=1.162–12.190). Adjusted by age, gender, maternal smoking history, and maternal history of diabetes, the differences remain significant ($P<0.05$). All results suggested that IL-6 gene rs1800795 variant held close relation to CHD susceptibility in Chinese Han people, while the mutant C allele and CC genotype heightened the onset of CHD.

For rs1800796 polymorphism, no significant differences were detected in both genotype and allele distributions between groups. The ancestral homozygous GG genotype frequency showed an increased trend in the case group compared with the control group (18.63% versus 11.22%), and the mutant CC genotype frequency decreased in the case group (35.29% versus 45.92%). The C and G allele frequencies were 58.33% and 41.67% in the case group, and 67.35% and 32.65% in the control group. Although G allele and GG genotype appeared more frequently in the case group, the differences were not statistical significance ($P>0.05$). When adjusted by age, gender, maternal smoking history, and maternal history of diabetes, the differences were still not significant. We speculated that IL-6 gene rs1800796 polymorphism might have no obvious association to CHD occurrence among Chinese Han people.

Discussion

CHD refers to a gross structural abnormality in heart or great vessels of actual functional significance. CHD is the most

common defect among birth defects, and it has been regarded as a dominant reason for noninfectious death within the first year of life among neonates [19]. With the increasing quality of care for preterm infants, the incidence of CHD has grown as well. CHD is generally a serious disease that has a huge economic burden for both patients' families and society. In recent years, many treatment methods related to coronary heart disease have also improved. For example, remifentanyl-based fast-track anesthesia has advantages that are helpful for CHD patients [20]. Besides, the exploration on CHD etiology has been widely investigated, although it remains unclear. Recently, new technologies have emerged to highlight the genetic factors that contribute to CHD.

In humans, the IL-6 gene is located at chromosome 7q21–24 and consisting of 4 introns and 5 exons. A number of SNPs have been identified in the IL-6 gene, and variants in its promoter area can modulate IL-6 degree [21]. Furthermore, higher serum IL-6 degree appears among CHD children. All evidence implies a potential for IL-6 variants to affect CHD.

Rs1800795 (–174G/C) and rs1800796 (–572C/G) are popular variants in the human IL-6 gene and are located at the gene's promoter area. Growing evidence shows the IL-6 variant rs1800795 participates in diverse cardiovascular illnesses, like acute coronary syndrome, coronary artery disease, and atherosclerotic vascular disease [22–24]. In our research, genotype and allele distributions for IL-6 variant rs1800795 were examined between CHD cases and controls. Accordingly, CC genotype and C allele appeared more frequently among CHD patients,

while CC genotype carriers showed 3.763-fold higher risk for CHD compared with GG genotype carriers. A previous study uncovered that among Chinese people, CC genotype and C allele of rs1800795 had a close association with increased coronary artery disease incidence, which has been validated in Caucasian populations [25,26]. In addition, rs1800795 polymorphism has an effect on blood pressure [27]. While evidence supports our study results, the molecular mechanisms of rs1800795 polymorphism on CHD onset risk is still unexplained. A major study reported that *IL6* rs1800795*C allele showed a significant relationship with increased IL-6 levels [28]. Furthermore, higher IL-6 levels have been detected in children with CHD. It might be a more reasonable explanation for the risk role of the CC genotype and C allele in CHD occurrence, supporting conclusions found in earlier research. But the hypothesis needs to be validated. Another SNP, rs1800796, was also analyzed in this study. Although its GG genotype and G allele was found more frequently in CHD children, the difference was not statistically significant. Hence, IL-6 rs1800796 polymorphism might have no obvious association with CHD among Chinese Han people. Fang et al. reported that IL-6 gene rs1800796 polymorphism was correlated with serum IL-6 levels [21]. Although no significant association of rs1800796 with CHD susceptibility was found in this study, these results should be verified in other studies.

There were several limitations to our study. Firstly, the sample size was relatively small, and the genetic association of IL-6 variation with specific types of CHD susceptibility was not evaluated. Secondly, our study population was from the single hospital, which might introduce bias to our results. Thirdly, the interactions between IL-6 variants with environment factors or other genetic factors in CHD were not explored in our study. In addition, the mechanisms underlying the function of IL-6 polymorphisms remains poorly understood. Therefore, further well-designed studies with expanded sample size are needed.

Conclusions

Results from our research found IL-6 variant rs1800795 had a close association with CHD incidence among Chinese Han people, with CC genotype and C allele elevating CHD susceptibility in Chinese.

Conflict of interest

None.

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