Cardiology in the Young (2016), **26**, 237–243
doi:10.1017/S1047951115000037

Original Article

Association between polymorphisms in *IL27* and risk for CHD in a Chinese population

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Abstract *Background:* IL-27, a member of the IL-12 family, has been involved in maternal tolerance to the foetus and successful pregnancy. Growing evidences indicate that IL-27 plays a crucial role in pregnancy. *Aim:* We carried out the present study in order to investigate whether polymorphisms in the *IL27* are associated with the risk for CHDs, including atrial septal defect and ventricular septal defect. *Patients and methods:* We conducted this case–control study among 247 atrial septal defect patients, 150 ventricular septal defect patients, and 368 healthy controls in a Chinese population using polymerase chain reaction-restriction fragment length polymorphism assay. *Results:* Significantly increased risk for atrial septal defect ($p = 0.001$, OR = 1.490, 95% CI = 1.178–1.887) and ventricular septal defect ($p = 0.004$, OR = 1.502, 95% CI = 1.139–1.976) was observed to be associated with the allele G of rs153109. In a dominant model, we have also observed that increased susceptibilities for atrial septal defect ($p < 0.01$, OR = 1.89, 95% CI = 1.35–2.63) and ventricular septal defect ($p < 0.01$, OR = 2.50, 95% CI = 1.67–3.85) were statistically associated with rs153109; however, no association was found between CHD risk and rs17855750 in the *IL27* gene. *Conclusion:* The 153109 of the *IL27* gene may be associated with the susceptibility to CHD, including atrial septal defect and ventricular septal defect.

Keywords: CHD; *IL27*; polymorphisms; genetic susceptibility; cytokine

Received: 18 September 2014; Accepted: 4 January 2015; First published online: 9 February 2015

CHD, WHICH IS DEFINED AS THE STRUCTURAL abnormality of the heart or blood vessels near the heart, present either at the time of birth or detected later on, is a common cause of peri-natal morbidity and mortality.[1,2] Most of the known causes of CHD are sporadic genetic changes, but genes regulating the complex developmental sequence have only been partly elucidated.[3] In addition, known ante-natal environmental factors include maternal infections – for example, Rubella – drugs such as alcohol, hydantoin, lithium, and thalidomide, and maternal illness – for example, diabetes mellitus, phenylketonuria, and systemic lupus erythematosus.[4]

Atrial septal defect is the third most common type of CHD, with an estimated incidence of 56/100,000 live births.[5] Included in this group of malformations are several types of atrial communications that allow shunting of blood between the systemic and the pulmonary circulations.[6] Most atrial septal defects are sporadic with no identifiable cause. Abnormalities in genes essential for cardiac septation have been associated with atrial septal defect.[7]

Ventricular septal defect is a common CHD, which accounts for up to 40% of cardiac anomalies.[8] In recent years, multiple genes, mainly genes encoding transcriptional factors, such as TBX5, NKX2.5, and GATA4, have been found to be important for

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cardiac development and are candidate genes for ventricular septal defect.[9,10] It seems that genetic factors may play an important role in the pathogenesis of ventricular septal defect.

Cardiac development is a complex biological process requiring the integration of cell commitment, morphogenesis, and excitation–contraction coupling.[11] In addition, there are a large number of cytokines involved in embryogenesis.[12] Previous research has suggested that immune mechanisms may play a critical role in the development of human congenital defects.[13]

IL-27 is a novel heterodimeric cytokine that consists of IL-27p28 and Epstein–Barr virus-induced gene 3 (EBI3).[14–16] IL-27, with its gene located on chromosome 16 (16p11), is a new IL-12 family member. It is an early product of activated antigen-presenting cells and triggers Th1 polarisation and IFN-γ production in synergy with IL-12. IL-27 mediates its biological effects through a signal-transducing receptor constituted by WSX-1, also known as TCCR, a class I cytokine receptor, and glycoprotein 130 (gp130), an IL-6-related receptor subunit.[17,18] The success of pregnancy is a complex process with a large number of immune cytokines involved in the process.[19] Immunohistochemistry studies showed that IL-27 was expressed at the interface and is involved in maternal tolerance to the foetus and successful pregnancy.[20] In addition, polymorphisms located on genes regulating the immune response may result in increased susceptibility and/or poorer prognosis in certain individual diseases, especially immune-related diseases.[21,22] As IL-27 plays an important role during embryogenesis, we assumed that polymorphisms in *IL27* may be associated with the risk for CHD.

The rs153109 and rs17855750 polymorphisms of IL-27p28 were recently identified. Previous studies have demonstrated their association with chronic obstructive pulmonary disease and allergic rhinitis;[23,24] however, to date, no studies have examined the role of polymorphisms within the *IL27* gene in the development of CHD. In the present study, we assumed that polymorphisms in *IL27* may affect the risk for CHD. We have identified two possible variation sites of *IL27*, including one exon (rs17855750) and one promoter (rs153109) sequence. To determine whether these polymorphisms are associated with susceptibility to CHD, we have analysed their frequencies in genomic DNAs isolated from CHD patients and healthy controls.

Material and methods

Patients

The present study was carried out with the approval of the ethics committee of the West China First University Hospital of Sichuan University, and all the patients gave their written informed consent to participate. A hospital-based case–control study was conducted, including 247 unrelated atrial septal defect patients ranging in age from 1 to 65 years (mean ± SD, 29.62 ± 16.34; male/female, 74/173) and 150 unrelated ventricular septal defect patients ranging in age from 1 to 53 years (mean ± SD, 16.13 ± 12.28; male/female, 69/81) between June 2008 and October 2012 at the First University Hospital of Sichuan University. The diagnoses of atrial septal defect and ventricular septal defect were based on the patient's history, physical examination, electrocardiogram, and echocardiogram studies. Clinical data were collected from the hospital record section. A group of control patients, including 368 healthy controls ranging in age from 12 to 48 years (mean ± SD, 26.69 ± 6.33; male/female, 135/233) was selected randomly from a routine health survey in the same hospital. Patients with any personal or family history of heart disease or other serious disease were intentionally excluded.

Genotyping

Genomic DNA of each individual was extracted from 200 µl of ethylenediaminetetraacetic acid-anticoagulated peripheral blood samples using a DNA isolation kit from Bioteke (Peking, China). The procedure was performed according to the instruction manual. The genotypes of the two single nucleotide polymorphisms – rs153109 and rs17855750 – selected were analysed using a polymerase chain reaction-restriction fragment length polymorphism assay. The primers used for amplification of the rs153109 polymorphism were F: 5'-CTGATCCTGACCTCACTCA ACGC-3' and R: 5'-CTGACTGGGACTGGGACTC AGC-3', and primers used for amplification of the rs17855750 polymorphism were F: 5'-ATCTCGCCA GGAAGCTGCGC-3' and R: 5'-CTGTTAGTGGGG GCCAGAAGGGA-3'. The polymerase chain reaction reactions were performed in a total volume of 25 µl, including 2.5 µl 10 × polymerase chain reaction buffer, 1.5 mmol/L $MgCl_2$, 0.15 mmol/L dNTPs, 0.5 µmol/L each primer, 100 ng of genomic DNA, and 1U of Taq DNA polymerase. The polymerase chain reaction conditions for rs153109 were as follows: 94°C for 4 minutes, followed by 32 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C, with a final elongation at 72°C for 10 minutes; and the polymerase chain reaction conditions for rs17855750 were as follows: 94°C for 4 minute, followed by 36 cycles of 30 seconds at 94°C, 30 seconds at 66°C, and 30 seconds at 72°C, with a final elongation at 72°C for 10 minutes. Polymerase chain reaction products of these two polymorphisms were digested overnight with a

specific restriction enzyme, Bstu I, and the digested polymerase chain reaction products were separated using 6% polyacrylamide gel and stained with 1.0 mg/ml argent nitrate: for rs153109, allele G was cuttable, yielding two fragments of 100 and 19 bp, allele A was uncuttable and the fragment is still 119 bp. For rs17855750, allele G was cuttable, yielding two fragments of 101 and 19 bp, allele T was uncuttable and the fragment is still 120 bp. Approximately 20% of the samples were randomly selected to carry out the repeated assays and the results were 100% concordant. The genotypes were confirmed by DNA sequencing analysis.

Statistical analysis

All data analyses were carried out using SPSS 13.0 statistical software (SPSS Inc., Chicago, Illinios, United States of America). Allele and genotype frequency of the *IL27* gene rs153109 and rs17855750 were obtained by directed counting, and the Hardy–Weinberg equilibrium was evaluated by the χ^2 test. Genotypic association tests in a case–control pattern assuming co-dominant, dominant, recessive, over-dominant, or log-additive genetic models were performed using SNPstats.[25] Odds ratio and respective 95% confidence intervals were reported to evaluate the effects of any difference between alleles and genotypes. A $p < 0.05$ was regarded as statistically significant.

Results

Both rs153109 and rs17855750 polymorphisms were successfully genotyped in 247 atrial septal defect patients and in 368 healthy control patients. Genotype distribution of these two polymorphisms in our cases and controls were consistent with the Hardy–Weinberg equilibrium. Allele frequencies of these two polymorphisms for 247 atrial septal defect patients and for 368 control patients are shown in Table 1. Significantly increased atrial septal defect risk was observed to be associated with the allele G of rs153109 locus ($p = 0.001$, OR = 1.490, 95% CI = 1.178–1.887); however, the allele frequency of rs17855750 polymorphism was not significantly different between atrial septal defect patients and controls ($p = 0.139$, OR = 0.747, 95% CI = 0.510–1.094)

As shown in Table 2, significantly increased atrial septal defect risk was found to be associated with the AG ($p < 0.01$, OR = 1.89, 95% CI = 1.32–2.70) and GG genotypes ($p < 0.01$, OR = 1.89, 95% CI = 1.15–3.03) of the rs153109 polymorphism in the co-dominant model, compared with the AA genotype. Compared with the homozygote AA/GG genotypes carriers, the AG heterozygote carriers have a 1.59-fold atrial septal defect risk in the over-dominant model ($p < 0.01$, OR = 1.59, 95% CI = 1.15–2.17). Moreover, in the dominant model, significantly increased atrial septal defect susceptibility was also observed to be associated with G allele carriers ($p < 0.01$, OR = 1.89, 95% CI = 1.35–2.63); however, for genotypic association analysis, no statistically significant difference was detected between atrial septal defect patients and control patients for the rs17855750 polymorphism.

To further investigate whether these two polymorphisms of the *IL27* gene are associated with other types of CHD, we also detected these two polymorphisms of the *IL27* gene on ventricular septal defect patients. Allele frequencies of these two polymorphisms for 150 ventricular septal defect patients and 368 controls are shown in Table 3. Significantly increased ventricular septal defect risk was observed to be associated with the G allele of rs153109 locus ($p = 0.004$, OR = 1.502, 95% CI = 1.139–1.976), but allele frequency of the rs17855750 polymorphism was not significantly different between ventricular septal defect patients and controls ($p = 0.809$, OR = 0.947, 95% CI = 0.590–1.518). As shown in Table 4, significantly increased ventricular septal defect risk was found to be associated with the AG genotype of the rs153109 polymorphism in the co-dominant model, compared with AA and GG genotypes ($p < 0.01$, OR = 2.86, 95% CI = 1.85–4.35). Compared with the homozygote AA/GG genotypes carriers, the AG heterozygote carriers have

Table 1. Data of selected polymorphisms in the *IL27* gene among patients with atrial septal defect and controls.

Polymorphisms	Allele	Patients with atrial septal defect [n = 247(%)]	Controls [n = 368(%)]	OR (95% CI)	p value
rs153109	A	284 (57.5)	492 (66.8)	**1.490 (1.178–1.887)**	**0.001**
	G	210 (42.5)	244 (33.2)		
rs17855750	T	439 (88.9)	673 (91.4)	0.747 (0.510–1.094)	0.139
	G	55 (11.1)	63 (8.6)		

CI = confidence interval; OR = odds ratio
No corresponds to the number of individuals
Boldfaced values indicate a significant difference at the 5% level

https://doi.org/10.1017/S1047951115000037 Published online by Cambridge University Press

Table 2. Genotype frequencies of selected polymorphisms in the *IL27* gene among patients with atrial septal defect and controls and their association with atrial septal defect risk.

				Logistic regression	
Genetic model	**Genotype**	**Patients with atrial septal defect (n = 247)**	**Controls (n = 368)**	**OR (95% CI)***	**p value**
Rs153109 A/G					
Co-dominant	A/A	79 (32%)	173 (47%)	1.00	**<0.01**
	A/G	126 (51%)	146 (39.7%)	**1.89 (1.32–2.70)**	
	G/G	42 (17%)	49 (13.3%)	**1.89 (1.15–3.03)**	
Dominant	A/A	79 (32%)	173 (47%)	1.00	**<0.01**
	A/G-G/G	168 (68%)	195 (53%)	**1.89 (1.35–2.63)**	
Recessive	A/A-A/G	205 (83%)	319 (86.7%)	1.00	0.21
	G/G	42 (17%)	49 (13.3%)	0.75 (0.48–1.17)	
Over-dominant	A/A-G/G	121 (49%)	222 (60.3%)	1.00	**<0.01**
	A/G	126 (51%)	146 (39.7%)	**1.59 (1.15–2.17)**	
Log-additive				**1.47 (1.16–1.85)**	**<0.01**
Rs17855750 T/G					
Co-dominant	T/T	199 (80.6%)	305 (82.9%)	1.00	0.0016
	T/G	41 (16.6%)	63 (17.1%)	1.00 (0.65–1.54)	
	G/G	7 (2.8%)	0 (0%)	0.00 (0.00–NA)	
Dominant	T/T	199 (80.6%)	305 (82.9%)	1.00	0.47
	T/G-G/G	48 (19.4%)	63 (17.1%)	0.86 (0.57–1.30)	
Recessive	T/T-T/G	240 (97.2%)	368 (100%)	1.00	3e-04
	G/G	7 (2.8%)	0 (0%)	0.00 (0.00–NA)	
Over-dominant	T/T-G/G	206 (83.4%)	305 (82.9%)	1.00	0.87
	T/G	41 (16.6%)	63 (17.1%)	1.04 (0.67–1.60)	
Log-additive				0.75 (0.52–1.10)	0.14

CI = confidence interval; OR = odds ratio
No corresponds to the number of individuals
Boldfaced values indicate a significant difference at the 5% level
*Adjusted by age

Table 3. Data of selected polymorphisms in the *IL27* gene among patients with ventricular septal defect and controls.

Polymorphisms	**Allele**	**Patients with ventricular septal defect [n = 150 (%)]**	**Controls [n = 368 (%)]**	**OR (95% CI)**	**p value**
rs153109	A	172 (57.3)	492 (66.8)	**1.502 (1.139–1.976)**	**0.004**
	G	128 (42.7)	244 (33.2)		
rs17855750	T	273 (91.0)	673 (91.4)	0.947 (0.590–1.518)	0.809
	G	27 (9.0)	63 (8.6)		

CI = confidence interval; OR = odds ratio
No corresponds to the number of individuals
Boldfaced values indicate a significant difference at the 5% level

a 2.56-fold ventricular septal defect risk in the over-dominant model ($p < 0.01$, OR = 2.56, 95% CI = 1.72–3.85). Moreover, in the dominant model, significantly increased ventricular septal defect susceptibility was also observed to be associated with G allele carriers ($p < 0.01$, OR = 2.50, 95% CI = 1.67–3.85); however, for genotypic association analysis, no statistically significant difference was detected between atrial septal defect patients and controls for the rs17855750 polymorphism.

Discussion

To our knowledge, this is the first study to investigate the association between the polymorphisms in the *IL27* gene and CHD risk. Our results demonstrated that the allele G of the rs153109 polymorphism may increase atrial septal defect and ventricular septal defect susceptibility. We have calculated the statistical power with "Power and Sample Size Calculation" software (version 3.0.43), and our study had >80% power to detect the association between this polymorphism of *IL27* and both atrial septal defect and ventricular septal defect susceptibility.The rs17855750 polymorphism, however, has no statistically significant differences between CHD patients, including atrial septal defect and ventricular septal defect patients, and healthy control patients.

CHD, a heart problem caused by the improper development of the heart during foetal development,

https://doi.org/10.1017/S1047951115000037 Published online by Cambridge University Press

Table 4. Genotype frequencies of selected polymorphisms in the *IL27* gene among patients with ventricular septal defect and controls and their association with ventricular septal defect risk.

				Logistic regression	
Genetic model	Genotype	Patients with ventricular septal defect (n = 150)	Controls (n = 368)	OR (95% CI)*	p value
Rs153109					
Co-dominant	A/A	39 (26%)	173 (47%)	1.00	<0.01
	A/G	94 (62.7%)	146 (39.7%)	**2.86 (1.85–4.35)**	
	G/G	17 (11.3%)	49 (13.3%)	0.65 (0.34–1.25)	
Dominant	A/A	39 (26%)	173 (47%)	1.00	<0.01
	A/G-G/G	111 (74%)	195 (53%)	**2.50 (1.67–3.85)**	
Recessive	A/A-A/G	133 (88.7%)	319 (86.7%)	1.00	0.54
	G/G	17 (11.3%)	49 (13.3%)	1.20 (0.67–2.16)	
Over-dominant	A/A-G/G	56 (37.3%)	222 (60.3%)	1.00	<0.01
	A/G	94 (62.7%)	146 (39.7%)	**2.56 (1.72–3.85)**	
Log-additive				**1.52 (1.14–2.00)**	<0.01
Rs17855750					
Co-dominant	T/T	124 (82.7%)	305 (82.9%)	1.00	0.29
	T/G	25 (16.7%)	63 (17.1%)	1.02 (0.62–1.70)	
	G/G	1 (0.7%)	0 (0%)	0.00 (0.00–NA)	
Dominant	T/T	124 (82.7%)	305 (82.9%)	1.00	0.95
	T/G-G/G	26 (17.3%)	63 (17.1%)	0.99 (0.60–1.63)	
Recessive	T/T-T/G	149 (99.3%)	368 (100%)	1.00	0.12
	G/G	1 (0.7%)	0 (0%)	0.00 (0.00–NA)	
Over-dominant	T/T-G/G	125 (83.3%)	305 (82.9%)	1.00	0.90
	T/G	25 (16.7%)	63 (17.1%)	1.03 (0.62–1.72)	
Log-additive				0.94 (0.58–1.54)	0.81

CI = confidence interval; OR = odds ratio
No corresponds to the number of individuals
Boldfaced values indicate a significant difference at the 5% level
*Adjusted by age

is always presented at birth.[4] It has been known as a major cause for infant mortality, but the aetiology of the vast majority of CHD is not fully addressed. Cardiac development is a complex biological process requiring the integration of cell commitment, morphogenesis, and excitation–contraction coupling.[11] Both genetic and environmental factors are suspected to be involved.

As we know, a large number of immune cytokines play an important role in either the progress of pregnancy or implantation.[26] Increased risk for atrioventricular septal defects has been observed to be associated with maternal urinary tract infections in the first trimester.[27] Moreover, dysregulation of the maternal immune response related to an elevation of cytokines important in inflammatory conditions is suggested to play a role in cardiac malformations during foetal development.[28] In addition, cytokines have been confirmed to be involved in various cardiovascular diseases.[29–31] Previous studies have suggested the potential association between the aberration in cytokine production and CHD risk.[28,32]

IL-27, a pro-inflammatory cytokine, composed of Epstein–Barr virus-induced gene 3 protein (EBI3) and p28, plays an important role in response to infection, and is expressed at a very high level at the human foetal–maternal interface.[16,20,33–36] Existing research has found that IL-27 acts directly on endothelial cells and inhibits angiogenesis by inducing the production of anti-angiogenic chemokines IP-10 and MIG.[37] Moreover, IL-27 produced by extravillous trophoblasts may prevent excess pro-angiogenic activity through a negative-feedback mechanism.[20] Taken together, there may be an association between IL-27 and angiogenesis during embryogenesis, and thus IL-27 may be involved in the pathogenesis of CHD.

Existing researches have suggested an important role genetic factors may play in the pathogenesis of CHD.[3,4] Polymorphisms in genes potentially influence susceptibility to CHD. Identification of human mutations that cause CHD offers a complementary approach to gene ablation studies and particularly fosters definition of gene defects that perturb later stages of cardiac development.[11] As IL-27 may play an important role in the pathogenesis of CHD, genetic variation in the *IL27* gene may be associated with risk for this disease. In the present study, our data demonstrated an association between rs153109 polymorphism and susceptibility to CHD, including atrial septal defect and ventricular septal defect. These findings suggested that the

https://doi.org/10.1017/S1047951115000037 Published online by Cambridge University Press

rs153109 polymorphism may be a contributor to the pathogenesis of CHD. This polymorphism might become a useful prognostic biomarker for CHD patients. Nonetheless, the precise role the *IL27* gene may have in the development of CHD remained unclear, further studies are necessary to confirm our findings and its possible mechanisms.

Although we detected the association between rs153109 polymorphism in *IL27* and CHD, it is worth mentioning that there are some limitations to our study. First, we did not detect the protein level of IL-27 in the peripheral blood and did not perform a functional analysis study, thus we could not draw a certain conclusion about the influence of these polymorphisms on the cytokine levels. Second, the number of patients in our study were limited. Further large-scale studies are necessary to confirm our findings.

In summary, the present study demonstrated that the allele G and GG genotype of the rs153109 polymorphism in the *IL27* gene contribute to increased CHD susceptibility, including atrial septal defect and ventricular septal defect, in Chinese Han population. Nevertheless, further studies are necessary to investigate the real association between these two polymorphisms and the risk for CHD, especially in ethnically disparate population.

Financial Support

This work was supported by the fund of Chongqing Population and Family Planning Science and Technology Research Institute (No.1304, No.1202); 2013cstc-jbky-01704; 2012cstc-jbky-01702; CSTC, 2009CA5001.

Conflicts of Interest

None.

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https://doi.org/10.1017/S1047951115000037 Published online by Cambridge University Press