

# IL-35 polymorphisms and cognitive decline did not show any association in patients with coronary heart disease over a 2-year period

## A retrospective observational study (STROBE compliant)

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### Abstract

Prior evidence suggested that inflammation and inflammatory cytokines polymorphisms might be essential in the development of coronary heart disease (CHD) and cognitive decline. The following study investigated the associations between interleukin-35 (*IL-35*) polymorphisms and cognitive decline in CHD patients over a 2-year period.

CHD patients were enrolled between January 2015 and January 2016. Cognitive function, including memory, orientation, verbal and attention were assessed using Telephone Interview for Cognitive Status-Modified (TICS-m) during a 2-year follow-up. Genotypes of the single nucleotide polymorphisms (SNPs), including rs2243115, rs568408, rs582054, rs583911, rs428253, rs4740 and rs393581 of *IL-35* were examined by MassArray (Sequenom). The differences of TICS-m score between 2-year interval were used to estimate the cognitive decline; linear regression model was used to analyze the association between *IL-35* polymorphisms and cognitive decline in CHD patients after a 2-year follow-up.

The mean age of study individuals was 60.58 ( $\pm 7.86$ ) years old. There were 255 (68.5%) males and 117 (31.5%) female patients. The TICS-m scores, including overall cognition score, verbal attention and memory scores gradually decreased over a 2 year follow up period ( $P < .001$ , respectively), whereas there was no difference in orientation function score between the 1-year and 2-year follow-up ( $P = .448$ ). Furthermore, after adjusting for age, sex, history of hypertension (HT) and Diabetes mellitus (DM), smoking, education, Therapy regimen (PCI, CABG, medication) left ventricular ejection fraction (LVEF), and the severity of coronary artery stenosis (Gensini score), no association was found between *IL-35* rs2243115, rs568408, rs582054, rs583911, rs428253, rs4740 genotypes and cognitive decline in CHD patients over a 2-year period.

Our data revealed that *IL-35* polymorphisms was not associated with cognitive decline in CHD patients over a 2-year period. Yet, further studies are needed to confirm the role of cytokine gene polymorphisms in cognitive decline among CHD patients.

**Abbreviations:** ACEI = Angiotensin-Converting Enzyme Inhibitor, ACS = acute coronary syndrome, AD = Alzheimer's disease, AMI = acute myocardial infarction, ARB = angiotensin receptor blocker, CABG = coronary artery bypass graft surgery, CDR = clinical dementia rating, CHD = coronary heart disease, COPD = chronic obstructive pulmonary disease, DM = diabetes mellitus, EB13 = the subunit Epstein-Barr virus (EBV)-induced gene 3, eGFR = estimated glomerular filtration Rate, FAQ = functional activities questionnaire, FBG = fasting blood glucose, HT = hypertension, HWE = Hardy-Weinberg Equilibrium, IADL = instrumental activities of daily living scale, *IL-35* = interleukin-35, LDL = low-density lipoprotein, LVEF = left ventricular ejection fraction, MAF = minor allele frequency, MDRD = modification of diet in renal disease, MI = myocardial infarction, MMSE = mini-mental state examination, NO = nitric oxide, NT-Pro BNP = N-Terminal Pro-brain natriuretic peptide, PCI = percutaneous coronary intervention, Scr = serum creatinine, SNP = single nucleotide polymorphism, TG = triglyceride, UAP = unstable angina pectoris.

**Keywords:** cognitive decline, coronary heart disease, EB13, IL-12A, IL-35, polymorphisms

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## 1. Introduction

Cognitive decline is a condition characterized by decline in mental or intellectual function involving memory, attention, orientation, language, which often lead to dementia.<sup>[1,2]</sup> Over the last decade, cognitive decline has become a serious public health problem which strongly affects the patients' health and daily life activities.

Coronary heart disease (CHD) is the major cause of death worldwide.<sup>[3]</sup> Recent evidence has suggested that the major risk factors related to vascular diseases, including hypertension, smoking, obesity, diabetes mellitus and hyperlipidemia are linked to cognitive decline and dementia.<sup>[4–6]</sup> Deckers et al reported that individuals with CHD have a 45% increased risk for developing a cognitive decline.<sup>[7]</sup> Moreover, Lisbeth et al found higher prevalence of dementia in CHD patients who underwent coronary artery bypass graft surgery (CABG).<sup>[8]</sup> Inflammatory cytokines have been suggested to be the molecules that link cardiovascular diseases and cognitive decline.<sup>[9,10]</sup> Furthermore, many studies have proved that SNPs of inflammatory cytokines, including *IL-18*, *IL-10*, *IL-6* polymorphisms have a vital role in CHD and are associated with the development of cognitive decline.<sup>[11–16]</sup>

*IL-35* is a heterodimer cytokine that belongs to the *IL-12* family. *IL-35*, which was identified in 2007,<sup>[17,18]</sup> is composed of the *p35* subunit of *IL-12* (*IL-12A*) and the subunit Epstein-Barr virus (EBV)-induced gene 3 (*EBI3*), which is secreted by CD4+ regulated cells (Treg), activated dendritic cells and macrophages.<sup>[19–21]</sup> Previous studies have suggested that *IL-35* participates in the progression of atherosclerosis.<sup>[22]</sup> In addition, our previous study has shown that plasma *IL-35* levels are dramatically decreased in CHD patients and are positively correlated with left ventricular ejection fraction (LVEF), suggesting that *IL-35* may be a potential novel biomarker for CHD.<sup>[23]</sup> The polymorphisms in *IL-12A*, which is the subunit of *IL-35*, were found to be related to the risk of Alzheimer's disease (AD); while, *IL-35* variants were reported to be associated with CHD recently.<sup>[24–26]</sup> However, current information on the relationship between *IL-35* polymorphisms and cognitive decline is still limited. In the present study, we aimed to investigate the effect of *IL-35* polymorphisms on cognitive decline within CHD patients over a 2-year period.

## 2. Materials and methods

### 2.1. Study population

Patients were consecutively recruited from The People's Hospital of Guangxi Zhuang Autonomous Region from January 2015 to January 2016. Eligible patients fulfilled the following criteria:

- (1) first-time diagnosis of acute myocardial infarction (AMI) characterized by elevation of myocardial injury specific biomarker (cTnT, cTnI and CK-MB) values with at least one value above the 99<sup>th</sup> percentile of the upper reference limit. AMI was accompanied with at least one of the following:
  - (a) typical symptoms of ischemia,
  - (b) significant ST-T wave changes in correlated leads, new or presumed new left bundle branch block or pathological Q waves,
  - (c) imaging evidence of new or presumed new loss of viable myocardium or regional wall motion abnormality<sup>[27]</sup>; or
- (2) first-time diagnosis as CHD according to WHO criteria.<sup>[28]</sup>

Patients with following situations were excluded:

- (1) other severe diseases such as malignant tumor, liver disease, autoimmune diseases (such as Grave's disease, systemic lupus erythematosus) and cardiogenic shock;
- (2) organic mental disorders and/or any neuropsychiatric disorders;
- (3) with drug abuse;
- (4) history of any kind of cerebrovascular disease and central nervous system disease;
- (5) language disorder, hearing impairment or could not cooperate with the evaluation of cognitive function; and
- (6) history of valvular or aortic surgery.

The Ethics Committee of The People's Hospital of Guangxi Zhuang Autonomous Region approved this study. Informed consent was obtained from all patients. A total of 80.87% (372/460) eligible subjects consented to participate in the present study; the screening process for participants is shown in Figure 1.

### 2.2. Covariates

Sociodemographic information was collected by same-trained investigators. Participants were divided into low-level, middle-level and high-level according to the education degree (primary school or less; high school; university or more, respectively). Medical histories as well as family history of dementia were also recorded.

Blood samples were extracted the following morning after admission. Biological variables such as fasting blood glucose (FBG), triglyceride (TG), low-density lipoprotein (LDL), N-Terminal Pro-brain natriuretic peptide (NT-Pro BNP), serum creatinine (Scr) at baseline were determined by same standard protocols in the department of clinical lab at the People's Hospital of Guangxi Zhuang Autonomous Region. Modification of Diet in Renal Disease (MDRD) Study Equation was used to calculate the estimated Glomerular Filtration Rate (eGFR).<sup>[29]</sup>

The CHD patients were categorized into PCI group, CABG group in addition to medication and medication group according to therapy regimen. The severity of coronary artery stenosis was assessed by Gensini score on the basis of the results of Coronary angiograph.<sup>[30]</sup>

### 2.3. Cognitive function evaluation

Cognitive function evaluation was assessed by the TICS-m developed by Brandt, Gallo and Breathier.<sup>[31]</sup> Previous studies have found a high correlation between face-to-face and telephone interview. In addition, TICS-m have shown significantly positive associations with Mini-Mental State Examination (MMSE), and significant negative associations with Clinical Dementia Rating (CDR), Instrumental Activities of Daily Living Scale (IADL) and Functional Activities Questionnaire (FAQ), which are widely used in assessment of cognitive function.<sup>[32]</sup> The TICS-m has 21 items and 12 sub-items with a maximum score of 50, representing memory, orientation, verbal and attention function respectively.<sup>[31,32]</sup> Cognitive function was assessed three times during the study:

- 1) within 48 hours after admission;
- 2) at the end of 1 year ( $\pm 7$  days) after the first assessment;
- 3) at the end of the 2-year follow up period ( $\pm 7$  days). All assessments were performed by the same well trained investigators via face-to-face or telephone interview.

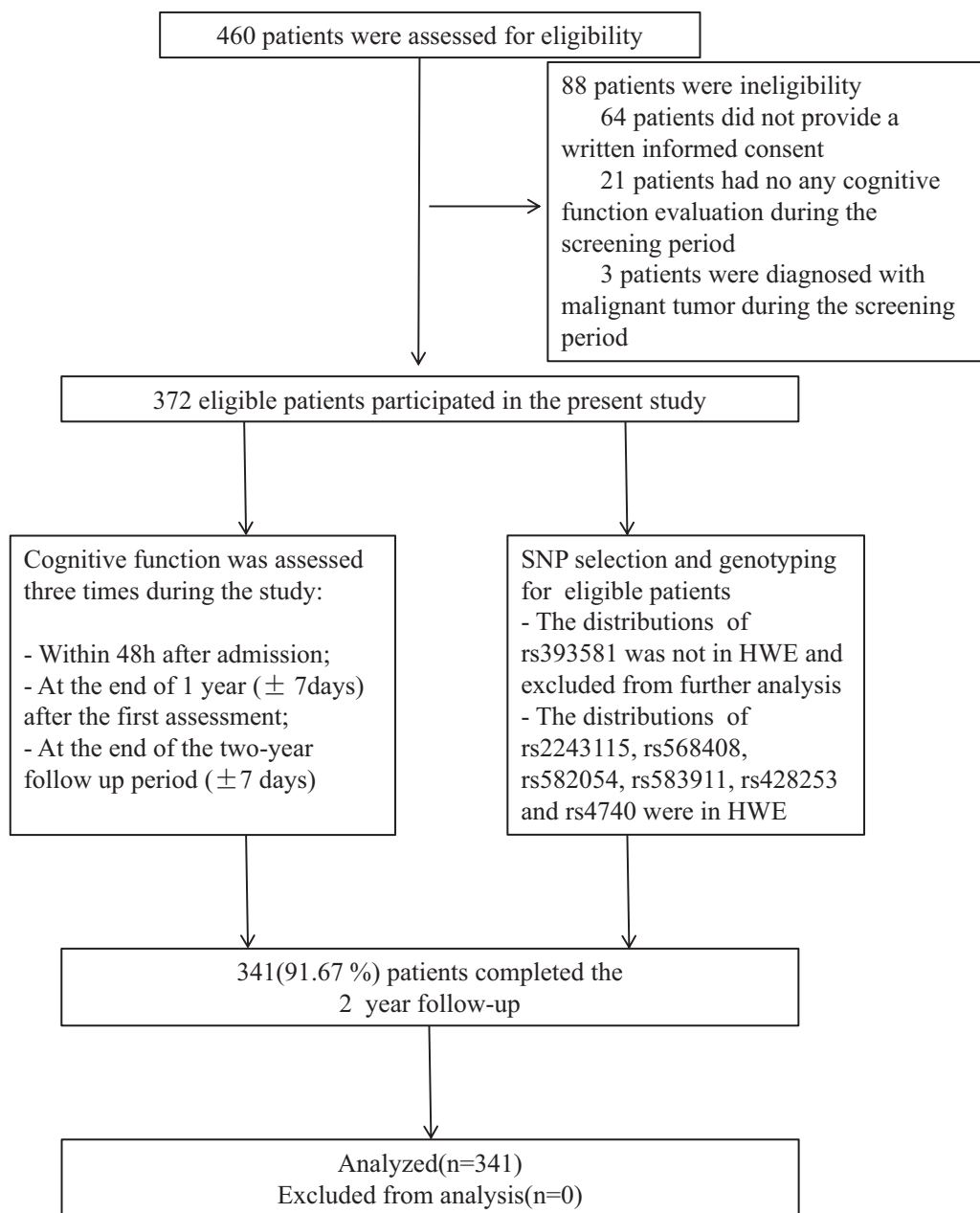


Figure 1. Flow chart.

#### 2.4. Single nucleotide polymorphism (SNP) selection and genotyping

SNPs were selected from the Hapmap database (<http://www.hapmap.org>) and Genomes database, according to following criteria:  $r^2 \geq 0.8$  and minor allele frequency (MAF)  $\geq 0.1$  in CHB data; promoter (5' near gene), 5'UTR, exon and 3'UTR SNPs were given priority. Finally, rs2243115, rs568408, rs582054, rs583911, rs428253, rs4740, and rs393581 were selected for the subsequent analysis. Genomic DNA was isolated from peripheral blood samples by Puregene kit (Gentra systems, Inc., Minneapolis, MN) following the manufacturer's instruction. Genotyping of rs2243115, rs568408, rs582054, rs583911, rs428253, rs4740 and rs393581 were determined by MassArray (Sequenom, Inc., San Diego, CA). The primers were designed by the MassArray

AssayDesign 3.1 software (Sequenom, Inc., San Diego, CA). The PCR was conducted in a reaction of 4  $\mu$ l PCR master mix and 1  $\mu$ l DNA (20ng/ $\mu$ l) in 384-well format. Finally, genotyping failed in 4(1.08%), 12(3.23%), 5(1.34%), 5(1.34%), 22(5.91%), 12(3.23%), and 5(1.34%) in rs2243115, rs568408, rs582054, rs583911, rs428253, rs4740, and rs393581 loci, respectively owing to DNA quantity or quality.

#### 2.5. Statistical analyses

All SNP alleles were examined for Hardy-Weinberg Equilibrium (HWE) using a chi-square test. Continuous variables were presented as the mean  $\pm$  SD or median with interquartile ranges according to Kolmogorov-Smirnov normality test. Categorical variables were expressed as percentages. The decline score of

TICS-m, including overall cognition score, memory, orientation, verbal and attention function scores between baseline and the end of follow up was used to evaluate cognitive decline. The association between *IL-35* polymorphisms and cognitive decline risk within 2 years in CHD patients was assessed by linear regression. In addition, the risk factors such as age, sex, history of HT and DM, smoking, Gensini score, education, therapeutic regimen (PCI, CABG, medication) and LVEF were also analyzed in the model. A *P* value < .05 or < .017 for multiple comparison of Kruskal-Wallis test were considered statistically significant. All statistical analyses were performed using the SPSS 17.0 (SPSS corp. Chicago, IL).

### 3. Results

#### 3.1. Genotype distributions of *IL-35* in CHD patients

The genotype distributions of rs2243115, rs568408, rs582054 and rs583911 in *IL-12A*, and rs428253, rs4740 and rs393581 in *EBI3* are shown in Table 1. The distributions of rs2243115, rs568408, rs582054, rs583911, rs428253, and rs4740 were in HWE (*P* = .60, *P* = .90, *P* = .76, *P* = .64, *P* = .50, *P* = .47, respectively), while the distribution of rs393581, which departed from HWE (*P* < .001), was excluded from further analysis.

#### 3.2. The baseline characteristics of study population

The baseline characteristics of the study population are presented in Table 2. The mean age of study subjects was 60.58 (± 7.86) years old; 68.5% were male and 31.5% were female. In addition, 172 (46.2%) participants were diagnosed with stable angina pectoris (SAP) while 200 (53.8%) were diagnosed with acute coronary syndrome (ACS), including myocardial infarction (MI) and unstable angina pectoris (UAP). Among those, 222 underwent Percutaneous coronary intervention (PCI), five received coronary artery bypass graft (CABG) on the basis of medication (such as antiplatelet, statin, Angiotensin-Converting Enzyme Inhibitor (ACEI)/Angiotensin Receptor Blocker (ARB), β-receptor blocker), and the remaining received medication only. The baseline of TICS-m scores are shown in Table 2.

#### 3.3. Genetic association of *IL-35* polymorphisms and cognitive decline in CHD patients over a 2-year period

The median follow-up of present study was 729 days with interquartile ranges from 729 days to 734 days. Furthermore, 91.67% (341/372) population completed the 2 year follow-up; the status of follow-up is presented in Figure 1. The results demonstrated that TICS-m scores, including overall cognition

**Table 2**

#### General characteristics of study population at baseline.

Variables	Study Population (n = 372)
Sex, Male/Female (N, %)	255/117 (68.5/31.5)
Age, y	60.58 ± 7.86
BMI, kg/m <sup>2</sup> *	23.07 ± 6.40
FBG, mmol/L*	5.51 ± 1.86
LDL, mmol/L*	2.95 ± 1.10
TC, mmol/L*	4.55 ± 1.38
TG, mmol/L*	1.75 ± 1.48
Scr, mmol/L*	81.69 ± 26.88
UA, μmol/L*	385.23 ± 108.07
LVEF, %*	57.87 ± 10.55
FS, %*	30.95 ± 6.64
Gensini	21.00 (10.00–40.00)
NT-proBNP, pg/ml	820.00 (86.74–2408.00)
Smoking (yes, %)	155 (42.1)
Drinking (yes, %)	97 (26.4)
CHD (N, %)	
SAP	172 (46.2)
ACS	200 (53.8)
Therapy <sup>#</sup> (N, %)	
PCI	222 (59.7)
CABG	5 (1.34)
Medication	145 (38.9)
Past history (yes, %)	
Hypertension	227 (61.5)
Diabetes	90 (24.4)
Family history of Dementia (yes, %)	18 (4.9)
Education (N, %)	
Low	60 (16.2)
Middle	208 (55.9)
High	104 (27.9)
TICS-m score (baseline)	
Total Score	35.00 (35.00–36.00)
Memory	7.00 (6.00–7.00)
Orientation	13.00 (12.50–13.00)
Verbal and attention	16.00 (15.25–17.00)

\* ACS = acute coronary syndrome, BMI = body mass index, CABG = coronary artery bypass grafting, CHD = coronary heart disease, CRP = C-reactive protein, FBG = fasting blood glucose, FS = fractional shortening, LDL = low density lipoprotein, LVEF = Left Ventricular Ejection Fraction, PCI = percutaneous coronary intervention, SAP = stable angina pectoris, Scr = Serum creatinine, TC = total cholesterol, TG = Triglyceride, UA = Uric Acid.

<sup>†</sup>*P* < .05.

score (TICS-m total score), verbal and attention and memory score were gradually decreased (*P* < .001, respectively), whereas no difference was found in the orientation function score between the 1-year to 2-year follow-up, the results are demonstrated in Table 3.

**Table 1**

#### Distribution of *IL-35* SNPs allele in study patients\*

Gene	SNPs	HomMaj (n/%)	HomHet (n/%)	HomMin (n/%)	HomMin/HomHet	HWE <i>P</i>
<i>IL-12A</i>	rs2243115 (TT/TG/GG)	295 (80.2)	70 (19.0)	3 (0.80)	73 (19.8)	.60
	rs568408 (GG/GA/AA)	278 (77.2)	77 (21.4)	5 (1.4)	82 (22.8)	.90
	rs582054 (AA/AT/TT)	183 (49.9)	154 (42.0)	30 (8.1)	184 (50.1)	.76
	rs583911 (GG/GA/AA)	191 (52.0)	145 (39.5)	31 (8.4)	176 (47.9)	.64
<i>EBI3</i>	rs428253 (GG/GC/CC)	210 (60.0)	125 (35.7)	15 (4.3)	140 (40.0)	.50
	rs4740 (GG/GA/AA)	99 (27.5)	173 (48.1)	88 (24.4)	261 (72.5)	.47
	rs393581 (GG/AG/AA)	2 (0.5)	365 (99.5)	0 (0)	365 (99.5)	<.001

\* HomMaj = homozygote of majority, Het = heterozygote, HomMin = homozygote of minority.

<sup>#</sup>HWE = Hardy-Weinberg equilibrium.

**Table 3**  
Telephone interview for cognitive status-modified (TICS-m) scores across the follow-up time-points\*.

	TICS-m Score Median (Interquartile Range)			P	P1 <sup>adj</sup> T0 vs T1	P2 <sup>adj</sup> T0 vs T2	P3 <sup>adj</sup> T1 vs T2
	T0	T1	T2				
TICS-m scores-Memory	7.00 (6.00–7.00)	6.00 (5.00–6.00)	5.00 (5.00–6.00)	<.001	<.001	<.001	<.001
TICS-m scores-Orientation	13.00 (12.50–13.00)	13.00 (13.00–13.00)	13.00 (13.00–13.00)	<.001	<.001	<.001	.45
TICS-m scores-Verbal and attention	16.00 (15.25–17.00)	15.00 (15.00–16.00)	15.00 (13.00–15.00)	<.001	<.001	<.001	<.001
TICS-m scores-Total	35.00 (35.00–36.00)	34.00 (33.00–35.00)	33.00 (31.00–34.00)	<.001	<.001	<.001	<.001

\* Kruskal-Wallis Test was used to analyze the TICS-m scores across the following times (at baseline (T0), 1 year after the first assessment (T1), 2 years after the first assessment (T2)).

P<sup>adj</sup>: The P values were adjusted by the comparison times (Bonferroni adjustment) and was considered statistical significance at .017.

To further investigate the effect of observed *IL-35* SNPs on cognitive decline in CHD patients, a risk factors such as age, sex, history of HT and DM, smoking, education, therapeutic regimen (PCI, CABG, medication), LVEF, the severity of coronary artery stenosis (Gensini score) were adjusted by linear regression. Yet, no association was found between *IL-35* rs2243115, rs568408, rs582054, rs583911, rs428253, rs4740 genotypes and cognitive decline in CHD patients over a 2- year period, which are shown in Table 4 and Supplemental Figure 1, <http://links.lww.com/MD/E595> (See Fig. Supplemental Content, which illustrates TICS-total Scores in different genotype between follow-up periods.).

#### 4. Discussion

Cognitive decline, which is commonly observed with neurological disorders, has a complex and heterogeneous pathogenesis.

Studies have suggested that patients with CHD have a higher risk of developing cognitive impairment.<sup>[17]</sup> In addition, growing evidences has shown that certain cytokines, including IL-6, IL-1 and TNF- $\alpha$  play an important role in the pathogenesis of cognitive decline, as well as CHD.<sup>[133]</sup> The inflammatory cytokines IL-1 and TNF- $\alpha$  that are produced by glial cells and can induce nitric oxide (NO), might take part in the pathogenesis of cognitive decline by causing neuronal injury.<sup>[134]</sup> Moreover, studies have discovered that the deficiency of p40 subunit of IL-12/IL-23 pathway or its receptor complex may decrease cerebral amyloid load, which is critical in AD. Furthermore, the same study suggested that the inhibition of IL-12/IL-23 pathway might reduce cognitive impairment of AD.<sup>[135]</sup>

Polymorphisms in genes that code for neurotransmitters, neuropeptides or cytokines are involved in the pathophysiology

**Table 4**  
the relationship between *IL-35* polymorphisms and  $\Delta$ TICS scores in study patient.

Variables	Unstandardized Coefficients		Standardized Coefficients Beta	95%CI for B		P*
	B	Std.Error		Lower bound	Upper bound	
$\Delta$ TICS Total						
Rs2243115	0.042	0.209	0.012	-0.369	0.453	.842
Rs568408	0.170	0.191	0.055	-0.207	0.546	.375
Rs582054	0.034	0.144	0.014	-0.249	0.316	.814
Rs583911	0.064	0.141	0.028	-0.214	0.343	.650
Rs428253	0.001	0.161	0.001	-0.315	0.318	.993
Rs4740	0.084	0.126	0.041	-0.163	0.331	.504
$\Delta$ TICS-m scores-Memory						
Rs2243115	-0.110	0.136	-0.049	-0.378	0.159	.423
Rs568408	-0.081	0.124	-0.040	-0.325	0.164	.518
Rs582054	-0.103	0.094	-0.067	-0.287	0.082	.275
Rs583911	-0.110	0.092	-0.074	-0.291	0.071	.232
Rs428253	-0.095	0.104	-0.058	-0.300	0.109	.359
Rs4740	0.043	0.082	0.032	-0.119	0.205	.601
$\Delta$ TICS-m scores-Orientation						
Rs2243115	-0.132	0.084	-0.095	-0.298	0.035	.120
Rs568408	0.038	0.078	0.030	-0.115	0.190	.626
Rs582054	-0.075	0.058	-0.080	-0.190	0.040	.199
Rs583911	-0.079	0.058	-0.085	-0.192	0.035	.172
Rs428253	0.025	0.065	0.024	-0.103	0.152	.705
Rs4740	0.054	0.050	0.066	-0.046	0.153	.289
$\Delta$ TICS-m scores-Verbal and attention						
Rs2243115	0.285	0.225	0.077	-0.158	0.729	.206
Rs568408	0.184	0.205	0.055	-0.220	0.588	.371
Rs582054	0.233	0.154	0.0093	-0.070	0.536	.131
Rs583911	0.271	0.151	0.109	-0.027	0.569	.074
Rs428253	0.033	0.171	0.012	-0.304	0.370	.847
Rs4740	0.073	0.135	0.033	-0.192	0.338	.588

the decline of TICS-m score between baseline and the end of follow up was calculated to estimate cognitive decline, Homozygote of majority as a reference.

\* The linear regression model was used to evaluate the cognitive decline between subjects' genotypes; age, sex, history of HT and DM, smoking, Gensini score, Education, Therapy regimen (PCI, CABG, medication) and LVEF were enter into model analysis.



of many neurological disorders. Inflammation cytokine genetic polymorphisms can regulate the immune response by affecting the expression of these cytokines, thereby participating in the evolution of cognitive decline. Previous studies showed that the C allele of the *IL-6-174G>C* (rs1800795) was associated with higher serum IL-6 levels and more severe cognitive decline compared with G allele.<sup>[36,37]</sup> Moreover, the homozygosity for the A allele of the *IL-10-1082G/A* polymorphism has been linked with higher risk of AD and reduced IL-10 levels in peripheral cells.<sup>[13]</sup> Nowadays, genetic diagnosis and therapy are widely used in the field of clinical practice; genetic marker is a potential stratified tool for screening high risk cognitive decline. In present study, we examined the association between *IL-35* polymorphisms and cognitive decline in CHD patients. *IL-35* belongs to *IL-12* family of heterodimeric cytokines and is comprised of subunits shared with *IL-12* and *IL-27*.<sup>[17,18]</sup> The *IL-12A* (p35) subunit binds to a p40 subunit to form *IL-12*, while the *EBI3* subunit binds to a p28 subunit to form *IL-27*. Studies have indicated that *IL-35* might suppress CD4+ effector T cells (Teff) activity and prevent the development of inflammatory diseases, while the inflammation was remarkably taken place in vulnerable brain region of Alzheimer's disease (AD) patient, which suggested that *IL-35* might be involved in the pathophysiology of cognitive decline.<sup>[19,33,38–40]</sup> Furthermore, previous researches indicated that *IL-12A* genetic variants could contribute to the risk of various inflammatory disorders by affecting the expression of mRNA via disrupting exonic splicing enhancers.<sup>[41]</sup> Additionally, Wang et al found that the frequencies of GT and GT+GG of *IL-12A* rs2243115 were significantly different from TT in chronic obstructive pulmonary disease (COPD), which further suggested that *IL-12A* rs2243115 polymorphism may contribute to genetic susceptibility to COPD.<sup>[42]</sup> Moreover, other studies indicated that the SNPs in *IL-12A* rs568404 might contribute to the risk of asthma and Graves' disease in Chinese population; the TT genotype of *IL-12A* rs568408 was related to significantly decreased late-onset AD risk.<sup>[26,43,44]</sup> Since *IL-12A* was a subunit of *IL-35* and recently *IL-35* polymorphisms were explored to be related to the risk of CHD,<sup>[24,25]</sup> we aimed to explore the *IL-35* polymorphisms on cognitive function in CHD patients. Although our results suggested that TICS-m scores including overall cognition score, verbal and attention and memory scores were gradually decreased, no significant difference of TICS-m scores decline over a 2-year period were observed between different genotypes in *IL-35* rs2243115, rs568408, rs582054, rs583911, rs428253 and rs4740 within participants in present study. Cognitive impairment progression is a long process regulated by many factors, which might be the reason why we did not found association between *IL-35* SNP and cognitive decline. In addition, our data were not consistent with some previous studies; this might be due different genetic background, lifestyle and number of patients that were examined in other studies.<sup>[45–47]</sup> Moreover, SNP might contribute to the susceptibility of disease by interacting with other loci.<sup>[26]</sup> As a heterodimer, more combined effect research need to be carried on in the future to explore the association of *IL-35* polymorphism and cognitive decline. Furthermore, many technologies of artificial intelligence are widely used in the field of Medicine study, Sun et al explored a predictive-trend-aware composition by using a time series prediction model and genetic algorithms to address multiple-generated service templates with varying process configurations.<sup>[48]</sup> Since cognitive impairment progression is a time dependent process, we should use a appropriate predictive-trend-aware composition to evaluate

the correlation between genetics and cognitive function in future research.

#### 4.1. Limitations

This study has few limitations. First, this was a clinical study, thus there might be some selection bias might exist in enrollment. Second, the sample size in our study was not large enough to investigate the association between *IL-35* genotypes and cognitive decline in CHD. Third, considering that cognitive decline is a longer process, the follow up time needs to be increased. Fourth, only six *IL-35* SNPs were analyzed in current study. More loci should be investigated in the future to verify the role of cytokine gene polymorphisms in cognitive decline.

#### 5. Conclusion

In this study, we did not find any significant association between *IL-35* genotypes and cognitive decline in CHD patients over a 2-year period. In the future, a study with a larger sample size, multi-institution and longer follow-up might be necessary to further explore the association between the selected SNPs polymorphisms and cognitive performance among CHD patients.

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#### Author contributions

**Conception and design of the study:** HRL and YS.

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**Data management and analysis:** YZL and YS

**Manuscript drafting/editing:** HRL and YS.

All authors read and approved the final manuscript.

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