Biochemical Genetics (2019) 57:129–146
https://doi.org/10.1007/s10528-018-9880-7

ORIGINAL ARTICLE

Association of *IL-8*-251 A/T rs4073 and *IL-10* rs1800872 -592C/A Polymorphisms and Coronary Artery Disease in North Indian Population

Naindeep Kaur[1] · Jagtar Singh[1] · S. Reddy[2]

Received: 27 November 2017 / Accepted: 20 July 2018 / Published online: 2 August 2018

Abstract

CAD (Coronary Artery Disease) morbidity is becoming an endemic worldwide. Recently, the role of pro- and anti-inflammatory cytokines in the development of atherosclerotic plaques has been explored, but the association of their genetic polymorphisms and CAD has yet not been established. The present study aimed to investigate the association of *IL-8*-251A/T (rs4073) and *IL-10* -592C/A (rs1800872) polymorphisms and the risk of CAD in North Indian population. 1000 subjects (500 angiographically confirmed CAD patients and 500 controls) were genotyped by ARMS-PCR. Results revealed a significant risk association of both the polymorphisms with CAD. The heterozygous and the mutant genotypes of *IL-8* rs4073 were both found to be associated with the risk of disease after adjusting for the confounders ($p_{adj} < 0.001$, OR_{adj} 3.121, 95% CI 1.926–5.056 and $p_{adj} < 0.001$, OR_{adj} 3.116, 95% CI 1.952–4.973, respectively), but only the mutant AA genotype of *IL-10* rs1800872 correlated with risk of disease with $p_{adj} < 0.001$, OR_{adj} 4.106, 95% CI 2.160–7.806). Stratifying the samples on the basis of gender revealed CAD in heterozygous and mutant in males ($p_{adj} < 0.001$, OR_{adj} 3.693, 95% CI 2.031–6.716; $p_{adj} < 0.001$, OR_{adj} 3.288, 95% CI 1.848–5.851, respectively) and only the mutant to be associated with risk of disease in females ($p_{adj} = 0.010$, OR_{adj} 2.867, 95% CI 1.284–6.404) for *IL-8* rs4073, whereas only the mutant genotype AA of *IL-10* rs1800872 associated with CAD risk in males ($p_{adj} < 0.001$, OR_{adj} 5.821, 95% CI 2.831–11.970). Stratified analysis based on age showed a significant higher risk in the heterozygous and mutant genotype in subjects below 40 years of age ($p_{adj} = 0.039$, OR_{adj} 5.052, 95% CI 1.081–23.602; and $p_{adj} = 0.025$, OR_{adj} 5.533, 95% CI 1.239–24.704, respectively) compared with the heterozygous and mutant genotype association of the risk of disease in subjects above 40 years of age ($p_{adj} < 0.000$, OR_{adj} 2.964, 95% CI 1.747–5.027; and $p_{adj} < 0.000$, OR_{adj} 2.859, 95% CI 1.716–4.762, respectively) in *IL-8* rs4073. For *IL-10* rs1800872, risk association was seen only in subjects above 40 years of age ($p_{adj} < 0.001$, OR_{adj} 5.049, and 95% CI 2.414–10.561). The present study exhibited associations of *IL-8*-251A/T (rs4073)

Extended author information available on the last page of the article

and *IL-10* -592C/A (rs1800872) with CAD in the North Indian population and also that the associations are gender and age dependent.

Keywords Coronary artery disease · North Indian population · *IL-8* · ARMS-PCR · Genetic polymorphism

Introduction

Coronary artery disease (CAD) is now said to be the major cause of deaths worldwide and possesses a multifactorial nature having a number of risk factors like gender, age, obesity, high blood pressure, altered lipid profiles, diabetes, smoking, tobacco, and alcohol intake involved along with the genetic factors (Hansson 2005; Yiannakouris et al. 2014). There is a rapid elevation in the prevalence of CAD in developing countries like India (Enas et al. 2001; Anand et al. 2000), and this increase can be attributed to the changes in lifestyle, industrialization, urbanization, strict endogamous marital patterns (Gaziano and Gaziano 2008). The two key features of CAD are atherosclerosis and inflammation. The buildup of plaque in the arterial wall subsequently leads to the recruitment of inflammatory players at the site of plaque. A number of genes, and their resulting proteins, are involved in this whole mechanism making it a tough task for researchers to elucidate the multiple complex interconnecting pathways involved in the whole process.

Inflammation is considered to be a key player in the development and progression of atherosclerosis (Hansson 2005; Zakynthinos and Pappa 2009). Multiple studies have reported the roles of both the pro-inflammatory and anti-inflammatory cytokines to be involved in the disease pathogenesis (Libby et al. 2011).

Interleukin-8 (IL-8) is a member of the CXC chemokines superfamily and maps on to chromosome 4q13-21 (Gerszten et al. 1999; Rigamonti et al. 2008). It behaves as a chemoattractant for neutrophils and macrophages, and studies have shown the involvement of IL-8 in innate and acquired immune responses, and as an inflammatory molecule in atherosclerotic plaque development (Boisvert et al. 2000; Simonini et al. 2000; Vogiatzi et al. 2008; Morris et al. 1992). IL-8 is found to be secreted from macrophages and foam cells in the atherosclerotic lesions (Wang et al. 1996) and signals the smooth muscle cells (SMCs) in the latter to proliferate and migrate to the intima of the vessel wall (Yue et al. 1994; Yue et al. 1993). Genetic studies have been conducted worldwide to find the relationship between *IL-8* gene polymorphisms and susceptibility to CAD (Vogiatzi et al. 2008; Wang et al. 2015; Ren and She 2015; Zakynthinos and Pappa 2009), but no study has been carried out on the North Indian population yet. Hence, we chose to genotype -251 A/T rs4073 polymorphism in *IL-8*, located in the promoter region and hence to see the association between the polymorphism and CAD in a North Indian population.

Another cytokine, interleukin 10 (IL-10), is an anti-inflammatory cytokine—secretd by Th2 lymphocytes, B cells, and monocytes—is reported to play a protective role in CAD (Madeshiya et al. 2017). *IL-10* gene maps on to junction between 1q31 and 1q32 (Koch et al. 2001). IL-10 has the capability to inhibit NK-κB, MMPs (matrix degrading

metalloproteinases) and also promotes the Th2 phenotype, and thus, modulates the process of vascular inflammation and the plaque stability (Heeschen et al. 2003). It inhibits the synthesis of a range of Th1-producing cytokines (IFN-γ, IL-2, TNF-β, IL-1, IL-6, and TNF-α) and deviates from its regulation to the Th2-related immune response (Fernández and Kaski 2002; Halvorsen et al. 2005; Kanda et al. 1996). Multiple studies report the association of *IL-10* gene polymorphisms with diabetes mellitus (Mohebbatikaljahi et al. 2009; Saxena et al. 2012), acute coronary syndrome (ACS) (Srikanth Babu et al. 2012), and arterial thrombotic diseases (Meuwissen et al. 2004; Marousi et al. 2011). Also the lower plasma levels of plasma IL-10 are linked with ACS and ischemic stroke (IS) (Heeschen et al. 2003; Anguera et al. 2002; Xie et al. 2013), but information in the North Indian population is still missing. We have attempted to genotype the *IL-10* promoter region polymorphism, 592C/A (rs1800872), and its correlation with CAD and other associated parameters such as age, gender, obesity, smoking, and drinking status, family history, and various biochemical tests in the North Indian population.

Materials and Methods

Study Population

A total of 1000 subjects aged 25–70 years (both male and female) from North India, viz. including Punjab, Haryana, Chandigarh, Himachal Pradesh, New Delhi, Rajasthan, Jammu and Kashmir, Uttarakhand, Uttar Pradesh, and Uttaranchal, were enrolled for the study. Patients angiographically confirmed for CAD (with more than 50% stenosis in at least one coronary artery) visiting Out Patient Department (OPD), Department of Cardiology at Postgraduate Institute of Medical Education and Research, Chandigarh between November 2014 and December 2015 were enrolled as cases. Exclusion criteria for the cases include rejecting the subjects with pregnancy, hypo or hyperthyroidism, acute or chronic infection, hepatic, renal or respiratory insufficiency and malignancy. 500 healthy individuals were enrolled as controls. The inclusion criteria for the controls was: no past history of cardiac disorder, chronic diseases such as AIDS, hepatitis, tuberculosis, diabetes, hypertension, hypo- or hyperthyroidism, malignancy, etc, or any other comorbid illness, nonpregnant females. Strict caution was taken so that none of the control was a smoker, drinker or a consumer of tobacco. Sampling of controls was done at blood donation camps. Written consent from both patients and controls was taken. Ethical clearance was received from Institutional Ethics Committee, Panjab University, Chandigarh, India and the work has been done strictly in accordance with the "Ethical Guidelines for Biomedical Research on Human Participants, 2006″ as proposed by Indian Council of Medical Research and Ministry of Health, Govt. of India.

Biometric and Biochemical Measurements

All the anthropometric parameters of the subjects were recorded in a detailed questionnaire, and also the family history, smoking and drinking habits, the presence of

any other disease, etc. were noted as well. Standard biochemical methods were used for the determination of lipid profile, fasting serum glucose, hsCRP, Apolipoprotein A1, Apolipoprotein B, and uric acid.

DNA Isolation and SNP Selection and Genotyping

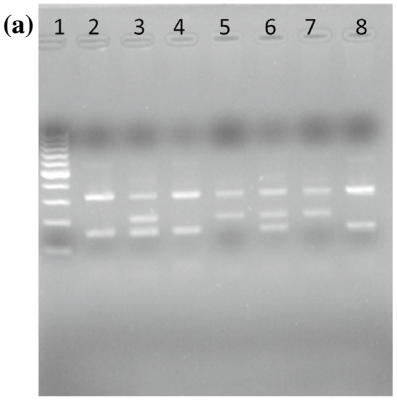
Blood samples were collected in EDTA-coated vials and stored at − 80 °C until genomic DNA was extracted. Saline-sodium citrate (SSC) method was used to isolate genomic DNA (Roe 1996) and the isolated DNA was checked on 0.8% agarose gel.

The amplification for both the selected polymorphisms was done using the tetra-primer amplification refractory mutation system-polymerase chain reaction (tetra-primer ARMS-PCR) method using the sequence specific primers given in the Table 1. For *IL-8* rs4073, PCR was carried out in a thermal cycler in a total volume of 25 µl containing 3 mM $MgCl_2$, 10X PCR buffer, 1 mg/ml BSA, 50 pmol of each primer, 0.125U Taq polymerase, 10 mM of each dNTP, and 2 µl genomic DNA. The PCR cycles comprised of initial denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 60 s, 58 °C for 60 s, 72 °C for 60 s, and final extension at 72 °C for 10 min. The PCR products were observed by agarose gel electrophoresis on 3% agarose gel and visualized by UV transillumination. The TT homozygote produced 169 bp, whereas AA produced 228-bp fragment. The two outer primers produced a common 349-bp fragment in each PCR (Fig. 1a).

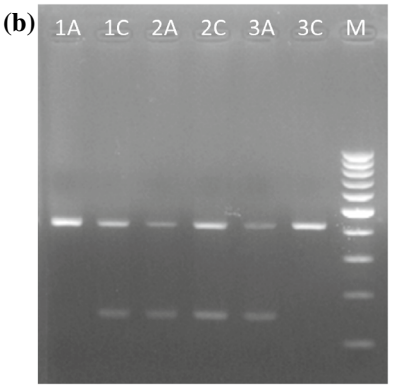
In case of *IL-10* rs1800872, the PCR reaction of 25 µl contained 2.5 mM $MgCl_2$, 10X PCR buffer, 1 mg/mL BSA, 50 pmol of each primer (either primer for C/A alleles), 0.125 U Taq polymerase, 10 mM of each dNTP and 2 µl genomic DNA. The PCR cycles consisted of initial denaturation at 95 °C for

Table 1 Primer sequences and fragment sizes for the single nucleotide polymorphisms (SNPs) genotyped using the ARMS-PCR method

Polymorphism	Primer sequence (5′–3′)	Fragment size
IL-8-251A/T (rs4073)	Forward inner primer (T allele): GTTATCTAG AAATAAAAAAGCATACAA Reverse inner primer (A allele): CTCATCTTT TCATTATGTCAGAG Forward outer primer: CATGATAGCATCTGT AATTAACTG Reverse outer primer: CACAATTTGGTGAAT TATCAAA	169 bp (T allele) 228 bp (A allele) 349 bp (two outer primers)
IL-10-592C/A (rs1800872)	Control (HGH) F 5′-CCTTCCAACCATTCC CTTA- 3′ Control (HGH) R 5′-TCACGGATTTCTGTT GTGTTTC- 3′ Common: TAACTTAGGCAGTCACCTTAGG IL10 -592 (C): ACATCCTGTGACCCCGCC TGTC IL10 -592 (A): ACATCCTGTGACCCCGCC TGTA	426 bp (internal control) 151 bp: C or A allele



Lane 1: 100bp ladder, Lane 2,4,8: homozygous wild AA genotype (349,169bp), Lane 3,6: heterozygous AT genotype (349,228,169bp), Lane 5,7: homozygous mutant TT genotype (349,228bp)



Lane 1A & 1C: homozygous wild CC genotype (426bp and 151bp), Lane 2A & 2C: heterozygous AC genotype (426 and 151bp), Lane 3A & 3C: homozygous mutant AA genotype (426 and 151bp), Lane M: 100bp ladder

Fig. 1 **a**: ARMS-PCR products of *IL-8*-251A/T rs4073 polymorphism on 3% agarose gel. Lane 1: 100 bp ladder; Lanes 2,4,8: homozygous wild AA genotype (349,169 bp); Lane 3,6: heterozygous AT genotype (349,228,169 bp); Lanes 5,7: homozygous mutant TT genotype (349,228 bp). **b:** ARMS-PCR products of *IL-10* -592C/A rs1800872 polymorphism on 3% agarose gel. Lanes 1A and 1C: homozygous wild CC genotype (426 bp and 151 bp); Lanes 2A and 2C: heterozygous AC genotype (426 and 151 bp); Lanes 3A and 3C: homozygous mutant AA genotype (426 and 151 bp); and Lane M: 100 bp ladder

4 min, followed by 35 cycles at 94 °C for 50 s, 58 °C for 40 s, 72 °C for 60 s, and final extension at 72 °C for 10 min. Separate PCR was performed out for both the alleles of one SNP and then the results were observed directly by electrophoresis on 3% agarose gels stained with EtBr and visualized by UV transillumination. If the sample has both the bands in their respective alleles, it is heterozygous genotype as both the alleles are amplified, and if only one band is present,

it is homozygous for that allele (wild or mutant). A 426-bp product indicated the internal control and 151-bp fragments indicated the CC or AA genotype (Fig. 1b).

Statistical Analysis

SPSS software version 20.0 (SPSS, Inc., Chicago, IL) and Epi Info version 3.4.7 (CDC, Atlanta, GA) were used for statistical analysis. Continuous variables were expressed as the mean ± standard deviation (SD). The difference between baseline characteristics in cases and controls was calculated by Chi square test. Multivariate logistic regression was used to analyze the association of SNP and the susceptibility to CAD adjusted for age and gender. Additionally, dominant, recessive, and over-dominant models were used to perform the association study. Stratified analysis for gender and age was also done. Odds ratio (OR) and 95% confidence interval (CI) were used for the assessment of risk factors, and $p < 0.05$ was considered as statistically significant for all tests.

Results

Baseline Characteristics

The baseline parameters of CAD patients and controls are demonstrated in Table 2. Our results show a statistically significant variation between CAD and control groups with regard to age, gender, waist to hip ratio, smoking, drinking, family history, lifestyle, occupation, diabetes, dyslipidemia, hypertension, diet, exercise, Apo A1, Apo B, VLDL, fasting blood sugar, uric acid (all p values < 0.05), LDL, TC, but not with BMI, hsCRP, HDL, triglycerides and total lipids.

Distribution of Polymorphisms

Distribution of allele frequencies of both the selected polymorphisms followed the Hardy–Weinberg Equilibrium. Genetic distribution of *IL-8* rs4073 (AA, AT and TT) between CAD patients and the controls and the allele frequencies is given in Table 3. The frequencies of AA, AT, and TT genotypes in CAD group were 39.8, 45, and 15.2%, respectively; the corresponding frequencies in the control group were 29.6, 39, and 31.4%, respectively. The frequency of the TT genotype was significantly higher in CAD patients than that of controls (31.4% *v/s.* 15.2%, $p < 0.001$). A statistically significant difference was also found in the frequency of A and T allele in patients and controls ($p < 0.001$, OR 1.71, 95% CI 1.43–2.06). We found TT genotype to be significantly higher in the case group than that in the control group ($p < 0.001$, OR 0.360, 95% CI 0.255–0.509) (Table 3).

Multiple logistic regression analysis revealed heterozygous genotype and the mutant genotype of rs4073 to be associated with a significant high risk of CAD ($p_{adj} < 0.001$, OR_{adj} 3.121, 95% CI 1.926–5.056; and $p_{adj} < 0.001$, OR_{adj} 3.116,

Table 2 Demographic characteristics of the studied population

Phenotypic traits	Controls *n* (%)	Cases *n* (%)	*p*
Age (mean ± SD; years)	50.95 ± 10.18	56.08 ± 9.55	**< 0.001***
Waist to hip ratio	0.91 ± 0.08	0.97 ± 0.16	0.001*
Blood pressure			
SBP (mmHg)	121.13 ± 9.92	133.67 ± 15.25	**0.001***
DBP (mmHg)	80.35 ± 7.07	90.81 ± 13.45	**0.046***
Gender			
Males	370 (74)	397 (79.4)	
Females	130 (26)	103 (20.6)	**0.043***
BMI (kg/m^2)			0.069
Underweight ≤ 18.5	5 (1)	13 (2.6)	
Normal weight = 18.5–24.9	265 (53)	283 (56.6)	
Overweight = 25–29.9	168 (33.6)	159 (31.8)	
Obese ≥ 30	62 (12.4)	45 (9)	
Smoking status			**< 0.001***
Nonsmoker	0	327 (65.4)	
Smoker	0	173 (34.6)	
Drinking status			**< 0.001***
NonDrinker	0	348 (69.6)	
Drinker	0	152 (30.4)	
Address			0.342
Rural	233 (46.6)	248 (49.6)	
Urban	267 (53.4)	252 (50.4)	
Family history			**< 0.001***
Nil	47 (89.4)	304 (60.8)	
Positive	53 (10.6)	196 (39.2)	
Lifestyle			**< 0.001***
Active	431 (86.2)	352 (70.4)	
Sedentary	69 (13.8)	148 (29.6)	
Occupation			**< 0.001***
Home sitter/retired	5 (1)	111 (22.2)	
Student	35 (7)	4 (0.8)	
Working	354 (70.8)	231 (46.2)	
Housewife	79 (15.8)	86 (17.2)	
Agriculturist	18 (3.6)	50 (10)	
Laborer	9 (1.8)	18 (3.6)	
Diabetes			**< 0.001***
Negative	0	353 (70.6)	
Positive	0	147 (29.4)	
Hypercholesterolemia		500 (100)	**< 0.001***
Negative	0		
Positive	0		
Hypertension		500 (100)	**< 0.001***

Table 2 (continued)

Phenotypic traits	Controls *n* (%)	Cases *n* (%)	*p*
Negative	0		
Positive	0		
Exercise			**< 0.001***
None	334 (66.8)	189 (37.8)	
Half an hour once	37 (7.4)	110 (22)	
Half an hour twice	85 (17)	78 (15.6)	
One hour once	0 (0)	28 (5.6)	
One hour twice	44 (8.8)	95 (19)	
Diet			**0.001***
Veg	425 (85)	385 (77)	
Non veg	75 (15)	115 (23)	
CPK MB	54.46 ± 45.81	34.36 ± 41.81	**< 0.001***
CPKNAC	135.76 ± 99.80	102.76 ± 79.10	**< 0.001***
APO A1	144.56 ± 26.41	121.09 ± 42.36	**< 0.001***
APO B	98.67 ± 36.54	65.94 ± 23.77	**< 0.001***
hsCRP	4.56 ± 0.20	2.35 ± 0.22	**< 0.001***
HDL-C	52.35 ± 3.12	88.06 ± 9.11	0.673
LDL-C	135.65 ± 22.33	76.54 ± 32.50	**0.005***
VLDL	48.57 ± 21.08	34.83 ± 20.74	**0.043***
FBG	82.73 ± 19.30	111.56 ± 39.59	**0.016***
URIC ACID	9.80 ± 3.45	6.26 ± 5.10	**0.006***
TC	275.76 ± 53.49	144.95 ± 37.22	**0.045***
TRIGLYCERIDES	198.23 ± 82.45	145.37 ± 65.62	0.468
TL	512.18 ± 116.05	436.05 ± 112.36	0.353

Statistically significant values are given in bold ($p < 0.05$)

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *hsCRP* high-sensitivity C-reactive protein (hs-CRP), *TC* total cholesterol, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low density lipoprotein cholesterol, *VLDL* very low density lipoprotein, *FBG* fasting blood glucose, *TL* total lipids

95% CI 1.952–4.973, respectively) after adjustment for confounding factors such as age and gender. Risk association with CAD was seen in all the three models, viz. dominant, recessive, and over-dominant models ($p_{adj} = 0.032$, OR_{adj} 1.526, 95% CI 1.037–2.246; $p_{adj} < 0.001$, OR_{adj} 3.118, 95% CI 2.043–4.760; and $p_{adj} = 0.015$, OR_{adj} 1.592, 95% CI 1.095–2.317, respectively) (Table 3). Further, stratified analysis on the basis of gender was done, as shown in Table 4. The AT and TT each significantly increased the risk of CAD in males ($p_{adj} < 0.001$, OR_{adj} 3.693, 95% CI 2.031–6.716; and $p_{adj} < 0.001$, OR_{adj} 3.288, 95% CI 1.848–5.851, respectively), whereas only the TT increased the CAD risk in females ($p_{adj} = 0.010$, OR_{adj} 2.867, 95% CI 1.284–6.404) (Table 4). Stratification of the samples was also done on the basis of age i.e. below 40 years and above 40 years (Table 5). Both the heterozygous and the homozygous mutants showed strong risk associations with the CAD risk

Table 3 Genotype and allele distributions for the patient and the control group

Genotypes	Controls	Cases	Crude analysis		Multiple logistic regression analysis	
	500(%)	500(%)	*p*	OR (95% CI)	p_{adj}	OR_{adj} (95% CI)
IL-8-251A/T (rs4073)						
AA	148 (29.6)	199 (39.8)	(Ref.)		(Ref.)	
AT	195 (39)	225 (45)	0.295	0.858 (0.644–1.243)	**< 0.001***	**3.121 (1.926–5.056)**
TT	157 (31.4)	76 (15.2)	**< 0.001***	**0.360 (0.255–0.509)**	**< 0.001***	**3.116 (1.952–4.973)**
Alleles						
A	491 (49.1)	623 (62.3)	(Ref.)			
T	509 (50.9)	377 (37.7)	**< 0.001***	**1.712 (1.43–2.06)**		
Dominant model	352 (70.4)	301 (60.2)	**0.001***	**0.636 (0.489–0.827)**	**0.032***	**1.526 (1.037–2.246)**
Recessive model	157 (31.4)	76 (15.2)	**< 0.001***	**0.392 (0.288-0.533)**	**< 0.001***	**3.118 (2.043-4.760)**
Overdominant model	195 (39)	225 (45)	0.055	0.781 (0.608–1.005)	**0.015***	**1.592 (1.095–2.317)**
IL-10 -592C/A (rs1800872)						
CC	233 (46.6)	166 (33.2)	(Ref.)		(Ref.)	
CA	176 (35.2)	300 (60)	**< 0.001***	**2.393 (1.822–3.142)**	0.630	1.168 (0.621–2.197)
AA	91 (18.2)	34 (6.8)	**0.004***	**0.524 (0.337-0.815)**	**< 0.001***	**4.106 (2.160–7.806)**
Alleles						
C	642 (64.2)	632 (63.2)	(Ref.)			
A	358 (35.8)	368 (36.8)	0.64	0.96 (0.79–1.15)		
Dominant model	267 (53.4)	334 (66.8)	0.051	0.870 (0.223–0.579)	**< 0.001***	**0.370 (0.253–0.541)**
Recessive model	91 (18.2)	34 (6.8)	**0.008***	**0.448 (0.248–0.812)**	**0.008***	**2.230 (1.232–4.037)**
Overdominant model	176 (35.2)	300 (60)	0.051	0.561 (0.453-1.326)	**< 0.001***	**3.624 (2.450-5.358)**

Statistically significant values are given in bold ($p < 0.05$)

Ref. reference, *OR* odds ratio, *CI* confidence interval, % frequency

in subjects below 40 years of age ($p_{adj} = 0.039$, OR_{adj} 5.052, 95% CI 1.081–23.602; and $p_{adj} = 0.025$, OR_{adj} 5.533, 95% CI 1.239–24.704, respectively). Also, in the subjects above 40 years of age, heterozygous and the homozygous mutant genotypes increased the CAD risk ($p_{adj} = 0.000$, OR_{adj} 2.964, 95% CI 1.747–5.027; and $p_{adj} < 0.000$, OR_{adj} 2.859, 95% CI 1.716–4.762, respectively).

Genetic distribution of *IL-10* rs1800872 (CC, CA and AA) between CAD patients and the controls and the allele frequencies is given in Table 3. The frequencies of CC, CA, and AA genotypes for cases were 33.2, 60, and 6.8%, respectively;

Table 4 Genotype and allele distributions for the gender stratified analysis for the polymorphisms after multiple logistic regression analysis

Polymorphisms	Male				Female			
Genotypes	Controls 370 (%)	Cases397 (%)	p_{adj}	OR_{adj} (95% CI)	Controls 130 (%)	Cases 103 (%)	p	OR_{adj} (95% CI)
IL-8-251A/T (rs4073)								
AA	107 (28.9)	164 (41.3)	(Ref.)		41 (31.5)	35 (34)	(Ref.)	
AT	147 (39.7)	179 (45.1)	**< 0.001***	**3.693 (2.031–6.716)**	48 (36.9)	46 (44.7)	0.061	2.189 (0.963–4.976)
TT	116 (31.4)	54 (13.6)	**< 0.001***	**3.288 (1.848–5.851)**	41 (31.5)	22 (21.3)	**0.010***	**2.867 (1.283–6.404)**
Alleles								
A	361	507	(Ref.)		130	116	(Ref.)	
T	379	287	**< 0.001***	**1.850 (1.500–2.229)**	130	90	0.589	1.208 (0.609–2.397)
Dominant model	263 (71.1)	233 (58.7)	**0.029***	**1.688 (1.056–2.698)**	89 (68.5)	68 (66)	(Ref.)	
Recessive model	116 (31.4)	54 (13.6)	**< 0.001***	**3.468 (2.049–5.871)**	41 (31.5)	22 (21.3)	**0.010***	**2.529 (1.243–5.147)**
Overdominant model	147 (39.7)	179 (45.1)	0.095	1.469 (0.935–2.307)	48 (36.9)	46 (44.7)	**< 0.001***	**3.468 (2.049–5.871)**
IL-10 -592C/A (rs1800872)								
CC	157 (42.4)	133 (33.5)	(Ref.)		76 (58.5)	33 (32)	(Ref.)	
CA	138 (37.3)	238 (59.9)	0.053	2.005 (0.990–4.063)	38 (29.2)	62 (60.2)	**0.020***	**0.172 (0.039–0.757)**
AA	75 (20.3)	26 (6.5)	**< 0.001***	**5.821 (2.831–11.970)**	16 (12.3)	8 (7.8)	0.915	0.915 (0.212-3.958)
Alleles								
C	452 (61)	504 (63.7)	(Ref.)		190 (73)	128 (60.9)	(Ref.)	
A	288 (39)	290 (36.3)	0.332	1.107 (0.900–1.361)	70 (27)	78 (39.1)	**0.011***	**0.604 (0.408–0.895)**
Dominant model	213 (57.6)	264 (66.5)	**0.003***	**0.504 (0.319–0.795)**	54 (41.5)	70 (68)	**< 0.001***	**0.186 (0.091–0.380)**
Recessive model	295 (79.7)	371 (93.5)	**< 0.001***	**0.286 (0.147–0.553)**	114 (87.7)	95 (92.2)	0.200	2.474 (0.619–9.888)
Overdominant model	138 (37.3)	238 (59.9)	**< 0.001***	**3.405 (2.121–5.467)**	38 (29.2)	62 (60.2)	**0.001***	**4.218 (2.102–8.463)**

Statistically significant values are given in bold ($p < 0.05$)

Ref. reference, *OR* odds ratio, *CI* confidence interval, % frequency

Table 5 Genotype and allele distributions for the age stratified analysis for the polymorphisms after multiple logistic regression analysis

Polymorphisms	Below 40 years				Above 40 years			
Genotypes	Controls 379 (%)	Cases 27 (%)	p_{adj}	OR_{adj} (95% CI)	Controls 121 (%)	Cases 473 (%)	p_{adj}	OR_{adj} (95% CI)
IL-8-251A/T (rs4073)								
AA	113 (29.8)	10 (37)	(Ref.)		35 (28.9)	189 (40)	(Ref.)	
AT	154 (40.6)	15 (55.6)	**0.039***	**5.052 (1.081–3.602)**	41 (33.9)	210 (44.4)	**< 0.001***	**2.964** (1.747–5.027)
TT	112 (29.6)	2 (7.4)	**0.025***	**5.553 (1.239–4.704)**	45 (37.2)	74 (15.6)	**< 0.001***	**2.857 (1.716–4.732)**
Alleles								
A	380	35	(Ref.)		118	588	(Ref.)	
T	378	19	**0.037***	**1.83 (1.00–3.39)**	131	358	**< 0.001***	**1.82 (1.360–2.440)**
Dominant model	266 (70.2)	17 (63)	0.419	1.398 (0.620–3.151)	86 (71.1)	301 (60.2)	**0.049***	**1.558 (1.001–2.424)**
Recessive model	112 (29.6)	2 (7.4)	**0.024***	**5.330 (1.240–2.905)**	45 (37.2)	74 (15.6)	**< 0.001***	**2.907 (1.844–4.582)**
Overdominant model	154 (40.6)	15 (55.6)	0.131	1.834 (0.835–4.028)	41 (33.9)	210 (44.4)	0.052	1.525 (0.996–2.335)
IL-10 -592C/A (rs1800872)								
CC	158 (41.7)	9 (33.3)	(Ref.)		75 (62)	157 (33.2)	(Ref.)	
CA	145 (38.3)	15 (55.6)	0.580	1.458 (0.383–5.544)	31 (25.6)	285 (60.3)	0.644	1.177 (0.588–2.356)
AA	76 (20.1)	3 (11.1)	0.141	2.595 (0.728–9.249)	15 (12.4)	31 (6.6)	**< 0.001***	**5.049 (2.414–10.561)**
Alleles								
C	461 (60.8)	33 (61.1)	(Ref.)		181 (75.4)	599 (63)	(Ref.)	
A	297 (39.2)	21 (38.9)	0.657	1.012 (0.574–1.783)	61 (25.6)	347 (27)	**< 0.001***	**0.581 (0.422–0.800)**
Dominant model	221 (58.3)	18 (66.7)	0.421	0.712 (0.311–1.628)	46 (38)	316 (66.8)	**< 0.001***	**0.318 (0.208-0.484)**
Recessive model	303 (79.9)	24 (88.9)	0.226	0.498 (0.146–1.699)	106 (87.6)	442 (93.4)	**0.014***	**0.429 (0.219–0.840)**
Overdominant model	145 (38.3)	15 (55.6)	0.089	1.984 (0.902–4.366)	31 (25.6)	285 (60.3)	**< 0.001***	**4.402 (2.789-6.947)**

Statistically significant values are given in bold ($p < 0.05$)

Ref. reference, *OR* odds ratio, *CI* confidence interval, % frequency

the corresponding frequencies in the control group were 46.6, 35.2, and 18.2%, respectively. The mutant genotype AA imparted risk toward CAD with OR_{adj} 4.106, 95% CI (2.160–7.806) and a highly significant p_{adj} after adjusting for confounders. Risk associations with CAD were seen in recessive and over-dominant models ($p_{adj} = 0.008$, OR_{adj} 2.230, 95% CI 1.232–4.037; $p_{adj} < 0.001$, OR_{adj} 3.624, 95% CI 2.450–5.358; and $p_{adj} < 0.001$*, respectively) (Table 3).

Gender-stratified analysis showed that only the mutant genotype AA is associated with CAD risk in males ($p_{adj} < 0.001$, OR_{adj} 5.821, 95% CI 2.831–11.970), whereas risk association in females was observed only under the overdominant model with $p_{adj} < 0.001$, OR_{adj} 4.218, 95% CI 2.102–8.463 (Table 4). Stratification of the samples was also done on the basis of age, i.e., below 40 years and above 40 years (Table 5). Risk association was seen only in subject above 40 years of age. The mutant AA genotype conferred a strong risk association with the CAD in subjects above 40 years of age with a significant $p_{adj} < 0.001$, OR_{adj} 5.049, and 95% CI 2.414–10.561. Upon analyzing the models of inheritance, risk association was seen in overdominant model with $p_{adj} < 0.001$, OR_{adj} 4.402 and 95% CI 2.789–6.947.

Discussion

CAD is becoming an epidemic with an alarming increase in both the developed and developing countries. CAD is well known to be a multifactorial disease i.e. having a genetic background along with numerous other contributing factors (Hansson 2005; Yiannakouris et al. 2014). However, not all the individuals exposed to the risk factors develop CAD, and also, it is not necessary that a person with genetic changes necessarily will have CAD, suggesting that there is interplay between all the factors that ultimately decide the disease occurrence and penetrance. The roles of genes and genetic polymorphisms have attracted the attention of researchers worldwide. In the past few years, research has been carried out to decipher the function of inflammation both in the atherosclerotic plaque and in the vessel wall (Libby et al. 2011; Hansson et al. 2015). This has led to the discovery of the role of cytokines in CAD, and it is this balance between anti-inflammatory and pro-inflammatory stimuli, which is important in the development and progression of the disease.

The −251 position (rs4073) of the *IL-8* gene and -592 position (rs1800872) of the *IL-10* gene, both located in the promoter region, were chosen for genotyping with ARMS-PCR. Till date none of the study has been conducted on the North Indian population to find the association of *IL-8* SNP and we are first to document it. One thousand individuals (500 controls and 500 angiographically proven CAD patients) of North Indian decent were genotyped to explore the association of both *IL-8* and *IL-10* polymorphisms with CAD.

Results revealed a significant association of *IL-8* with CAD in our population. The previously available literature states contradicting results. Two studies carried out on Chinese population states the association of this polymorphism with CAD. In study by (Zhang et al. 2011) on 675 patients and 636 controls revealed a strong association with $p = 0.004$, OR 1.30 and 95% CI (1.12–1.53). Also they reported the correlation of increased serum levels of IL-8 in MI subjects revealing

that the polymorphism might affect IL-8 expression. Similarly (Zhang, R., et al., IL-8-251A/T polymorphism contributes to CAD susceptibility in a Chinese population. Genetics and molecular research: GMR (2017) in his study on 217 patients and 245 control subjects showed that the heterozygous and the mutant genotypes were at an elevated risk compared to the wild ones (OR 1.59, 95% CI (1.01–2.57), and $p=0.040$; and OR 2.06, 95% CI (1.21–3.52), and $p=0.005$, respectively). Also they analyzed the different genetic models and reported the risk association under dominant model with (OR 1.75, 95% CI (1.13–2.73) and $p=0.008$ and also under recessive model with a risk OR 1.54, 95% CI (1.02–2.37) and $p=0.040$. These results stand in support of our study as our results also report a risk association in both dominant and recessive models with significant p values (Table 3). Also (Zhang, R., et al., *IL-8*-251A/T polymorphism contributes to coronary artery disease susceptibility in a Chinese population. Genetics and molecular research: GMR 2017) performed the Chi square tests and revealed significant differences between the two groups *w.r.t.* gender, BMI, hypertension, diabetes smoking, TC and LDL-C which is in consensus for our results also (Table 2). A study on Swedish population by (Velásquez et al. 2014) also showed risk association under additive (OR 1.2) and recessive models (OR 1.3). However, (Vogiatzi et al. 2008) in his study on Caucasian Greeks population, enrolled 241 CAD patients and 157 controls and concluded that the polymorphism is linked to a reduced risk among patients with ACS, whereas no significant difference could be observed in the CAD group. Yang et al. (2015), however, showed no association of the polymorphism and the risk of disease in his study on 410 patients and 410 controls belonging from China. Similarly, (Ren and She 2015) also supported the above said observations and found no association in the Chinese population.

Analyzing the results obtained for *IL-10* rs1800872 -592C/A polymorphism revealed a significant association with the mutant AA genotype with an OR 4.106. Studies have been conducted in subjects from different ethnicities worldwide suggesting its diverse role in the pathogenesis of CAD. The PROSPER study conducted in Ireland, Scotland, and Netherlands on 5804 subjects revealed a significant risk association with the coronary events (OR 1.21, 95% CI (1.04–1.36) (Trompet et al. 2007). Similarly in study carried out on Mexican patients (389 ACS patients and 302 healthy controls) a highly significant $p=0.000$ with an OR 1.48 was observed (Fragoso et al. 2011). Another epidemiological study carried on 1652 Chinese individuals, reports (AA vs. AC + CC genotype, OR 1.60, 95% CI (1.060–2.390) significantly associated with ischemic stroke even after controlling for covariates (Xie et al. 2013). Also in a case–control study conducted on 249 patients and 132 unaffected controls belonging to China also revealed the risk of CAD in the patients with A gene (AA + CA) was 2.449 times greater than those without ($p=0.012$) (Jin et al. 2013). (Yu et al. 2012) conducted a study in a Korean population (313 control and 173 patients), and reported that the *IL-10* -592 C/A polymorphism might be associated with ischemic heart disease. In context to North Indian population, (Madeshiya et al. 2017) carried out a study on 384 patients and 386 controls and found the mutant allele A of *IL-10* –592C > A polymorphism to be higher among the cases (40.1%) when compared to controls (34.2%) and the dominant model showed an association with the disease with OR 1.35, 95% CI (1.01–1.80) and $p=0.04$. But

in our work we observed only a marginal difference in the minor allele frequency among controls and cases (35.8% v/s 36.8%) and a protective association was seen in dominant model whereas risk association was reported in recessive model showing that two copies of the mutant allele are required for the disease manifestation.

In contrast to our findings, the meta-analysis by (Xuan, Y., et al., Association between 3 IL-10 Gene polymorphisms and cardiovascular risk of disease: systematic review with meta-analysis and trial sequential analysis. Medicine 2016) showed no relationship of this polymorphism and CVD risk. Similarly, no significant association between the *IL-10* -592 C/A polymorphism and CAD risk was observed by (Koch et al. 2001; Wang et al. 2015; Yao et al. 2016). However a study carried out in Kolkata, India (Biswas et al. 2014) on 500 MI patients and 500 controls revealed no association of mean plasma levels of IL-10 and the three genotypes but revealed a protective association with an OR 0.697 and $p = 0.014$ (Biswas et al. 2014). Such differences in results might occur due to the different populations being studied, different criteria for the selection of patients and control subjects, difference in the sample sizes and different techniques used for genotyping the samples.

Both the selected polymorphisms are located in the promoter region of the gene and influence the binding of transcription factors and thus are able to regulate the gene expression (Hacking et al. 2004) (Biswas et al. 2014; Ohyauchi et al. 2005). Many studies document the promoter region polymorphism regulating the transcription activity and hence, the serum levels (Hull et al. 2000) (Ohyauchi et al. 2005; Taguchi et al. 2005; Hildebrand et al. 2007). But we have not done the study at the expression level and cannot say more to it.

Our study also revealed a gender-specific association of *IL-8* rs4073 with CAD. Strong associations are observed in case of males in dominant and recessive models and in females in the homozygous mutant genotype and under recessive model after multiple logistic regression (Table 4). (Velásquez et al. 2014) conducted a study on Swedish population and found only the male specific increased risk of myocardial infarction. In case of *IL-10* rs1800872 C/A, risk association of the SNP was observed only in males with homozygous mutant genotype. Till date no study has been conducted to find a gender-specific link between the *IL-8* and *IL-10* polymorphisms and CAD, and ours to the best of our knowledge is the first to report it. The novel results point toward the roles of some sex-related entities or the gender-specific hormones and their interaction with *IL-8*, which lead to CAD, but the exact phenomenon yet need to be deciphered, and a link needs to be established.

We stratified our population on the basis of age, viz. below 40 years and above 40 years, and strong risk association was seen with *IL-8* rs4073 in below 40-year group after adjusting for the confounders. For *IL-10* rs1800872 C/A, highly significant risk association was reported in the subjects above 40 years of age. India is a developing country and is undergoing a rapid socioeconomic shift along with a changing lifestyle, modernization, urbanization, and industrialization, which has automatically led to an increase in the number of people affected with diabetes, hypertension, metabolic syndrome, and dyslipidemia (Gupta 2004) (Mohan et al. 2007; Prabhakaran et al. 2007). Epidemiologic studies on the premature CAD group in Indians have revealed dyslipidemia, diabetes, smoking, and lack of physical activity to be the major players in the predisposition of the individuals to CAD (Mohan

et al. 2001) (Gupta et al. 2002) (Goel et al. 2003). Also, parental history of premature CAD is found to be an imperative, consistent, independent, and global determinant of CAD risk in future (Chow, C.K., et al., Parental History and Myocardial Infarction Risk Across the World 2011).

Strengths and Limitations

A major strength of this study is that it was performed in a well-characterized population of individuals. All the patients recruited were angiographically confirmed. Only those subjects were taken as controls that underwent a close examination by the doctor and were recommended as controls. Strict inclusion and exclusion criteria were followed for the recruitment of both the case and control selection, and it was ensured that all the recruited subjects belong to the North Indian descent. Our study documents the allelic and genotypic frequencies, and their associations with CAD in the North Indian population and also gender- and age-specific associations are shown for the first time. However, there are certain limitations to our study. The serum levels of both IL-8 and IL-10 levels were not quantified. We have genotyped only one polymorphisms of both the genes, but there may be many other polymorphisms which may be imparting risk of disease; and also linkage studies could have been done so as to elucidate the complete involvement of these polymorpshisms in the disease pathogenesis. Moreover, the study has been done at the genetic level only, but future studies at transcriptional and translational levels are required to validate and authenticate the link between the polymorphism and the disease. Gender-specific associations were observed, thereby demanding the quantification of various sex-specific hormones which would have led to a better interpretation of the results augmenting the hormonal data. Future studies on large sample size, multiple SNPs of both the genes, and the linkage studies are warranted that will likely open ways for a clearer and more robust risk assessment of CAD in the North Indian population.

Conclusion

To conclude, we can state that ours is a novel study revealing that *IL-8* rs4073 -251A/T and *IL-10* rs1800872 -592C/A have a significant association with the CAD risk in the North Indian population and that the risk is age and gender dependant also. Substantial variations are seen in the SNP studies across the world making it a necessity to perform the SNP studies on the worldwide level so that the exact genotypic and allelic frequencies in a particular population can be documented, and hence, the data can therefore be used in new arena of personalized medicine, identification of genetic and molecular markers, and also in detecting individuals with high and low risk. Also the inter and intragenic interactions are still not clear and studies need to be done to find out the various interconnecting pathways which will allow us to deduce the exact mechanism of the development and the progression and of the disease. Our results point toward the need of adapting a healthy lifestyle,

healthy diet, and routine physical activity so as to overcome the premature CAD risk. The factors that are well within our control and can be modified should first be targeted. In summary, we state that these polymorphisms in the promoter region are associated with CAD susceptibility in the North Indian population. Future research is needed to better elucidate the functional significance of the polymorphisms, and hence, to clarify the mechanism that explains epidemiologic associations involving these pro- and anti-inflammatory genes.

Acknowledgements All the authors are grateful to the participants (control subjects and patients) involved in this study.

Compliance with Ethical Standards

Conflict of interest All authors declared that they have no competing interests.

References

Anand SS et al (2000) Differences in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic groups (SHARE). Lancet 356(9226):279–284

Anguera I et al (2002) Elevation of serum levels of the anti-inflammatory cytokine interleukin-10 and decreased risk of coronary events in patients with unstable angina. Am Heart J 144(5):811–817

Biswas S, Ghoshal PK, Mandal N (2014) Synergistic effect of anti and pro-inflammatory cytokine genes and their promoter polymorphism with ST-elevation of myocardial infarction. Gene 544(2):145–151

Boisvert WA, Curnss LK, Terkeltaub RA (2000) Interleukin-8 and its receptor CXCR2 in atherosclerosis. Immunol Res 21(2–3):129–137

Chow CK et al (2011) Parental History and Myocardial Infarction Risk Across the World. J Am Coll Cardiol. https://doi.org/10.1016/j.jacc.2010.07.054

Enas EA et al (2001) Coronary artery disease in women. Indian Heart J 53(3):282–292

Fernández RP, Kaski JC (2002) Interleukin-10 and coronary disease. Rev Esp Cardiol 55(07):738–750

Fragoso JM et al (2011) Alleles and haplotypes of the interleukin 10 gene polymorphisms are associated with risk of developing acute coronary syndrome in Mexican patients. Cytokine 55(1):29–33

Gaziano TA, Gaziano JM (2008) Epidemiology of cardiovascular disease. In: Fauci AS, Braunwald E, Kasper DL (eds) Harrison's Principals of Internal Medicine, 17th edn. McGraw-Hill, New York, p 9

Gerszten RE et al (1999) MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature 398(6729):718–723

Goel PK et al (2003) A tertiary care hospital-based study of conventional risk factors including lipid profile in proven coronary artery disease. Indian Heart J 55(3):234–240

Gupta R (2004) Trends in hypertension epidemiology in India. J Hum Hypertens 18(2):73–78

Gupta R et al (2002) Prevalence of coronary heart disease and risk factors in an urban Indian population: Jaipur Heart Watch-2. Indian Heart J 54(1):59–66

Hacking D et al (2004) Increased in vivo transcription of an IL-8 haplotype associated with respiratory syncytial virus disease-susceptibility. Genes Immun 5(4):274

Halvorsen B et al (2005) Interleukin-10 enhances the oxidized LDL-induced foam cell formation of macrophages by antiapoptotic mechanisms. J Lipid Res 46(2):211–219

Hansson GK (2005) Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 352(16):1685–1695

Hansson G, Libby P, Tabas I (2015) Inflammation and plaque vulnerability. J Intern Med 278(5):483–493

Heeschen C et al (2003) Serum level of the antiinflammatory cytokine interleukin-10 is an important prognostic determinant in patients with acute coronary syndromes. Circulation 107(16):2109–2114

Hildebrand F et al (2007) Association of IL-8-251A/T polymorphism with incidence of acute respiratory distress syndrome (ARDS) and IL-8 synthesis after multiple trauma. Cytokine 37(3):192–199

Hull J, Thomson A, Kwiatkowski D (2000) Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. Thorax 55(12):1023–1027
Jin H, Wang Y, Xu L (2013) Association of interleukin 10 gene-592C/A polymorphism with coronary artery disease. Zhonghua yi xue yi chuan xue za zhi = Zhonghua yixue yichuanxue zazhi = Chinese. J Med Genet 30(6):724–728
Kanda T et al (1996) Interleukin-8 as a sensitive marker of unstable coronary artery disease. Am J Cardiol 77(4):304–307
Koch W et al (2001) Interleukin-10 and tumor necrosis factor gene polymorphisms and risk of coronary artery disease and myocardial infarction. Atherosclerosis 159(1):137–144
Libby P, Ridker PM, Hansson GK (2011) Progress and challenges in translating the biology of atherosclerosis. Nature 473(7347):317–326
Madeshiya AK et al (2017) Association of IL-10 gene (−1082A > G, −819C > T and −592C > A) polymorphism and its serum level with metabolic syndrome of north Indian subjects. J Genet 96(1):53–64
Marousi S et al (2011) Functional inflammatory genotypes in ischemic stroke: could we use them to predict age of onset and long-term outcome? Stroke Res Treat. https://doi.org/10.4061/2011/792923
Meuwissen M et al (2004) Role of plaque inflammation in acute and recurrent coronary syndromes. Neth Heart J 12(3):106
Mohan V et al (2001) Prevalence of coronary artery disease and its relationship to lipids in a selected population in South India: the Chennai Urban Population Study (CUPS No. 5). J Am Coll Cardiol 38(3):682–687
Mohan V et al (2007) Epidemiology of type 2 diabetes: Indian scenario. Indian J Med Res 125(3):217
Mohebbatikaljahi H et al (2009) Study of interleukin-10 promoter region polymorphisms (−1082A/G, −819T/C and −592A/C) in type 1 diabetes mellitus in Turkish population. J Genet 88(2):245–248
Morris SW et al (1992) Assignment of the genes encoding human interleukin-8 receptor types 1 and 2 and an interleukin-8 receptor pseudogene to chromosome 2q35. Genomics 14(3):685–691
Ohyauchi M et al (2005) The polymorphism interleukin 8–251 A/T influences the susceptibility of Helicobacter pylori related gastric diseases in the Japanese population. Gut 54(3):330–335
Prabhakaran D et al (2007) Differences in the prevalence of metabolic syndrome in urban and rural India: a problem of urbanization. Chronic Illness 3(1):8–19
Ren B, She Q (2015) Study on the association between IL-1β, IL-8 and IL-10 gene polymorphisms and risk of coronary artery disease. Int J Clin Exp Med 8(5):7937
Rigamonti E et al (2008) Induction of CXCR2 receptor by peroxisome proliferator-activated receptor γ in human macrophages. Arterioscler Thromb Vasc Biol 28(5):932–939
Roe BA, Crabtree JS, Khan AS (1996) DNA isolation and sequencing, vol 11. Wiley, Hoboken
Saxena M et al (2012) An interleukin-10 gene promoter polymorphism (−592A/C) associated with type 2 diabetes: a North Indian study. Biochem Genet 50(7–8):549–559
Simonini A et al (2000) IL-8 is an angiogenic factor in human coronary atherectomy tissue. Circulation 101(13):1519–1526
Srikanth Babu BM et al (2012) Cytokine gene polymorphisms in the susceptibility to acute coronary syndrome. Genet Test Mol Biomark 16(5):359–365
Taguchi A et al (2005) Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan. Cancer Epidemiol Prev Biomark 14(11):2487–2493
Trompet S et al (2007) Genetic variation in the interleukin-10 gene promoter and risk of coronary and cerebrovascular events. Ann N Y Acad Sci 1100(1):189–198
Velásquez IM et al (2014) Association of interleukin 8 with myocardial infarction: results from the Stockholm Heart Epidemiology Program. Int J Cardiol 172(1):173–178
Vogiatzi K et al (2008) Interleukin 8 and susceptibility to coronary artery disease: a population genetics perspective. J Clin Immunol 28(4):329–335
Wang N et al (1996) Interleukin 8 is induced by cholesterol loading of macrophages and expressed by macrophage foam cells in human atheroma. J Biol Chem 271(15):8837–8842
Wang S et al (2015a) Effect of IL-1β, IL-8, and IL-10 polymorphisms on the development of myocardial infarction. Genet Mol Res 14(4):12016–12021
Wang B-J et al (2015b) Association between three interleukin-10 gene polymorphisms and coronary artery disease risk: a meta-analysis. Int J Clin Exp Med 8(10):17842
Xie G et al (2013) Relationship of serum interleukin-10 and its genetic variations with ischemic stroke in a Chinese general population. PLoS ONE 8(9):e74126

Xuan Y et al (2016) Association between 3 IL-10 Gene polymorphisms and cardiovascular disease risk: systematic review with meta-analysis and trial sequential analysis. Medicine. https://doi.org/10.1097/MD.0000000000002846

Yang H et al (2015) Association of interleukin gene polymorphisms with the risk of coronary artery disease. Genet Mol Res 14(4):12489–12496

Yao X-L et al (2016) Association between IL-10 gene promoter polymorphisms and the development of coronary artery disease in a Chinese population. Int J Clin Exp Pathol 9(5):5673–5679

Yiannakouris N et al (2014) Additive influence of genetic predisposition and conventional risk factors in the incidence of coronary heart disease: a population-based study in Greece. BMJ Open 4(2):e004387

Yu G-I et al (2012) Association of promoter region single nucleotide polymorphisms at positions − 819C/T and − 592C/A of interleukin 10 gene with ischemic heart disease. Inflamm Res 61(8):899–905

Yue T-L et al (1993) Interleukin-8 is chemotactic for vascular smooth muscle cells. Eur J Pharmacol 240(1):81–84

Yue T-L et al (1994) Interleukin-8. A mitogen and chemoattractant for vascular smooth muscle cells. Circ Res 75(1):1–7

Zakynthinos E, Pappa N (2009) Inflammatory biomarkers in coronary artery disease. J Cardiol 53(3):317–333

Zhang R et al (2017) IL-8-251A/T polymorphism contributes to coronary artery disease susceptibility in a Chinese population. Genet Mol Res. https://doi.org/10.4238/gmr16018224

Zhang X et al (2011) Interleukin-8 gene polymorphism is associated with acute coronary syndrome in the Chinese Han population. Cytokine 56(2):188–191

Affiliations

Naindeep Kaur[1] · Jagtar Singh[1] · S. Reddy[2]

✉ Jagtar Singh
jagtar@pu.ac.in

[1] Department of Biotechnology, Panjab University, Chandigarh 160014, India

[2] Department of Cardiology, Postgraduate Institute of Medical Education and Research, Chandigarh, India