Association of Polymorphisms at *LDLR* Locus with Coronary Artery Disease Independently from Lipid Profile

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Abstract- Coronary artery disease (CAD) is the leading cause of mortality in many parts of the world. Genome-wide association studies (GWAS) have identified several genetic variants associated with CAD in Low-density lipoprotein receptor *(LDLR*) locus. This study was evaluated the possible association of genetic markers at *LDLR* locus with CAD irrespective to lipid profile and as well as the association of these SNPs with severity of CAD in Iranian population. Sequencing of 2 exons in *LDLR* gene (Exon 2, 12) and part of intron 30 of *SMARCA4* gene include rs1122608, was performed in 170 Iranian patients angiographically confirmed CAD and 104 healthy controls by direct sequencing. Sullivan's scoring system was used for determining the severity of CAD in cases. Our results showed that homozygote genotypes of rs1122608 (P<0.0001), rs4300767 (P<0.005) and rs10417578 (p<0.007) SNPs have strong protective effects on the CAD. In addition, we found that rs1122608 (GT or TT) was at higher risk of three vessel involvement compared to single vessels affecting (P=0.01).

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Introduction

Coronary artery disease (CAD) is the most common type of heart disease and leading cause of morbidity and mortality among men and women of almost all racial and ethnic groups (1,2). It is a complex disease influenced by many different environmental and heritable risk factors (3,4). However, many of the current traditional and novel risk factors such as lipoprotein-cholesterol, elevated low density lipoprotein cholesterol, high blood pressure, smoking, obesity and diabetes are unable to fully predict who is at risk for high density (5).

CAD is estimated that be heritable between 40 to 60 percent, yet the genetic mechanisms underlying it are poorly understood (6,7). CAD progression involves a complex series of events involving multiple biological pathways and genes (8,9). Genetic epidemiologic studies have suggested that certain genetic variants; including polymorphisms in the different genes are associated with an increased prevalence of CAD in high- or low-risk subjects. These variations may each have a small effect but cumulatively influence a sizable proportion of the myocardial infarction (MI) and CAD risk (10,11). Elucidating the genetic determinants would improve risk assessment and provide better measures for prevention and treatment.

There are several gene variants described at *LDLR* locus which some of them show the strongest association with *LDL*-cholesterol (*LDL-C*) levels among different populations (12,13). The rs2228671 in exon 2 *LDLR* has been shown to have a strong association with *LDL-C* level; the T allele of this SNP is associated with decreased *LDL-C* and in consequence with the decreased CAD risks (14). Thers688 within exon 12 of *LDLR* gene has been reported to alter the *LDL-C* level (T allele) and also splicing efficiency (15). Finally, several genome wide association studies have been reported that G allele of rs1122608 in intron 30 of *SMARC1* gene adjusted to *LDLR* gene, is associated with the high level

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of *LDL-C* and in consequence with increased risk of MI (12,13).

It is noteworthy that the allele frequencies and association analysis can vary widely between European Caucasians, African, Americans, Asians, Hispanics, and other ethnic groups and associations found in one ethnic group may not translate to the same association in other ethnic groups (16).

Coronary angiography is the gold standard to evaluate the severity of CAD. In other way, association of the single nucleotide polymorphisms with the severity of coronary arteries has been demonstrated (17-19). Hence, we pursued association between *LDLR* locus polymorphisms with severity of CAD by accredited coronary scoring systems and evaluation of the possible direct associations of these SNPs with CAD irrespective to *LDL-C.*

Materials and Methods

We enrolled 274 participants (170 cases and 104 controls), who undergone a coronary angiography examination from Shahid Rajaie Hospital, Tehran, Iran.

Inclusion criteria for the cases were: 1) Age at diagnosis of CAD in patients, 55 years or younger in men and 65 or younger in women, 2) At least 50% of stenosis in one of major epicardial coronary arteries which have been confirmed by angiography, and also absence of other diseases. Our control samples were selected among those who had undergone angiography for reasons other than CAD and have normal coronary arteries (17,20).

All participants have been asked for complete clinical history such as history of MI, diabetes, hypercholesterolemia, hypertension, smoking, BMI. Information on demographic characteristics, lifestyle behaviors, diet and so on was obtained using a structured questionnaire. The study protocol was approved by the Ethics Committee of

University of Social Welfare and Rehabilitation Sciences for Medical Research, Tehran, Iran and informed written consent was obtained from all subjects.

Genotyping

Genomic DNA was extracted from nucleated fresh blood using the salting-out method (21). The genomic regions of selected SNPs were amplified by touchdown polymerase chain reaction (PCR). Three SNPs tagging the *SMARCA4-LDLR* gene locus were selected (i.e., rs688, rs2228671, and rs1122608). For rs688 and rs2228671 polymorphisms, genotyping was performed as follows: briefly, the rs688 polymorphism (*LDLR* 1773C/T) was detected by PCR using the following primers: forward 5- CTC ACA TGT GGT TGG AGC TG -3 and reverse 5′- CGT TCA TCT TGG CTT GAG TG -3′. The rs2228671 polymorphism (*LDLR* 81C/T) was detected by PCR using the following primers: forward, 5′- TTG GCA GGA AAT AGA CAC AGG -3′; and reverse, 5′- TGA GAC CAG AAA TTC AAG ACC -3′. Also, the rs1122608 polymorphism (*SMARCA4* 5330G/T) was detected by PCR using the following primers: forward, 5′- GAT CCT GTG ATT TCT GCC TCT -3′; and reverse, 5′- TCT CAC TCC CCA CCA AGA AC -3′. Genotyping of these SNPs was performed by Big Dye Terminators (Applied Biosystems, 3130 Genetic Analyzer, and Foster City, CA).

Coronary angiography and scoring

All participants had undergone coronary angiography using Judkins technique, and coronary angiograms were estimated by two experienced Cardiologists. They enrolled any CAD>50%, at least one major coronary artery with ≥50% stenosis, and those with less than 10% stenosis were considered as control. Consequently, quantitative angiographic has been scored based on Sullivan vessel system (22-24). Coronary angiograms assess were done without the knowledge of the genotype status.

Sullivan's scoring system was used for quantitative estimation of atherosclerotic disease in the coronary artery tree. Vessel score was computed based on the number of coronary arteries with ≥75% stenosis reduction in lumen diameter, then, was ranged from 0 to 3.

Statistical analysis

Continuous Variables were tested for normality with Kolmogorov-Smirnov, and because all of them were skewed, were expressed as median and interquartile range. Categorical variables were expressed as proportions. Differences between continuous variables were analyzed using Mann-Whitney U test. Proportions were compared using the $\chi 2$-test and Fisher's exact test. We assessed independent SNP predictors of the coronary artery disease and number of diseased vessels with binary and multinomial logistic regression analysis, using variables that were associated with these outcomes in the univariate analysis including gender, age and smoking status. All the analyses were made using Stata V.11 software. P value less than 0.05 was considered statistically significant.

Results

A total of 274 participants consisting of 170 cases and 104 controls were genotyped for 3 SNPs in *LDLR* locus. Of them, 60.6% were male aged 51.15±10.14 years. Coronary angiography revealed CAD in 170 patients, of which 62 (22.3%) had a single vessel diseases affected, 48(17.8%) had two and the remaining 55(20.4%) had three vessel diseases affected. About 13 SNPs have been genotyped with our designed primers. In addition, a novel SNP (C>G) was found in intron 31 of *SMARCA4* gene in the *LDLR* locus.

As illustrated in table 1, frequency of male gender (76.5%), high *LDL* status (52.4%), history of previous MI(27.6%), academic level education (11.4%), oil consumption (46.7%), smoking (44.6%), familial history of CAD (72.9%) in CAD patients were more than those in normal participants. These cases also were younger (mean age: 49.7 vs. 53.5 years) and heavier (mean BMI: 27.7 vs. 27.3) than controls. However, only differences in gender, age, history of MI, serum HDL and smoking status between two groups were statistically significant (*P* value <0.001).

Table 1. Characteristics of the study population with and without CAD

Characteristic		CAD- free (%) (n=104)	CAD (%) (n=170)	p- value
Male gender		34.62	76.47	<0.0001
High LDL		51.92	52.94	0.9
History ofMI		1.92	27.65	<0.0001
Marital status	Single	1.01	4.12	0.26
	Married	91.92	91.18	
	Widowed/divorced	7.07	4.71	
Educational level	Illiterate/elementary	69.79	58.68	
	Diploma	21.88	29.94	0.2
	Academic	8.33	11.38	
Blood group	A	25.27	28.95	
	B	24.18	23.68	0.9
	AB	15.38	13.82	
	O	35.16	33.55	
High vegetable consumption		96.7	95.27	0.6
High oil consumption		41.94	46.71	0.4
Smoking	yes	22	44.12	<0.0001
Exercise	yes	47.47	44.12	0.6
Diabetes mellitus	yes	23.47	24.24	0.9
Fars Ethnicity		39.39	36.47	0.2
Close parental relativeness		5.88	7.1	0.6
Family history of CAD		61.39	72.94	0.9
Hypertension		23.08	15.88	0.6
Age (median-interquartile range)		51(46-60)	50(45-54)	0.001
BMI (median-interquartile range)		26.8(24.2-29.7)	27.6(25.1-30.1)	0.3
LDL(median-interquartile range)		97.5(75-120)	93(70-120)	0.8
HDL(median-interquartile range)		40(35-49)	37(32-45)	0.04
TG(median-interquartile range)		120(92-161)	137(106-197)	0.1

The homozygote TT and heterozygote GT genotypes were less frequent in CAD group than in the control group in rs1122608 SNP (47.3% vs. 56.9%) and rs7259278 SNP (9.4% vs. 22.3%), but these differences were statistically significant only in the latter one (*P*=0.003). Carriers of G allele (AG or GG) in rs1529729, rs3745677and rs172488 SNPs, were higher in CAD patients than in normal participants, although none of these differences were statistically significant (74.6% vs. 71.6%, *P*=0.6; 5.3% vs. 4.95%, *P*=0.9 and 2.35% vs. 1.9%, *P*=0.8, respectively). These carriers were significantly less represented among CAD patients in rs4300767and rs7259278 SNPs (16% vs. 27.45%, *P*=0.02; 9.4% vs. 22.3%, *P*=0.003, respectively). In rs10417578, rs10411252 and rs1799898 SNPs, CT and TT genotypes were significantly less frequent in CAD patients than in healthy participants (8.3% vs. 21.6%, *P*=0.002; 8.3% vs. 19.6%, *P*=0.006 and 20.6vs. 35.9%, *P*=0.005, respectively). While no significant differences in these genotypes among two groups were observed in rs222867 and rs688 SNPs (*P*=0.08 and *P*=0.2, respectively). Finally, lower rs2738447 AC-CC genotype frequencies in participants with CAD (52.35%) compared to CAD-free individuals (58.25%) were not statistically significant (*P*=0.3) (Table 2) (Figure 1).

Table 2. Distribution and association between different SNPs and CAD

SNPs	Genotype	CAD-free (n=104)	CAD (n=170)	P	Crude OR	P value	95% CI		Adjusted OR *	p-value	95% CI	
	GG	43.14	52.66	0.004	1	--	--	--	1	--	--	--
rs1122608	GT	41.18	43.2	--	0.85	0.6	0.50	1.45	1.03	0.9	0.56	1.87
	TT	15.69	4.14	--	0.22	0.002	0.08	0.56	0.13	<0.0001	0.05	0.41
	GT+TT	56.86	47.34	0.1	0.68	0.1	0.41	1.12	0.73	0.3	0.42	1.29
	AA	28.43	25.44	0.003	1	--	--	--	1	--	--	--
rs1529729	AG	42.16	60.36	--	1.60	0.1	0.9	2.88	1.90	0.06	0.97	3.71
	GG	29.41	14.2	--	0.54	0.09	0.26	1.10	0.58	0.2	0.25	1.31
	AG+GG	71.57	74.56	0.6	1.16	0.6	0.67	2.02	1.35	0.3	0.73	2.53
	AA	72.55	84.02	--	1	--	--	--	1	--	--	--
rs4300767	AG	22.55	15.38	0.02	0.59	0.09	0.31	1.10	0.69	0.3	0.33	1.41
	GG	4.9	0.59	--	0.10	0.04	0.01	0.90	0.05	0.01	0.005	0.52
	AG+GG	27.45	15.98	0.02	0.50	0.02	0.27	0.91	0.53	0.07	0.27	1.05
	CC	78.43	91.72	--	1	--	--	--	1	--	--	--
rs10417578	CT	12.75	7.69	0.001	0.52	0.1	0.22	1.16	0.86	0.7	0.33	2.23
	TT	8.82	0.59	--	0.06	0.007	0.01	0.46	0.06	0.01	0.007	0.60
	CT+TT	21.57	8.28	0.002	0.33	0.003	0.16	0.67	0.49	0.09	0.22	1.12
	CC	80.39	91.72	-	1	--	--	--	1	--	--	--
rs10411252	CT	13.73	6.51	0.02	0.41	0.04	0.18	0.95	0.68	0.4	0.26	1.79
	TT	5.88	1.78	--	0.26	0.06	0.06	1.08	0.43	0.3	0.09	2.05
	CT+TT	19.61	8.28	0.006	0.37	0.008	0.17	0.77	0.60	0.2	0.26	1.40
rs2228671	CC	78.22	86.47	0.08	1	--	--	--	--	--	--	--
	CT	21.78	13.53	--	0.56	0.08	0.29	1.07	0.58	0.1	0.28	1.20
	AA	95.05	94.71	--	1	--	--	--	1	--	--	--
rs3745677	AG	3.96	4.12	0.9	1.04	0.9	0.29	3.65	0.99	0.9	0.24	4.07
	GG	0.99	1.18	--	1.19	0.9	0.10	13.3	1.95	0.6	0.14	27.04
	AG+GG	4.95	5.29	0.9	1.07	0.9	0.34	3.29	1.15	0.8	0.33	4.09
rs2745678	AA	97.03	99.41	--	1	--	--	--	--	--	--	--
	AG	2.97	0.59	0.1	0.19	0.1	0.02	1.88	0.29	0.3	0.02	3.90
	CC	39.81	32.35	--	1	--	--	--	1	--	--	--
rs688	CT	45.63	46.47	0.3	1.25	0.4	0.72	2.15	1.30	0.4	0.69	2.42
	TT	14.56	21.18	--	1.78	0.1	0.86	3.69	1.92	0.1	0.85	4.32
	CT+TT	60.19	67.65	0.2	1.38	0.2	0.83	2.30	1.46	0.2	0.82	2.61
	CC	64.08	79.41	--	1	--	--	--	1	--	--	--
rs1799898	CT	32.04	17.65	0.02	0.44	0.006	0.25	0.79	0.40	0.006	0.21	0.77
	TT	3.88	2.94	--	0.61	0.4	0.15	2.35	0.61	0.5	0.13	2.8
	CT+TT	35.92	20.59	0.005	0.46	0.006	0.27	0.80	0.42	0.007	0.23	0.79
	GG	77.67	90.59	--	1	--	--	--	1	--	--	--
rs7259278	GT	14.56	5.88	0.01	0.34	0.01	0.14	0.80	0.42	0.08	0.16	1.12
	TT	7.77	3.53	--	0.38	0.09	0.13	1.16	0.66	0.5	0.18	2.35
	GT+TT	22.33	9.41	0.003	0.36	0.004	0.18	0.72	0.49	0.08	0.23	1.10
rs172488	AA	98.06	97.65	--	1	--	--	--	--	--	--	--
	AG	1.94	2.35	0.8	1.21	0.8	0.22	6.76	1.15	0.8	0.12	10.92
	AA	41.75	47.65	--	1	--	--	--	1	--	--	--
rs2738447	AC	29.13	25.88	0.6	0.77	0.4	0.43	1.40	0.94	0.8	0.48	1.87
	CC	29.13	26.47	--	0.79	0.4	0.44	1.43	0.87	0.7	0.45	1.70
	AC+CC	58.25	52.35	0.3	0.78	0.3	0.48	1.29	0.91	0.7	0.52	1.59

Based on gender, age and smoking status

The frequencies of CT-GT and TT genotypes in rs1122608, rs1799898 and rs10417578 SNPs were increased by the number of diseased vessels and frequencies of CT, TT and AC genotypes in rs2738447, and rs688 SNPs were decreased by increasing the number of diseased vessels. Although these differences were not statistically significant (p- value was 0.06 for rs1122608, 0.6 for rs1799898, 0.9 for rs10417578, 0.6 for rs2738447and 0.4 for rs688) (Table 3).

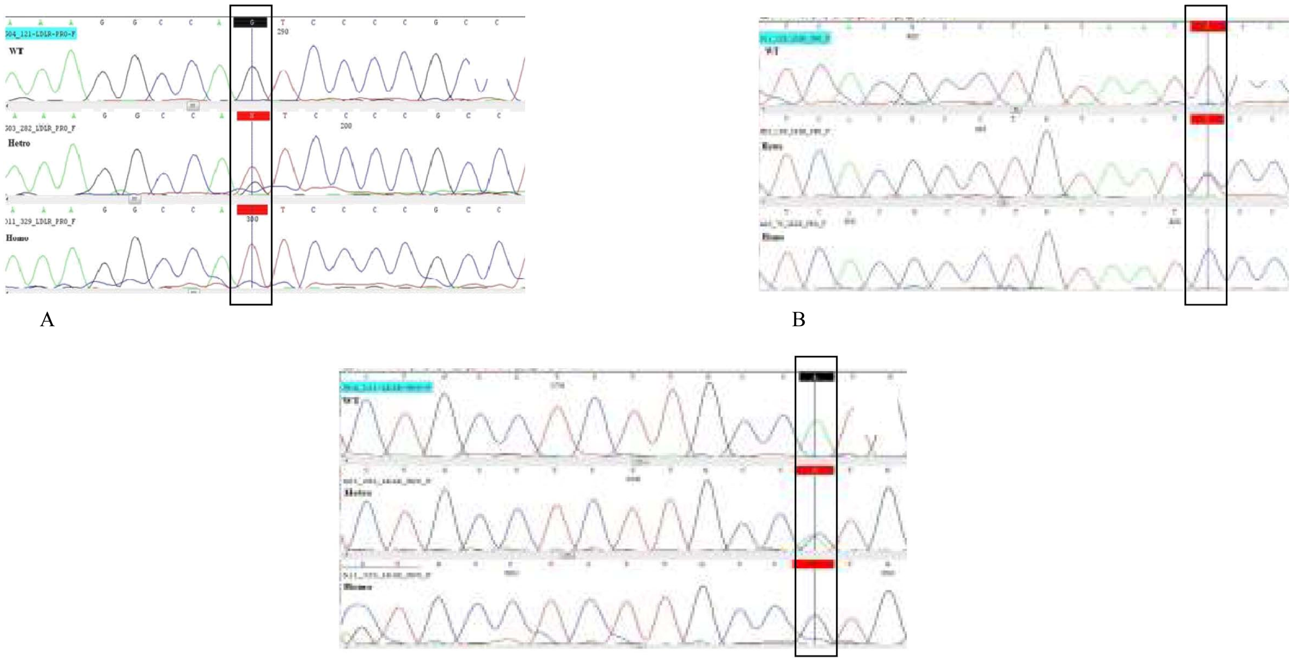

Figure 1. Sequencing analysis is shown the detection of rs1122608, rs4300767 and rs10417578 polymorphisms in SMARCA4 gene. Direct sequencing chromatograms of rs1122608 (A), rs10417578 (B) and rs4300767 (C) SNPs as examples including changes in wild type, heterozygote and homozygote genotypes

Table 3. Frequency and association of different SNP genotypes according to diseased vessel count

SNP genotypes	1 Vessel(%)	2 vessel(%)	3 vessel(%)	P value	Crude OR*	P. value	95% CI	Crude OR**	P value	95% CI	Adj OR*	P value	95% CI	adj OR**	P value	95% CI
rs1122608 GT+TT	37.1	47.92	59.26	0.06	1.56	0.2	0.72-3.35	2.46	**0.01**	1.17-5.21	1.63	0.2	0.71-3.70	2.48	**0.02**	1.13-5.43
rs2738447 AC+CC	56.45	50	47.27	0.6	0.77	0.5	0.36-1.64	0.69	0.3	0.33-1.43	0.56	0.2	0.24-1.29	0.53	0.1	0.23-1.16
rs7259278 GT+TT	12.9	6.25	9.09	0.5	0.45	0.2	0.11-1.79	0.67	0.5	0.21-2.20	0.65	0.6	0.15-2.88	0.93	0.9	0.26-3.29
rs1799898 CT+TT	17.74	20.83	25.45	0.6	1.22	0.7	0.47-3.16	1.58	0.3	0.65-3.85	1.39	0.5	0.49-3.92	1.89	0.2	0.73-4.88
rs688 CT+TT	72.58	64.58	61.82	0.4	0.68	0.4	0.30-1.55	0.61	0.2	0.28-1.33	0.61	0.3	0.25-1.47	0.54	0.1	0.24-1.24
rs3745677 AG+GG	6.45	2.08	5.45	0.6	0.30	0.3	0.03-2.85	0.83	0.8	0.18-3.91	0.36	0.4	0.03-3.84	1.08	0.9	0.20-5.70
rs10411252 CT+TT	6.45	8.33	7.41	0.9	1.31	0.7	0.31-5.56	1.16	0.8	0.27-4.88	1.28	0.7	0.25-6.67	0.90	0.9	0.18-4.47
rs10417578 CT+TT	6.45	8.33	9.26	0.9	1.31	0.7	0.31-5.56	1.47	0.5	0.37-5.81	1.16	0.8	0.22-5.98	1.05	0.9	0.23-4.80
rs4300767 AG+GG	14.52	12.5	20.37	0.6	0.84	0.7	0.28-2.55	1.50	0.4	0.57-3.96	0.87	0.8	0.27-2.79	1.50	0.4	0.54-4.14
rs1529729 AG+GG	72.58	68.75	81.48	0.3	0.83	0.7	0.36-1.90	1.66	0.2	0.68-4.03	1.08	0.8	0.44-2.64	2.10	0.1	0.81-5.46
rs2228671 CT	12.9	18.75	10.91	0.5	1.55	0.4	0.55-4.39	0.82	0.7	0.27-2.55	1.79	0.3	0.58-5.53	0.89	0.8	0.27-2.95
rs17248882 AG	1.61	4.17	1.82	0.7	2.65	0.4	0.23-30.1	1.13	0.9	0.06-18.5	1.59	0.7	0.11-22.2	0.66	0.8	0.03-13.2
rs2745678	1.61	0	0	0.4	-	-	-	-	-	-	-	-	-	-	-	-

* OR between SNPs and 2 vessels affecting
**OR between SNPs and 3 vessel affecting

Univariate analysis

Univariate logistic regression analyses showed significant associations between rs4300767 (OR=0.5, *P*=0.02), rs10417578 (OR=0.33, *P*=0.003), rs10411252 (OR=0.37, *P*=0.008), rs1799898 (OR=0.46, *P*=0.006) and rs7259278 (OR=0.36, *P*=0.004) with CAD development. There were no significant associations between CAD and pooled risk genotypes of rs1122608 (*P*=0.1), rs1529729 (*P*=0.6), rs2228671 (*P*=0.08), rs3745677 (*P*=0.9), rs2745678 (*P*=0.1), rs688 (*P*=0.2), rs172488 (*P*=0.8) and rs2738447 (*P*=0.3) (Table 1). Only GG-GT genotype of 1122608 SNP was associated with more than two-fold increase in risk of three vessels involvement relative to a single vessel affecting (OR=2.46, *P*=0.01). Although the presence of these genotypes increased risk of double vessel disease more than 50%, the association was not statistically significant (OR=1.56, *P*=0.2). There was no significant association between other SNPs and number of diseased vessels (Table 3).

Multivariate analysis

When we performed a multivariate logistic regression analysis of the relationship between genotypes and CAD risk with age, gender and smoking status, the above associations were changed. Considering the GG genotype carriers as the reference group, in rs1122608 SNP, only TT genotype was associated with a highly significant 87% decreased risk of CAD (OR=0.13, p<0.0001). GT or combined genotype GT-TT did not have a significant association with CAD (*P*=0.9 and 0.3, respectively).

Moreover, homozygote GG genotype in rs4300767, homozygote TT genotype in rs10417578 and combined heterozygote and homozygote CT+TT genotype in rs1799898 SNPs were associated with decreased risk of CAD compared to AA, CC and CC genotypes as reference groups respectively (OR=0.05, *P*=0.01; OR=0.06, *P*=0.01 and OR=0.42, *P*=0.007, respectively). In addition, OR between AG genotype in rs1529729 SNP and CAD development and also OR between GT genotype in rs7259278 SNP and CAD were 1.90 and 0.49, respectively, which were borderline significant (*P*=0.06 and 0.08, respectively) indicating a 90% increase and 51% decrease in risk of CAD (Table 2).

Although no associations were observed between CAD and presence of SNPs such as rs10411252 (OR=0.60, *P*= 0.2), rs2228671 (OR=0.58, *P*= 0.1), rs2745678 (OR=0.29, *P*= 0.3) and rs2738447 (OR=0.91, *P*= 0.7), none of them were statistically significant. Moreover, ORs between CAD and rs3745677 (1.15), rs688 (1.46) and rs172488 (1.15), were more than one, indicating positive associations, but all of them were non-significant too (*P*= 0.8, 0.2 and 0.8, respectively) (Table 2).

Similar to the univariate analysis, GT and TT genotypes of rs1122608 SNP were associated with about significant 2.5 fold increase only in the risk of three vessels involvement (OR=2.48, *P*=0.02) relative to a single vessel disease, whereas its 63% increase in the risk of two vessel involvement was not statistically significant(OR=1.63, *P*=0.2). Finally, no significant associations were found among the number of diseased vessels and the other SNPs (Table 3).

Regarding the new novel SNP(C/G) found in *SMARCA4* gene, the frequency of CG genotype was higher in CAD patients than in healthy participants (2.9% vs. 0, *P*=0.08) associated with a more than 70% increase in the risk of CAD development (OR=1.72), but that was not statistically significant (*P*=0.6).

Discussion

Our study shows that SNPs at the *LDLR* locus are associated with CAD and its severity. We also report for the first time, a novel single nucleotide change in *SMARCA4* gene. Although most of the SNPs were significantly associated with CAD in the univariate analysis, controlling for some demographic and behavioral characteristics, few of them lost their statistically significant association suggesting the confounding effect of gender, age and smoking status on the association between SNPs and CAD.

It has been shown that homozygote genotypes of rs1122608 (G/T), rs4300767 (A/G) and rs10417578(C/T) SNPs seemed to have strong protective effects on the CAD, i.e. these genotypes decreased the risk of CAD about 87%, 95% and 94%, respectively. No significant association was found between heterozygote genotypes of these SNPs and risk of CAD, and when we combined homozygote and heterozygote genotypes of the SNPs as pooled risk genotypes, borderline significant protective associations were detected between carriership of rs4300767 G allele and rs10417578 T allele which could be highly significant if the sample size was more. Moreover, AG genotype of rs1529729 SNP was associated with a borderline significant 90% increased risk of CAD which introduces this genotype as an independent risk factor of the coronary artery disease in the enough sample size.

Unlike the results of this study, Nicola Martinelli et al., (25) found no association between rs1122608 and CAD and also the study was conducted by Linnea (26) detected a significant 15% increase in the risk of CAD by this SNP.

We also found that patients with CT or TT genotypes of rs1799898 SNP had a lower risk of CAD. It means that this SNP might be a protective factor for CAD.

Although our univariate analyses demonstrated a significant protective effect of the carriership of rs7259278 T allele on CAD, adjusting for other variables showed only a borderline significant association which may be due to small sample size.

In the current study, we found harmful effects of different genotypes of rs688 SNP and protective effects of genotypes of rs2738447 SNP on the, but these associations were not statistically significant. That was in contrast to the results of Martinelli et al. study which reported rs688 SNP as a risk factor for CAD (25).

Results of crude and adjusted multinomial logistic regression analyses showed that of the SNPs investigated in the current study, only patients with GT or TT genotypes of rs1122608 SNP were at higher risk of three vessel involvement compare to single vessels involvement. We did not observe any association between other SNPs and severity of CAD.

As discussed previously, one of the most important findings in our study was detection of a new C>G change in 31 intronic region of *SMARCA4* gene for the first time which had been occurred only in CAD patients with a high level of serum *LDL* with a borderline significant difference of frequency between CAD patients and healthy participants. So it is necessary to be further investigated among Iranian people.

Our study shows that SNPs at the *LDLR* locus are associated with CAD and its severity. We also report for the first time, a novel single nucleotide change in *SMARCA4* gene.

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References

1. KulloIJ, Ding K. Mechanisms of disease: the genetic basis of coronary heart disease. Nat Clin Pract Cardiovasc Med 2007;4(10):558-69.
2. World Health Organization. The World Health Report, 2002: reducing risks, promoting healthy life. Geneva, 2002.
3. Franchini M, Peyvandi F, Mannucci PM. The genetic basis of coronary artery disease: from candidate genes to whole genome analysis. Trends Cardiovasc Med 2008;18(5):157-62.
4. Padmanabhan S, Hastie C, Prabhakaran D. Genomic approaches to coronary artery disease. Indian J Med Res 2010;132(5):567-78.
5. Musunuru K, Kathiresan S. Genetics of Coronary Artery Disease. Annu Rev Genomics Hum Genet 2010;11(1):91-108.
6. Girelli D, Martinelli N, Peyvandi F. Genetic architecture of coronary artery disease in the genome-wide era: implications for the emerging ''golden dozen'' loci. SeminThrombHemost 2009;35(7):671-82.
7. Weissglas-Volkov D, Pajukanta P. Genetic causes of high and low serum HDL-cholesterol. J Lipid Res 2010;51(8):2032-57.
8. Fishbein MC. The vulnerable and unstable atherosclerotic plaque. Cardiovasc Pathol 2010;19(1):6-11.
9. Bui QT, Prempeh M, Wilensky RL. Atherosclerotic plaque development. Int J Biochem Cell Biol 2009;41(11):2109-13.
10. Samani NJ, Erdmann J, Hall AS, et al. Genome wide association analysis of coronary artery disease. N Engl J Med 2007;357(5):443-53.
11. Roberts R, Stewart AF. Genes and coronary artery disease: where are we? J Am Coll Cardiol 2012;60(18):1715-21.
12. Kathiresan S, Willer CJ, Peloso GM, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet 2009;41(1):56-65.
13. Kathiresan S, Voight BF, Purcell S, et al. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet 2009;41(3):334-41.
14. Linsel-Nitschke P, Götz A, Erdmann J, et al. Lifelong reduction of *LDL*-Crelated to a common variant in the *LDL*-receptor gene decreases the risk of coronary artery disease: a MendelianRandomisation study. PLoS One 2008;3(8):e2986.
15. Zhu H, Tucker HM, Grear KE, et al. A common polymorphism decreases low-density lipoprotein receptor exon 12 splicing efficiency and associates with increased cholesterol. Hum Mol Genet 2007;16(14):1765-72.
16. Kittles RA, Weiss KM. Race, ancestry, and genes: Implications for defining disease risk. Ann Rev Genomics Hum Genet 2003;4(1):33-67.
17. Patel RS, Su S, Neeland IJ, et al. The chromosome 9p21 risk locusis associated with angiographic severity and progression of coronary artery disease. Eur Heart J 2010;31(24):3017-23.
18. Anselmi M, Garbin U, Agostoni P, et al. Plasma levels of

oxidized-low-density lipoproteins are higher in patients with unstable angina and correlated with angiographic coronary complex plaques. Atherosclerosis 2006;185(1):114-20.

19. Sadeghi M, Heidari R, Mostanfar B, et al. The relation between ankle-brachial index (ABI) and coronary artery disease severity and risk factors: an angiographic study. ARYA Atherosclerosis 2011;7(2):68-73.
20. Lei W, Yi-tong Ma, Xiang X, et al. Association of MMP-9 gene polymorphisms with acute coronary syndrome in the Uygur population of China. World J Emerg Med 2011;2(2):104-10.
21. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16(3):1215.
22. Sullivan DR, Marwick TH, Freedman SB. A new method of scoring coronary angiograms to reflect extent of coronary atherosclerosis and improve correlation with major risk factors. Am Heart J 1990;119(6):1262-7.
23. Garcia-Moll X, Coccolo F, Cole D, et al. Serum Neopterin and Complex Stenosis Morphology in Patients With Unstable Angina. J Am Coll Cardiol 2000;35(4):956-62.
24. Anselmi M, Garbin U, Agostoni P, et al. Plasma levels of oxidized-low-density lipoproteins are 2010 with angiographic coronary complex plaques. Atherosclerosis 2006;185(1):114-20.
25. Martinelli N, Girelli D, Lunghi B, et al. Polymorphisms at *LDLR* locus may be associated with coronary artery disease through modulation of coagulation factor VIII activity and independently from lipid profile. Blood 2010; 116(25):5688-97.
26. Baudhuin LM. Genetics of coronary artery disease: focus on genomewide association studies. Am J Transl Res 2009;1(3):221-34.