Association of Polymorphisms at \textit{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 (\textit{LDLR}) locus. This study was evaluated the possible association of genetic markers at \textit{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 \textit{LDLR} gene (Exon 2, 12) and part of intron 30 of \textit{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 (\textit{P}<0.0001), rs4300767 (\textit{P}<0.005) and rs10417578 (\textit{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 (\textit{P}=0.01).

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Keywords: Coronary artery disease; \textit{LDLR} locus; Single nucleotide polymorphism; \textit{SMARCA4} gene

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 \textit{LDLR} locus which some of them show the strongest association with \textit{LDL}-cholesterol (\textit{LDL-C}) levels among different populations (12,13). The rs2228671 in exon 2 \textit{LDLR} has been shown to have a strong association with \textit{LDL-C} level; the T allele of this SNP is associated with decreased \textit{LDL-C} and in consequence with the decreased CAD risks (14). Thers688 within exon 12 of \textit{LDLR} gene has been reported to alter the \textit{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 \textit{SMARC1} gene adjusted to \textit{LDLR} gene, is associated with the high level
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 the χ²-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.
Association of polymorphisms at ...

**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>
<thead>
<tr>
<th>Characteristic</th>
<th>CAD-free (%)</th>
<th>CAD (%)</th>
<th>$p$-value</th>
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<tr>
<td>Male gender</td>
<td>34.62</td>
<td>76.47</td>
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<td>High LDL</td>
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<td>52.94</td>
<td>0.9</td>
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<tr>
<td>History of MI</td>
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<td>Marital status</td>
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<td>Married</td>
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<tr>
<td>Widowed/divorced</td>
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<td>46.71</td>
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<td>&lt;0.0001</td>
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<td>Diabetes mellitus yes</td>
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<td>24.24</td>
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<td>Family history of CAD</td>
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<td>Hypertension</td>
<td>23.08</td>
<td>15.88</td>
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<td>Age (median-interquartile range)</td>
<td>51(46-60)</td>
<td>50(45-54)</td>
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<td>BMI (median-interquartile range)</td>
<td>26.8(24.2-29.7)</td>
<td>27.6(25.1-30.1)</td>
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<td>LDL (median-interquartile range)</td>
<td>97.5(75-120)</td>
<td>93.7(70-120)</td>
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<td>HDL(median-interquartile range)</td>
<td>40(35-49)</td>
<td>37(32-45)</td>
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<td>TG(median-interquartile range)</td>
<td>120(92-161)</td>
<td>137(106-197)</td>
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</table>

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 (52.3%) compared to CAD-free individuals (58.25%) were not statistically significant ($P$=0.3) (Table 2) (Figure 1).
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 rs2738447 and 0.4 for rs688) (Table 3).

### Table 2. Distribution and association between different SNPs and CAD

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<tr>
<th>SNPs</th>
<th>Genotype</th>
<th>CAD-free (n=104)</th>
<th>CAD (n=170)</th>
<th>P</th>
<th>Crude OR</th>
<th>P value</th>
<th>95% CI Adjusted OR</th>
<th>p-value</th>
<th>95% CI Adjusted OR</th>
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<td>0.004</td>
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<td>--</td>
<td>--</td>
<td>1</td>
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<td>--</td>
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<tr>
<td></td>
<td>CT</td>
<td>32.04</td>
<td>0.004</td>
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<td>0.44</td>
<td>0.006</td>
<td>0.25</td>
<td>0.79</td>
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<td>--</td>
<td>1</td>
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<td>--</td>
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<tr>
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<td>0.004</td>
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<td>0.01</td>
<td>0.14</td>
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<tr>
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<td>TT</td>
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<td>0.004</td>
<td>1</td>
<td>0.93</td>
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</tr>
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<td>0.004</td>
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<td>--</td>
<td>1</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
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<td>AA</td>
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<td>0.22</td>
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<td>0.004</td>
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<td>--</td>
<td>1</td>
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</tr>
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<td>AC+CC</td>
<td>58.25</td>
<td>0.004</td>
<td>1</td>
<td>0.78</td>
<td>0.3</td>
<td>0.48</td>
<td>1.29</td>
<td>0.91</td>
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</tbody>
</table>

Based on gender, age and smoking status
Association of polymorphisms at …

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

<table>
<thead>
<tr>
<th>SNP genotypes</th>
<th>1 Vessel (%)</th>
<th>2 vessel (%)</th>
<th>3 vessel (%)</th>
<th>P value</th>
<th>Crude OR*</th>
<th>P value</th>
<th>95% CI</th>
<th>Crude OR**</th>
<th>P value</th>
<th>95% CI</th>
<th>Adj OR*</th>
<th>P value</th>
<th>95% CI</th>
<th>Adj OR**</th>
<th>P value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1122608 GT+TT</td>
<td>37.1</td>
<td>47.92</td>
<td>59.26</td>
<td>0.06</td>
<td>1.56</td>
<td>0.2</td>
<td>0.72-3.35</td>
<td>2.46</td>
<td>0.01</td>
<td>1.17-5.21</td>
<td>1.63</td>
<td>0.2</td>
<td>0.71-3.70</td>
<td>2.48</td>
<td>0.02</td>
<td>1.13-5.43</td>
</tr>
<tr>
<td>rs2738447 AC+CC</td>
<td>56.45</td>
<td>50</td>
<td>47.27</td>
<td>0.6</td>
<td>0.77</td>
<td>0.5</td>
<td>0.36-1.64</td>
<td>0.69</td>
<td>0.3</td>
<td>0.33-1.43</td>
<td>0.56</td>
<td>0.2</td>
<td>0.24-1.29</td>
<td>0.53</td>
<td>0.1</td>
<td>0.23-1.16</td>
</tr>
<tr>
<td>rs7259278 GT+TT</td>
<td>12.9</td>
<td>6.25</td>
<td>9.09</td>
<td>0.5</td>
<td>0.45</td>
<td>0.2</td>
<td>0.11-1.79</td>
<td>0.67</td>
<td>0.5</td>
<td>0.21-2.20</td>
<td>0.65</td>
<td>0.6</td>
<td>0.15-2.88</td>
<td>0.93</td>
<td>0.9</td>
<td>0.26-3.29</td>
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<td>20.83</td>
<td>25.45</td>
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<td>1.22</td>
<td>0.7</td>
<td>0.47-3.16</td>
<td>1.58</td>
<td>0.3</td>
<td>0.65-3.85</td>
<td>1.39</td>
<td>0.5</td>
<td>0.49-3.92</td>
<td>1.89</td>
<td>0.2</td>
<td>0.73-4.88</td>
</tr>
<tr>
<td>rs688 CT+TT</td>
<td>72.58</td>
<td>64.58</td>
<td>61.82</td>
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<td>0.68</td>
<td>0.4</td>
<td>0.30-1.55</td>
<td>0.61</td>
<td>0.2</td>
<td>0.28-1.33</td>
<td>0.61</td>
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<td>0.25-1.47</td>
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<td>rs24567 A+GG</td>
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<td>2.08</td>
<td>5.45</td>
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<td>0.30</td>
<td>0.3</td>
<td>0.03-2.85</td>
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<td>0.8</td>
<td>0.18-3.91</td>
<td>0.36</td>
<td>0.4</td>
<td>0.03-3.84</td>
<td>1.08</td>
<td>0.9</td>
<td>0.20-5.70</td>
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<tr>
<td>rs10411252 CT+TT</td>
<td>6.45</td>
<td>8.33</td>
<td>7.41</td>
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<td>1.31</td>
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<td>0.25-6.67</td>
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<td>0.9</td>
<td>0.18-4.47</td>
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<tr>
<td>rs10417578 CT+TT</td>
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<td>8.33</td>
<td>9.26</td>
<td>0.9</td>
<td>1.31</td>
<td>0.7</td>
<td>0.31-5.56</td>
<td>1.47</td>
<td>0.5</td>
<td>0.37-5.81</td>
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<td>0.22-5.98</td>
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<tr>
<td>rs4300767 AG+GG</td>
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<td>12.5</td>
<td>20.37</td>
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<td>0.84</td>
<td>0.7</td>
<td>0.28-2.55</td>
<td>1.50</td>
<td>0.4</td>
<td>0.57-3.96</td>
<td>0.87</td>
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<td>68.75</td>
<td>81.48</td>
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<td>0.7</td>
<td>0.36-1.90</td>
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<td>0.68-4.03</td>
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<td>1.55</td>
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<td>0.55-4.39</td>
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<td>0.27-2.55</td>
<td>1.79</td>
<td>0.3</td>
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<td>0.9</td>
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</table>

* 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 (Table 2).

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 carrihership 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 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.

Acknowledgment

We are grateful to our patients and their families for their participation in this study.

References


