Introduction

The influence of heritable materials in the pathogenesis of the myocardial infarction (MI) has been proved in many independent studies. Recently, genome-wide association studies (GWAS) improved the way of exploring the susceptible loci and genetic polymorphisms contributing to the pathogenesis of MI. Performing a large-scale GWAS in the Japanese population, Hirokawa et al introduced PLCL2 and AP3D1-DOT1L-SF3A2 as the two novel candidate genes for MI. Phospholipase C like 2 gene (PLCL2, OMIM#614276) located on the short arm of human chromosome 3 at 3p24.3 cytogenetic band and the coding information of the gene recorded in 12 exons. PLCL2 is abundantly expressed in skeletal muscles, but also detectable in lymphocytes and platelets, Brain, liver, thymus, and kidney. The catalytically inactive PLCL2 enzyme binds to inositol 1,4,5-trisphosphate [ins(1,4,5)P3] via the receptors existing on endoplasmic reticulum (ER) membrane and allows calcium release by forming vesicles. Alterations in PLCL2 can interfere with normal calcium homeostasis which results in abnormal proliferation, migration, and contraction of vascular smooth muscle cells (VSMCs) as the main events leading to atherosclerosis. Furthermore, the tissue distribution of the PLCL2 (e.g. lymphocytes and platelets; crucial components of atherosclerosis) may reflect the participation of this gene in atherosclerosis complex process through inflammation and immune responses.

The unique features of the PLCL2 in calcium signaling pathway and subsequently in atherosclerosis led us to speculate that it may associate with myocardial infarction. To investigate this association, we compared the genotype distribution of the rs4618210A>G intron variant between two groups of MI patients and healthy controls.

Materials and Methods

Study population

This hospital-based case-control study composed of 300 cases with MI and 300 healthy volunteers as a control group. MI patients were enrolled among all the eligible subjects who admitted by chest pain in the Heart Clinic of the Vali-e-Asr hospital, Fasa, Iran during a period of six months prior to blood sampling between March and August 2016. Control subjects were at the same age and sex as the cases. Control subjects declared that they did not have a previous sign of heart problem or MI. MI patients without any age limitation and both genders were recruited among those with positive result of the troponin measurement. Patients with Diabetes mellitus, hypertension, smoking, and family history of heart diseases were included in the study to evaluate the association of these conditions with the risk of MI. Systolic blood pressure higher than 140...
mmHg or diastolic blood pressure higher than 90 mmHg and fasting blood glucose level >126 mg/dL or dependency to insulin or other hypoglycemic drugs were considered as hypertension and diabetes mellitus respectively. Family history only checked among first and second relatives. Smoking status was defined as current smokers (positive group) and never smokers (negative group). Regarding the families with more than one affected patients with MI, only one patient was included. Patients who had recently performed heart surgery as well as patients with more than one MI were excluded from the study. Information about the lipid profile (LDL, HDL, and TG) of all subjects in both case and control groups was documented by checking the laboratory test result. Demographic characteristics including age, gender, weight, and height were asked at the time of sampling.

Molecular analysis
Following DNA extraction using standard salting out method, amplification refractory mutation system -polymerase chain reaction (ARMS PCR) method was used to amplify the genomic region containing the rs4618210A>G variant and its flanking sequences. The PCR mix prepared following the Yekta Tajhiz Azma kit instruction. The primers for ARMS PCR designed using primer 1 software after some modifications as follows (polymorphic alleles are bold and underlined); forward outer (FO): 5’ GAGTCCCTTTTGTCTGCCTTG 3’; reverse outer (RO): 5’ CCTCCTGCTTGGTCTTTTTCCATA 3’; reverse inner (RI) for A-allele: 5’ C C C T A A A C A T G A G T T T T G T C T T T T T A T T 3’; and RI for G-allele: 5’ CCCTAAACAATGAGTTTTGTGTCTCGCCTTG 3’. The amplification reaction was done in the thermal condition including pre-denaturation at 95°C for 5 minutes, 30 cycles of consecutive steps of denaturation at 95°C for 45 sec, annealing at 61°C for 45 seconds and extension for 45 seconds, and the final extension for 5 minutes at 72°C. The specified interaction of primers resulted in the non-allele specific product with 601 bp in length and the allele-specific product of 353 bp on 2.5% agarose gel (Figure 1).

Statistical analysis
SPSS version 19 applied to statistically analyze the data. Quantity variables were presented as mean ± standard deviation (SD) and the differences between case and control groups was assessed using t test. Frequency and percent were used to represent categorical variables. The accordance of the observed genotype frequencies with Hardy-Weinberg equilibrium was tested using the Chi-square test. The association between PLCL2 rs4618210 genotypes and MI was examined accounting the odds ratio (OR) and confidence interval (95%CI) in a monovariate logistic regression model. Logistic regression also used to explore the probable influence of some risk factors on the risk of MI. P values of less than 0.05 considered statistically significant in all analyses.

Results
Baseline characteristics of the study subjects
The mean age of the MI cases (63.20 ± 11.07 year) revealed no significant difference to the mean age of the controls (62.25 ± 12.06 year) with the P value of 0.32. The male to female ratio was 1.46 in patient group. Most of the MI cases were in the age above 50 years. The mean of the body mass index (BMI), Low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride (TG) was significantly higher in cases compared to controls (all P values ≤ 0.001) (Table 1).

Association analysis
Allele frequencies and genotype distributions in cases and controls are shown in Table 2. Deviation from Hardy-Weinberg equilibrium was not seen in the control group (χ² = 0.89, df = 1, P = 0.34). The frequency of the AG genotype was significantly higher in cases compared to controls (70% vs. 52.7%) and resulted in an increased risk of MI (OR: 1.91; 95%CI: 1.24 – 2.93; P = 0.003). Carriers of at least one G allele (GG + AG vs. AA) had a higher risk of MI (OR: 1.56; 95%CI: 1.03 – 2.36; P = 0.037). The frequency of the G allele in control and case groups was 51.65% and 49.7% respectively. The difference between the frequencies of the G allele between groups did not reach statistical difference (OR: 0.92; 95%CI: 0.73 – 1.16; P = 0.32).

Table 1. Demographic and anthropometric characteristics and lipid profile of cases and controls

| Variable                | Cases    | Controls | P   |
|-------------------------|----------|----------|-----|
| Total                   | 300      | 300      | -   |
| Age (Mean ± SD)         | 63.20 ± 11.07 | 62.25 ± 12.06 | 0.32 |
| Age at diagnosis (Mean ± SD) | 61.13 ± 9.46 | -       | -   |
| Sex ratio (male: female) | 178:122  | 174:126  | 0.74 |
| Weight                  | 71.80 ± 4.46 | 69.46 ± 8.00 | ≤ 0.001* |
| BMI, kg/m²              | 42.36 ± 1.99 | 40.49 ± 3.34 | ≤ 0.001* |
| LDL, mg/dL              | 86.73 ± 11.38 | 76.45 ± 11.42 | ≤ 0.001* |
| HDL, mg/dL              | 41.79 ± 6.32 | 39.14 ± 10.73 | ≤ 0.001* |
| TG, mg/dL               | 179.05 ± 38.00 | 139.59 ± 33.34 | ≤ 0.001* |

Abbreviations: SD, standard deviation
*Student t test
*Statistically significant.

Figure 1. Genotyping of the PLCL2 rs4618210 polymorphism using ARMS PCR
**PLCL2 rs4618210A>G polymorphism and MI**

**Table 2. Alleles and genotypes distributions of the PLCL2 rs4618210 polymorphism in cases and controls**

| SNP        | Genotypes | Variables | Cases n (%) | Controls n (%) | P       | OR (95% CI) |
|------------|-----------|-----------|-------------|----------------|---------|-------------|
| rs4618210  | GG        |            | 44 (14.7)   | 76 (25.3)      | 0.492   | 0.83 (0.49 – 1.41) |
|            | AG        |            | 210 (70)    | 158 (52.7)     | 0.003*  | 1.91 (1.24 – 2.93) |
|            | GG+AG     |            | 254 (84.7)  | 234 (78)       | 0.037*  | 1.56 (1.03 - 2.36) |
|            | A         | Alleles   | 302 (50.3)  | 290 (48.35)    | -       | Reference   |
|            | G         | Alleles   | 298 (49.7)  | 310 (51.65)    | 0.488   | 0.92 (0.73 - 1.16) |

*Logistic regression analysis
*Statistically significant

**Table 3. Association of the risk factors with MI**

| Risk factor | Condition | MI Number (%) | Controls Number (%) | P value* |
|-------------|-----------|---------------|---------------------|----------|
| Hypertension| No        | 6 (2)         | 297 (99)            | ≤ 0.001* |
|             | Yes       | 294 (98)      | 3 (1)               |          |
| Diabetes    | No        | 138 (46)      | 298 (99.3)          | ≤ 0.001* |
|             | Yes       | 16 (54)       | 2 (0.7)             |          |
| Smoking     | No        | 133 (44.3)    | 298 (99.3)          | ≤ 0.001* |
|             | Yes       | 167 (55.7)    | 2 (0.7)             |          |
| Family history | No     | 3 (1)        | 291 (97)            | ≤ 0.001* |
|             | Yes       | 297 (99)      | 9 (3)               |          |

*Logistic regression analysis
*Statistically significant

\[ P = 0.488. \]

The frequency of hypertension in MI groups was significantly higher than controls and the data support the increased in the risk of MI among patients with hypertension \((P \leq 0.001)\). Diabetes mellitus, family history for heart diseases and smoking were also risk factor for MI \((P \leq 0.001)\) (Table 3).

**Discussion**

Most of the predisposing loci known for their association with MI contained the genes of the immune system, inflammation and lipid metabolism.2,11-13 Hirokawa et al2 in their large GWAS found two novel genes for their association with MI. A strong association with the protective influence of the minor G-allele of the PLCL2 rs4618210A>G intronic variant and the occurrence of MI was found in the Japanese population \((P = 2.60 \times 10^{-6}; \text{OR: } 0.91)\). Qian et al14 investigated the possible influence of the PLCL2 rs4618210 polymorphism in the pathogenesis of CAD in the Chinese population. They showed a higher frequency for G allele among cases with CAD compared to controls, with more than one-fold greater risk of CAD among carriers of G-allele. The distribution of the genotypes showed no significant difference between cases with CAD and controls, while the genotype frequencies significantly differed in the CAD patients with MI compared to controls \((P < 0.05)\).

The results of our study focusing on the association of the mentioned SNP with MI was consistent with the pattern of relationship reported by Hirokawa et al. However, no significant difference was found between the frequencies of the alleles between groups. In our present study, the frequency of the minor G-allele in cases with MI was higher than the alternate frequency in Japan but lower than the estimated frequency in China. While the frequency of this allele in controls was higher than the reported frequencies in both populations. The discrepancies may result from the inter-population heterogeneity, ethnic dependency and partly by anthropometric characteristics of the recruited individuals.

MI is a complex heterogeneous disease and making a connection between the mechanisms in which the PLCL2 gene contribute in the pathogenesis of MI seems absolutely difficult. To achieve the best logical explanation for this association, we flashback to some previous reports investigating the role of PLCL2 gene in the pathobiology of human diseases. In a GWAS by Mells et al an association between PLCL2 rs1372072 polymorphism and risk of Primary biliary cirrhosis was found.15 In the other study, Sawcer et al investigated the cell-mediated immune mechanism in multiple sclerosis and reported an increased risk of disease in carriers of PLCL2 rs9821630A>G polymorphism.16 An increase in the risk of rheumatoid arthritis was shown for PLCL2 rs4535211 polymorphism.17 In recent studies, Arisimendi et al and Tsoi et al found an association between PLCL2 rs1372072 and systemic sclerosis,18 and PLCL2 rs4685408 and Psoriasis19 respectively. All the above-mentioned disorders passing through generations as multifactorial complex diseases and considered as autoimmune diseases. Then, it is probable that PLCL2 gene plays its role as a promoter of the immune reactions leading to atherosclerosis.

**Conclusion**

Our findings implied that rs4618210 might contribute to the etiology of MI, but further investigations in other population are inevitable to make a valid conclusion. Further investigation considering the association between PLCL2 rs4618210 polymorphism and the severity of the disease is suggested which our study limitation is.
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Competing interest
None.

Ethical approval
The study was approved by the local committee in our department at Islamic Azad University, Arsanjan Branch. (16030503942005) All subjects signed the written informed consent form prior to blood sampling. The study was approved by the local committee in our department.

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References
1. Topol EJ, Smith J, Plow EF, Wang QK. Genetic susceptibility to myocardial infarction and coronary artery disease. *Hum Mol Genet.* 2006;15 Spec No 2:R117-123. doi:10.1093/hmg/ddl183
2. Erdmann J, Linsel-Nitschke P, Schunkert H. Genetic causes of myocardial infarction: new insights from genome-wide association studies. *Dtch Arztebl Int.* 2010;107(40):694-699. doi:10.3238/arztebl.2010.0694
3. Ge WH, Lin Y, Li S, Zong X, Ge ZC. Identification of biomarkers for early diagnosis of acute myocardial infarction. *J Cell Biochem.* 2018;119(1):650-658. doi:10.1002/jcb.26226
4. Han Y, Dorajoo R, Chang X, Wang L, Khor CC, Sim X, et al Genome-wide association study identifies a missense variant at APOA5 for coronary artery disease in Multi-Ethnic Cohorts from Southeast Asia. *Sci Rep.* 2017;7(1):17921. doi:10.1038/s41598-017-18214-z
5. Hirokawa M, Morita H, Tajima T, Takahashi A, Ashikawa K, Miya F, et al A genome-wide association study identifies PLCL2 and AP3D1-DOT1L-SF3A2 as new susceptibility loci for myocardial infarction in Japanese. *Eur J Hum Genet.* 2015;23(3):374-380. doi:10.1038/ejhg.2014.110
6. Otsuki M, Fukami K, Kohno T, Yokota J, Takenawa T. Identification and characterization of a new phospholipase C-like protein, PLC-L2. *Biochem Biophys Res Commun.* 1999;266(1):97-103. doi:10.1006/bbrc.1999.1784
7. Ghigo A, Laffargue M, Li M, Hirsch E. PI3K and calcium signaling in cardiovascular disease. *Circ Res.* 2017;121(3):282-292. doi:10.1161/circressa.117.310183
8. Putney JW, Tomita T. Phospholipase C signaling and calcium influx. *Adv Biol Regul.* 2012;52(1):152-164. doi:10.1016/j.advenzreg.2011.09.005
9. Takenaka K, Fukumi K, Otsuki M, Nakamura Y, Kataoka Y, Wada M, et al Role of phospholipase C-L2, a novel phospholipase C-like protein that lacks lipase activity, in B-cell receptor signaling. *Mol Cell Biol.* 2003;23(20):7329-7338. doi:10.1128/mcb.23.20.7329-7338.2003
10. Uzun F, Erturk M, Cakmak HA, Kalkan AK, Akturk IF; Yalcin AA, et al Usefulness of the platelet-to-lymphocyte ratio in predicting long-term cardiovascular mortality in patients with peripheral arterial occlusive disease. *Postepy Kardiol Interwencyjnej.* 2017;13(1):32-38. doi:10.5114/ pic.2017.66184
11. Seidi A, Mirzaahmadi S, Mahmoodi K, Soleiman-Soltanpour M. The association between NFKB1 -94ATTG ins/del and NFKB1A 826C/T genetic variations and coronary artery disease risk. *Mol Biol Res Commun.* 2018;7(1):17-24. doi:10.22099/mbrc.2018.28261.1302
12. Ghaznavi H, Kiani AA, Soltanpour MS. Association study between DNA methylation and genetic variation of APOE gene with the risk of coronary artery disease. *Mol Biol Res Commun.* 2018;7(4):173-179. doi:10.22099/mbrc.2018.30955.1352
13. Dai X, Wiernek S, Evans JP, Runge MS. Genetics of coronary artery disease and myocardial infarction. *World J Cardiol.* 2016;8(1):1-23. doi:10.4330/wjc.v8.i1.1
14. Qian Q, Ma Z, Chen L, Tang C, Ma G, Chen Z. Association of PLCL2 and AP3D1-DOT1L-SF3A2 variants with the risk of coronary artery disease and its clinical phenotypes in a Chinese population. *Int J Clin Exp Med.* 2016;9(7):14684-14690.
15. Mells GF, Floyd JA, Morley KI, Cordell HJ, Franklin CS, Shin SY, et al Genome-wide association study identifies 12 new susceptibility loci for primary biliary cirrhosis. *Nat Genet.* 2011;43(4):329-332. doi:10.1038/ng.789
16. Sawcer S, Hollenhal G, Pirinen M, Spencer CC, Patzopoulos NA, Moutsianas L, et al Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature.* 2011;476(7359):214-219. doi:10.1038/nature10251
17. Bowes J, Ho P, Flynn E, Ali F, Marzo-Ortega H, Coates LC, et al Comprehensive assessment of rheumatoid arthritis susceptibility loci in a large psoriatic arthritis cohort. *Ann Rheum Dis.* 2012;71(8):1350-1354. doi:10.1136/annrheumdis-2011-200802
18. Arismendi M, Giraud M, Ruzehaji N, Dieudé P, Koumakis E, Ruiz B, et al Identification of NF-kB and PLCL2 as new susceptibility genes and highlights on a potential role of IRF8 through interferon signature modulation in systemic sclerosis. *Arthritis Res Ther.* 2015;17(1):71. doi:10.1186/s13075-015-0572-y
19. Tsai LC, Spain SL, Ellinghaus E, Stuart PE, Capon F, Knight J, et al Enhanced meta-analysis and replication studies identify five new psoriasis susceptibility loci. *Nat Commun.* 2015;6:7001. doi:10.1038/ncomms8001