Journal of Cardiovascular Disease Research

Journal of Cardiovascular Disease Research, 12 (1)
ISSN: 0975-3583, 0976-2833
DOI: 10.31838/jcdr.2021.12.01.16

Polymorphisms in *CCL2* and *IL6* genes in men with unstable atherosclerotic plaques in the coronary arteries

Striukova Evgeniia Vital'evna[1]*, Maksimov Vladimir Nikolaevich[1], Ragino Yulia Igorevna[1], Polonskaya Yana Vladimirovna[1], Murashov Ivan Sergeevich[2], Volkov Aleksandr Mihajlovich[2], Kurguzov Aleksej Vital'evich[2], Chernjavskii Aleksandr Mihajlovich[2], Kashtanova Elena Vladimirovna[1]

[1]Institution of Internal and Preventive Medicine, Federal Research Center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Russian Federation, Novosibirsk
[2]The Federal State Budgetary Institution "National Medical Research Center named academician E.N. Meshalkin" of the Ministry of Health of the Russian Federation, Novosibirsk

***Corresponding author:** Striukova Evgeniia Vital'evna Institution of Internal and Preventive Medicine – Branch of the Federal Research Center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Russian Federation, Novosibirsk. Address: 175/1 B.Bogatkova str., Novosibirsk , Russian Federation, 630089. E-mail: stryukova.j@mail.ru

Received: 17 November 2020; Accepted: 3 February 2021; Published: 28 April 2021

Abstract

Objective. To study polymorphisms of the *CCL2* and *IL6* genes to find their associations with the presence of unstable atherosclerotic plaques in the coronary arteries (CA) and with protein levels of MCP-1, IL-6, factor XII, and endothelin-1 in blood.

Materials and Methods. The study included 101 men aged 40–70 years with coronary atherosclerosis. According to histological analysis of plaques all men were divided into two groups: 40 (39.6%) with stable atherosclerotic plaques and 61 (60.4%) with stable and unstable plaques in the CA. Biochemical studies were performed by enzyme immunoassay. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism sites.

Results. Single nucleotide polymorphisms rs1024611 of the *CCL2* gene and rs1800795 of the *IL6* gene are not associated with the presence of unstable atherosclerotic plaques in CA. Levels of biochemical inflammatory markers MCP-1 and IL-6 do not differ in groups with different genotypes of *CCL2*and *IL6* genes. Differences were found in the groups of patients with stable and unstable plaques in factor XII and endothelin-1 levels in patients with different *CCL2* and *IL6* genotypes.

Conclusion. Single nucleotide polymorphisms rs1024611 of the *CCL2* gene and rs1800795 of the *IL6* gene are not associated with the presence of unstable atherosclerotic plaques in CA.

Keywords: endothelial dysfunction factors, rs1024611, rs1800795, atherosclerotic plaques

Introduction

It is now known that in addition to various factors that are of great importance in the development of the atherosclerotic focus: increased blood concentrations of total cholesterol, markers of inflammation, and endothelial dysfunction, smoking, obesity, type 2 diabetes, and genetic factors also contribute to the development of atherosclerosis.[1–3]

Modern high-tech methods of molecular genetic analysis allow discovering the influence of some endothelial dysfunction and inflammation genes polymorphisms on the progress of the atherosclerotic process.

This combination of traditional and updated technology has made the atherosclerotic process progress toward accuracy. This accuracy is beneficial, especially for young patients, in whom the risk factors are weakly expressed. Among inflammatory markers, the increased concentrations of MCP-1 and IL-6 are associated with atherosclerotic events. Polymorphisms of genes encoding these proteins can affect their structure, concentration, or function; and thereby lead to an aggravation of endothelial dysfunction and severity of atherosclerosis. Hence, this study examined *CCL2* (rs1024611, 2518A>G) and *IL6* (rs1800795, 174C>G) genes polymorphisms and their associations with the presence of unstable atherosclerotic plaques in coronary arteries (CA) in men with coronary atherosclerosis and endothelial dysfunction biomarkers (monocytic chemoattractant protein-1 [MCP-1]) or/and inflammation markers (interleukin-6 [IL-6]). Furthermore, because of minimal knowledge about elaborate regulation mechanisms of endothelial function, inflammation, and hemostasis, additional biochemical parameters like factor XII and endothelin-1 (ET-1) in patients with different *CCL2* and *IL6* genotypes were also analyzed.

Materials and Methods

The case-control study was conducted in the framework of combined scientific research of Institution of Internal and Preventive Medicine – Branch of the Federal Research Center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, (IIPM - Branch of the IC&G SB RAS) and FSBI, E.Meshalkin National Medical Research Center of Ministry of Health of the Russian Federation. The study was approved by the Local Ethics Committees of both institutions and written informed consent was obtained from all study participants before the data and sample collection.

The study included 101 men 40–70 years of age with coronary angiographic verified coronary atherosclerosis and stable angina pectoris II-II FC, without acute coronary syndrome (ACS), admitted to the Clinic of the FSBI E.Meshalkin National Medical Research Center of Ministry of Health of Russian Federation for coronary bypass surgery through endarterectomy from coronary artery/arteries for intraoperative indications. The material of endarterectomy was transversely divided into fragments containing atherosclerotic plaques for histological studies. Histological analysis of fragments of the intima/media of the CA after standard hematoxylin-eosin and Van Gieson's staining was investigated on a binocular microscope Axiostar Plus (C. Zeiss, Germany) with a digital photo output. The Waksman criteria were used to differentiate the atherosclerotic plaques into Stable or vulnerable.[4] Based on the histological conclusion, all study participants were divided into two groups: Group 1: 40 (39,6%) with only stable atherosclerotic plaques in CA and Group 2: 61 men (60.4%) with stable and unstable plaques in CA.

Exclusion criteria were ACS less than six months, acute inflammatory conditions, exacerbation of chronic inflammatory diseases, active liver diseases, chronic renal disease, and cancers.

Biochemical analysis

Biochemical analysis was performed by enzyme immunoassay with standard test-systems ELISA on analyzer Multiscan EX (Finland) before coronary artery bypass surgery. Blood was drawn from all the participant's vein in the morning after an overnight fast to obtain plasma and serum.

The plasma was used to determine Factor XII (AssayPro, St. Charles, MO). The blood serum was used to test the inflammatory and endothelial dysfunction markers:: MCP-1 (Bender Medsystems, Wien, Austria), IL-6 (Bender Medsystems), and ET-1 (Biomedica, Wien, Austria).

DNA was isolated from the peripheral venous blood by the standard phenol-chloroform extraction methodology. All polymorphisms were genotyped using polymerase chain reaction-restriction fragment length polymorphism sites (PCR-RFLP).

For PCR-amplification of the polymorphic regions of *CCL2* (rs1024611, *CCL2* promoter polymorphism), the following primers were used: 5'-CAGCATCACTCATAGAATCC-3' and 5'-AGTATCTGGAATGCAGGCTC-3'. PCR reactions were performed in reaction mixture containing 75 mM Tris hydrochloride (TRIS HCl; pH 9,0), 20 mM ammonium sulphate ($(NH_4)_2SO_4$), 0.01% Tween-20, 3 mM ($MgCl_2$), 2μl of each primer, 0.2 mM deoxyribonucleotide triphosphate (dNTPs), 2 μl DNA, one unit of Taq DNA polymerase, and water for volume adjustment to 25 μl. The amplification reactions were carried out as follows: 33 cycles including denaturation at 95°C for 30 sec,

Table 1 Restriction enzyme digestion patterns of polymerase chain reaction-amplified DNA.

Polymorphism	Molecular sizes of restriction fragments (bp)		
	Common homozygote	Heterozygote	Rare homozygote
MCP1-2518 A/G	476	476, 305, 171	305, 171
IL6 -174 G/C	110	110, 137, 130	137, 130

the annealing of primers at 63°C for 30 sec, and extension at 75°C for 30 sec. PCR amplified products were digested with restriction endonuclease Pvu II (SibEnzyme, Russia).[5] Molecular sizes of the restriction fragments are shown in Table 1.

For PCR-amplification of the polymorphic regions *IL6* (rs1800795), the following primers were used: 5'-AGCCTGTTAATCTGGTCACTGAAAA-3' and 5'-TGTGCAATGTGACGTCCTTTAGAAT-3'. PCR reactions were performed in reaction mixture containing 75 mM TRIS HCl (pH 9,0), 20 mM $(NH_4)2SO_4$, 0.01%, Tween-20 2mM $MgCl_2$, 1 µl of each primer, 0.2 mM dNTPs, 2 µl of DNA, one unit of Taq DNA polymerase, and water added to a final volume of 14 µl. The amplification reactions were carried out as follows: 30 cycles including denaturation at 95°C for 30 sec, the annealing of primers at 57°C for 30 sec, and extension at 75°C for 30 sec. PCR amplified products were digested with restriction endonuclease Hinf I (SibEnzyme, Russia). Molecular sizes of the restriction fragments are shown in Table 1.

Statistical analysis

Statistical processing of the results was carried out using SPSS 20.0 software for Windows (SPSS, Chicago, IL). The frequency of genotypes and alleles of studied polymorphisms in Group 2 men was determined. The study groups were compared by frequencies of genotypes and alleles was carried out using Pearson's chi-squared test. In the case of four-field tables, two-tailed Fisher's exact test with Yates continuity correction was applied. $P < 0.05$ was significant.

The normal distribution of biochemical parameters was verified using the Kolmogorov-Smirnov test. Under normal distribution, analysis of variance test was performed. For nonnormal distribution, the Kruskall-Wallis test and the Mann-Whitney test were used. For nominal and ordinal data scales, crosstables and Pearson's chi-squared test with likelihood adjustment was used. $P < 0.05$ was significant.

Results

No statistically significant differences were revealed between groups by frequencies of genotypes and alleles of single nucleotide polymorphisms of *CCL2* (rs1024611) and *IL6* (rs1800795) (Table 2).

We did not observe any significant differences in levels of biochemical parameters between groups with different genotypes ($P > 0.05$; Table 3).

Considering the pleiotropic effects of genes and lack of knowledge elaborate regulation mechanisms of endothelial function, inflammation, and hemostasis, additional biochemical parameters like factor XII and ET-1were analyzed in patients with different *CCL2* and *IL6* genotypes.

Regarding factor XII levels, patients with unstable plaques had higher levels of the factor XII in

Table 2 Frequencies of genotypes and alleles of *MCP-1* (rs 1024611) and *IL-6* (rs1800795) single nucleotide polymorphisms.

Single nucleotide polymorphism	Genotype/allele	Group with stable plaques		Group with unstable plaques		P value
		n	%	n	%	
CCL2 (rs1024611)	AA	17	45.9	27	46.6	0.954
	AG	18	48.6	28	48.3	0.972
	GG	2	5.4	3	5.2	0.960
	A		70.3		70.7	0.951
	G		29.7		29.3	0.951
IL6 (rs1800795)	CC	8	21.1	13	22	0.909
	CG	17	44.7	27	45.8	0.921
	GG	13	34.2	19	32.2	0.837
	C		43.4		44.9	0.902
	G		56.6		55.1	0.902

comparison with those with only stable plaques with AG genotype of *CCL2* gene (129,51 [81,76; 160,80] vs 49,64 [34,53; 83,34], P=0.001). Also, patients with unstable plaques had higher levels of the factor XII in comparison with patients with stable plaques with GG genotype of *IL6* gene were revealed (133,49 [76,22; 157,46] vs 43,06 [25,92; 59,83], P = 0.001). No significant differences between patients with other genotypes were revealed (Table 4).

Regarding ET-11 level, patients with stable plaques had higher levels of ET-1in comparison with those with unstable plaques with GG genotype of *IL6* gene (0,58 [0,33; 0,86] vs 0,24 [0,13; 0,67], P = 0.043). Among patients with unstable plaques, differences in the level of ET-1between patients with different genotypes were revealed (P = 0.043). No statistically significant differences between patients with other genotypes were revealed. Neither in the group with stable plaques nor the group with unstable ones did we find any differences in ET-1 levels in patients with different *CCL2* genotypes (Table 5).

Discussion

MCP-1

The MCP-1 designated as *CCL2* belongs to the family of chemotactic cytokines. It acts as an agonist of monocytes, memory T-cells, and basophils.[6]

MCP-1 is encoded by a member of the small inducible gene family. It has been shown to play role in monocyte chemotaxis at the sites of damage and

Table 3 Blood levels of biochemical parameters depending on the genotype in men with stable and unstable plaques.

Biochemical parameter	Genotype	General group	Group with stable plaques	Group with unstable plaques	P value
		Median [quartiles]			
MCP-1, pg / ml	AA (*CCL2*)	487.43 [352.55; 620,38]	404,21 [343,07; 611, 41]	563,86 [370,24; 626,90]	0.302
	AG (*CCL2*)	514,61 [352,82; 711,81]	429,69 [343,48; 629,81]	557,066 [361,83; 836,10]	0.347
	GG (*CCL2*)	470,46 [401,25; 983,78]	821,73 [497,62]	415,27 [387,23]	0.333
IL-6, pg / ml	CC (*IL6*)	5,00 [1,40; 16,99]	1,87 [1,27; 16,99]	5,88 [1,77; 18,06]	0.646
	CG (*IL6*)	4,34 [1,47; 15,00]	8,25 [1,84; 20,6]	4,34 [1,27; 12,88]	0.309
	GG (*IL6*)	8,63 [2,65; 21,85]	10,38 [2,92; 34,98]	8,50 [1,55; 16,00]	0.628

Table 4 Blood levels of factor XII depending on the CCL2 and IL6 genotype in men with stable and unstable plaques.

Biochemical parameter	Genotype	General group	Group with stable plaques	Group with unstable plaques	P value (between groups with stable and unstable plaques)
		Median [quartiles]			
Factor XII, µg/ml	AA (*CCL2*)	90,26 [43,73; 144,00]	59,67 [34,89; 129,00]	126,67 [48,82; 149,23]	0.198
	AG (*CCL2*)	89,01 [53,84; 147,24]	49,64 [34,53; 83,34]	129,51 [81,76; 160,80]	*0.001*
	GG (*CCL2*)	–	–	–	–
Factor XII, µg/ml	CC (*IL6*)	124,00 [67,24; 159,34]	107,13 [58,78; 160,00]	133,13 [67,24; 160,35]	0.750
	CG (*IL6*)	89,35 [39,78; 145,17]	72,67 [37,76; 149,50]	110,34 [66,67; 146,33]	0.313
	GG (*IL6*)	66,43 [35,60; 140,37]	43,06 [25,92; 59,83]	133,49 [76,22; 157,46]	*0.001*

Table 5 Blood levels of endothelin-1 depending on the *CCL2* and *IL6* genotype in men with stable and unstable plaques.

Biochemical parameter	Genotype	General group	Group with stable plaques	Group with unstable plaques	P value (between groups with stable and unstable plaques)
		Median [quartiles]			
Endothelin-1, pmol/l	AA (*CCL2*)	0,51 [0,25; 0,81]	0,50 [0,30; 0,68]	0,51 [0,25; 0,89]	0.999
	AG (*CCL2*)	0,53 [0,23; 0,85]	0,63 [0,34; 0,89]	0,32 [0,20; 0,79]	0.272
	GG (*CCL2*)	0,90 [0,34]	—	—	—
Endothelin-1, pmol/l	CC (*IL6*)	0,49 [0,28; 0,87]	0,51 [0,34; 0,88]	0,29 [0,21; 0,86]	0.285
	CG (*IL6*)	0,64 [0,29; 0,90]	0,58 [0,17; 0,76]	0,66 [0,31; 1,31]	0.259
	GG (*IL6*)	0,34 [0,23; 0,84]	0,58 [0,33; 0,86]	0,24 [0,13; 0,67]	*0.019*

infection. MCP-1 is mainly expressed by inflammatory and endothelial cells. The level of MCP-1 expression increases after proinflammatory stimuli and tissue damage associated with atherosclerotic lesions. It has been reported to play pivotal roles in the pathogenesis of atherosclerosis. In particular monocytes and macrophages containing MCP-1 affect the growth of other cell types in atherosclerotic foci.[7]

The *CCL2* gene was localized in the human chromosome 17 in the somatic cell analysis. *In situ* hybridization confirmed this and an additionally localized gene - 17q11.2-q21.1.[8] It has been demonstrated that the single nucleotide polymorphism –2518 A> G (rs1024611) of the *CCL2* gene in the promoter region can modulate MCP-1 expression levels.[9] The *CCL2*-2518 G allele is associated with increased production both in the MCP-1 transcript and in the protein vs. allele 2518 A.

A 2015 meta-analysis confirms that *CCL2* gene polymorphism 2518 A / G is associated with coronary artery disease (CAD), the G allele may also be a genetic risk factor for CAD. The overall frequency of the G allele in the control group significantly varies in different ethnic groups (0.43 and 0.27 in Asian and non-Asian, respectively). Indicating that genotype distribution was significantly associated with ethnicity.[10] Further large-scale, well-designed studies are still needed to confirm the results of the meta-analysis.

However, this study did not find differences in the genotypes of rs1024611 polymorphism of the *CCL2* gene, which may be explained by the fact that all analyzed patients had diagnosed CAD with multifocal lesions of CA.

IL-6

IL-6 is the main proinflammatory and procoagulant cytokine. They are produced by various cell types including activated monocytes, macrophages, endothelial cells, adipocytes, and Th2-cells. IL-6 production is initiated by infections. Elevated levels of IL-6 are detected under chronic inflammatory conditions associated with a higher risk of CAD. IL-6 enhances the inflammatory cascade by stimulating the liver's synthesis of acute-phase proteins, like C-reactive protein and fibrinogen. The other functions of IL-6 include activation of endothelial cells, activation of the hypothalamic-pituitary-adrenal system, stimulation of lymphocytes, proliferation, differentiation, and oxidation of lipoproteins.[11] Hence, IL-6 may play a central role in the initiation and progression of atherosclerotic plaques.[12]

The *IL6* gene is located on chromosome 7p21 and encodes the protein IL-6, which is one of the most active cytokines, involved in the implementation of the immune and inflammatory response.[13]

Many studies explored the associations between the *IL6* gene polymorphism and CAD progression. Research conducted in the Asian population found that 174G/C, C572G, rs8034928, and rs11556218 single nucleotide polymorphisms of the *IL6* gene were significantly associated with a higher risk of CAD.[14] In another study in the European population, it was proved that the homozygous genotype T/T of polymorphism C(-260)T of the gene *IL6* was more often isolated at the first clinical manifestation of CAD.[15] Dietel et al.[16] proved an association between *IL6* polymorphism and atherosclerotic lesion of vessels.

A large meta-analysis including 19 studies (9417 patients with CAD vs. 15,982 patients in the control group) shown that patients with allele –174C compared to –174G had a 4% higher risk of developing CAD. This study also provided conclusive evidence of an association between increased IL-6 and CAD development.[17]

This study did not find differences between patients with stable and unstable plaques in frequencies of the rs1800795 polymorphism genotypes of the *IL6* gene.

ET-1 and Factor XII

ET-1 is a vasoconstricting peptide consisting of 21 amino acids. It was first detected in the cells of the aortic endothelium in a pig. Analysis of the human genome showed that ET belonged to the ET family, consisting of ET-1, ET-2, and ET-3. The tissue distribution of the ET family genes expression differs from each other. Humans have ET-1 in many organs, such as the brain, kidneys, lungs, uterus, placenta, and endothelium cells. The matrix RNA of ET-2 is concentrated in the jejunum, adrenal glands, brain, pancreas, and renal medulla. ET-1 is proved to play the role of a neuropeptide in the central nervous system. The functions of ET-2 and ET-3 (except the vasoconstrictor one) are still poorly understood. ETs are biological activity after binding to specific receptors on the membranes of target cells.[18] The study by Zhou et al. suggested that ET-1 is an independent marker of cardiovascular outcomes risk in patients with stable CAD.[19]

Coagulation factor XII is a proenzyme of serine protease, factor XIIa. Factor XII converts to factor XIIa in the autoactivation process, induced by contact with charged surfaces. They are one of the key substances in fibrin formation, while upon its deficiency, increased bleeding is not observed. [20]

The primary main function of Factor XII involves the activation of the intrinsic coagulation pathway and kallikrein-kinin system. But previous studies have shown that they may also directly regulate cellular responses. Factor XII/XIIa was found to induce expression of inflammation mediators, promote cells proliferation, and enhance migration of neutrophils and lung fibroblasts.[21] They play a critical role in the formation of atherosclerotic lesions. Act as an inductor of proinflammatory cytokines in antigen-presenting mouse cells.[22]

This study revealed differences in factor XII concentrations in bloodstream between the genotypes of the *CCL2* and *IL6* genes. It also recorded the differences ET-1 between groups with stable and unstable plaques with genotype GG of the *IL6* gene.

There is a limited amount of research available on the associations between *CCL2* and *IL6* genes and various inflammatory cytokines, endothelial dysfunction markers, and coagulation factors. In Online Mendelian Inheritance in Man (OMIM – An Online Catalog of Human Genes and Genetic Disorders), these genes are associated with several heterogeneous phenotypes that highlight pleiotropy and require immense research. Indirect support of this assumption is the associations we found. But this needs to be replicated on large independent samples.

Conclusion

Single nucleotide polymorphisms rs1024611 of the *CCL2* gene and rs1800795 of the *IL6* gene are not associated with the presence of unstable atherosclerotic plaques in CA. Levels of MCP-1 and IL-6 (biochemical inflammatory markers) do not differ in groups with different genotypes of *CCL2* and *IL6* genes.

Differences were found in the patients with stable and unstable plaques in factor XII and ET-1 levels in patients with different *CCL2* and *IL6* genotypes.

Author Contributions

Y.I. Ragino conceived and designed the study. I.S. Murashov, A.M. Volkov, A.V. Kurguzov, and A.M. Chernjavskii operated and collected samples. E. V. Striukova, Y.V. Polonskaya, and E.V. Kashtanova conducted the biochemical analysis. V.N. Maximov and E. V. Striukova conducted the genetic analysis. They were also involved in the acquisition, analysis, interpretation of the data, and manuscript writing. All authors read and approved the final manuscript.

Funding

The study was performed in the framework of the budget theme of the State assignment No AAAA-A17-117112850280-2, as part of the budget theme for supporting bioresource collections under the state task no. 0324-2017-0048, with the financial support of RFBR Grant No. 19-015-00055a. The funding body had no role in the study design, data collection, analysis, interpretation of data, and manuscript writing.

Conflict of Interest

The authors declare that they have no conflict of interests.

Ethical Approval

All procedures performed in studies involving human participants were under the ethical standards of both institutions and the national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Local Ethics Committees of both institutions.

Informed Consent

The data and samples were collected after written informed was consent obtained from all study participants.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available because they are part of a larger dataset reported separately but are available from the corresponding author on reasonable request.

References

1. Kalayi Nia S, Ziaee S, Boroumand MA, Sotudeh Anvari M, Pourgholi L, Jalali A. The impact of vascular endothelial growth factor +405 C/G polymorphism on long-term outcome and severity of coronary artery disease. J Clin Lab Anal . 2017 ;31(4):e22066.
2. Abraham G, Havulinna AS, Bhalala OG, Byars SG, De Livera AM, Yetukuri L, et al. Genomic prediction of coronary heart disease. Eur Heart J. 2016; 37 (43): 3267–78.
3. de Vries MA, Trompet S, Mooijaart SP, Smit RAJ, Böhringer S, Castro Cabezas M, et al. Complement receptor 1 gene polymorphisms are associated with cardiovascular risk. Atherosclerosis. 2017;257:16–21.
4. Waksman Ron, Patrick W. Serruys, Schaar J. Handbook of the vulnerable plaque. 2nd ed. London: CRC Press; 2007. 1–48 p.
5. Dechkum N, Hananantachai H, Patarapotikul J, Ohashi J, Krudsood S, Looareesuwan S, et al. Monocyte chemoattractant protein 1 (MCP-1) gene polymorphism is not associated with severe and cerebral malaria in Thailand . Jpn J Infect Dis. 2006;59(4):239–44.
6. Charo IF, Taubman MB. Chemokines in the pathogenesis of vascular disease. Circ Res. 2004;95: 858–66.
7. Lin J, Kakkar V, Lu X. Impact of MCP -1 in atherosclerosis. Curr Pharm Des . 2014;20(28):4580–8.
8. Reference SNP (refSNP) Cluster Report: rs1024611 [cited 2020 Sep 8]. Available from: https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?do_not_redirect&rs=rs1024611
9. Rovin BH, Lu L, Saxena R. A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. Biochem Biophys Res Commun. 1999;259(2):344–8.
10. Cai G, Zhang B, Weng W, Shi G, Huang Z. The associations between the MCP-1 −2518 A/G polymorphism and ischemic heart disease and ischemic stroke: A meta-analysis of 28 research studies involving 21,524 individuals. Mol Biol Rep. 2015 Apr 8;42(5):997–1012.
11. Schuett H, Luchtefeld M, Grothusen C, Grote K, Schieffer B. How much is too much? Interleukin-6 and its signalling in atherosclerosis. Thromb Haemost. 2009 Aug;102(2): 215–22.
12. Hartman J, Frishman WH. Inflammation and atherosclerosis: A review of the role of interleukin-6 in the development of atherosclerosis and the potential for targeted drug therapy. Cardiol Rev. 2014 May-Jun;22(3):147–51.
13. Reference SNP (refSNP) Cluster Report: rs1800795 [cited 2020 Sep 8]. Available from: https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?do_not_redirect&rs=rs1800795
14. Li YY, Zhou CW, Xu J, Qian Y, Wang XM. Interleukin-6 C-572G gene polymorphism and coronary artery disease in asian: A meta-analysis of 2511 subjects. Int J Clin Exp Med. 2015;8(6):8995–9003.
15. Rizzello V, Liuzzo G, Trabetti E, Di Giannuario G, Brugaletta S, Santamaria M, et al. Role of the CD14 C(-260)T promoter polymorphism in determining the first clinical manifestation of coronary artery disease. J Cardiovasc Med. 2010;11(1):20–5.
16. Dietel B, Cicha I, Voskens CJ, Verhoeven E, Achenbach S, Garlichs CD. Decreased numbers of regulatory T cells are associated with human atherosclerotic lesion vulnerability and inversely correlate with infiltrated mature dendritic cells. Atherosclerosis. 2013;230(1):92–9.
17. Niu W, Liu Y, Qi Y, Wu Z, Zhu D, Jin W. Association of interleukin-6 circulating levels with coronary artery disease: a meta-analysis implementing mendelian randomization approach. Int J Cardiol. 2012 May 31;157(2):243–52.
18. Arinami T, Ishikawa M, Inoue A, Yanagisawa M, Masaki T, Yoshidaj MC, et al. Chromosomal assignments of the human endothelin family genes: The endothelin-I gene (EDN I) to 6p23-p24, the endothelin-2 gene (EDN2) to Ip34, and the endothelin-3 Gene (EDN3) to 20q1 3.2-q 1 3.3. Am J Hum Genet. 1991; 48(5):990–6.
19. Zhou BY, Guo YL, Wu NQ, Zhu CG, Gao Y, Qing P, et al. Plasma big endothelin-1 levels at admission and future cardiovascular outcomes: A cohort study in patients with stable coronary artery disease. Int J Cardiol. 2017 Mar 1;230:76–9.
20. Renné T, Schmaier AH, Nickel KF, Blombäck M, Maas C. In vivo roles of factor XII [Internet]. Blood. 2012;120: 4296–303.
21. Didiasova M, Wujak L, Schaefer L, Wygrecka M. Factor XII in coagulation, inflammation and beyond. Cell Signal. 2018; 51: 257–65.
22. Vorlova S, Koch M, Manthey HD, Cochain C, Busch M, Chaudhari SM, et al. Coagulation factor XII induces pro-inflammatory cytokine responses in macrophages and promotes atherosclerosis in mice. Thromb Haemost. 2017;117(1):176–87.